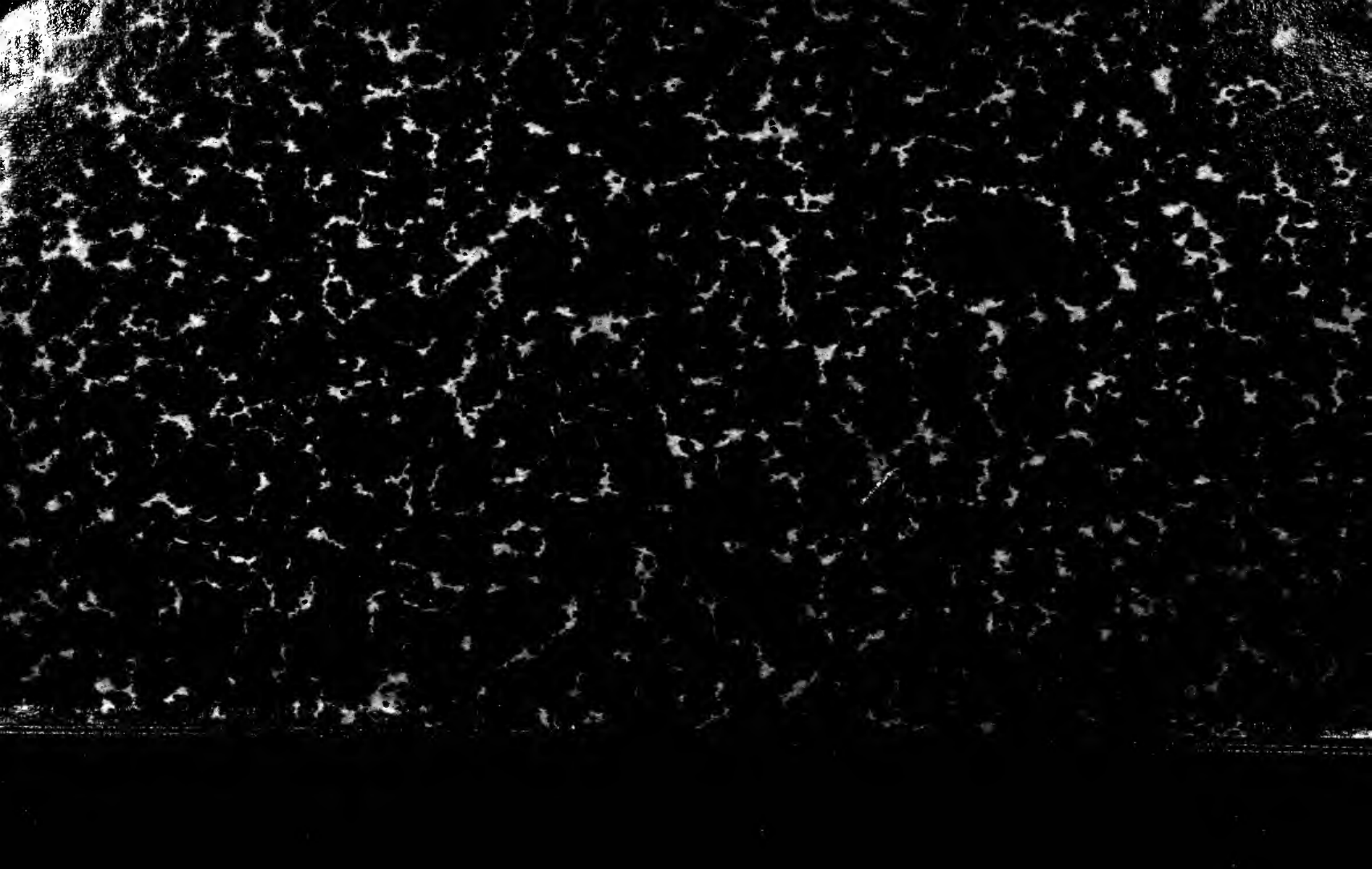

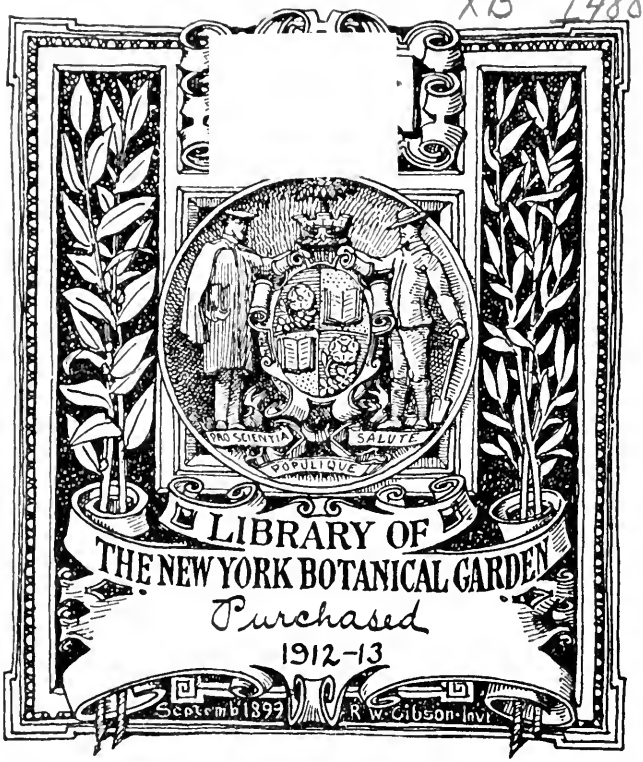
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WITH EIGHT PORTRAITS, EIGHT PLATES AND TWO ADDITIONAL ILLUSTRATIONS

NEW YORK
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VOLUME II SEPTEMBER, 1912 No. 5

IN MEMORIAM

ERNST SCHULZE

Born July 31, 1840 Died June 15, 1912

The death of Ernst Schulze is an irreparable loss to biology. Wherever the biochemistry of plants is appreciated, Schulze's death causes profound sorrow. Schulze was one of the founders of our present exact biochemical investigation. His researches in phytochemistry are classical and they have been charged with fundamental ideas that continue to influence research in this great field.

Dr. Ernst Schulze, professor of agricultural chemistry in the Eidgenössischen Technischen Hochschule at Zürich, was born July 31, 1840, in the hamlet of Bovenden, near Göttingen. In 1858 Schulze studied chemistry under Wöhler in Göttingen and also spent a semester with Bunsen in Heidelberg. In 1861 he was assistant to Lehman, and subsequently to Geuther, at the Chemical Institute in Jena. His scientific activity began at the Agricultural Experiment Station in Weende, under the direction of Henneberg. In 1871 Schulze was appointed director of the newly founded Agricultural Experiment Station in Darmstadt. Even while he was at Weende, his ability had attracted the attention of the Eidgenössischen institution. In June, 1872, he was called to Zürich, where his activities continued fruitfully for forty years.

Schulze's first important research was published with the collaboration of his friend Märcker, in 1870, in the *Journal für Landwirtschaft*. In this paper it was shown that the principles of pro-

tein metabolism, as they were stated by Voit on the basis of experiments on carnivorous animals, applied to the ruminants as well. In Zürich, Schulze brought his researches in *animal* physiology to an end by a thorough investigation of wool-fat. He succeeded in preparing typical cholesterol in a pure state and in isolating an isomer, isocholesterol.

Since 1872 Schulze had concerned himself exclusively with phytochemical research; and forty years of activity in this field fortified the conclusion that plants and animals contain the same classes of substances and that the chemical composition of animals is in many ways identical with that of plants.

Schulze developed new methods for the quantitative determination of nitrogenous substances and showed how to separate them in pure forms from the complex mixtures in plant juices and extracts. With his collaborators Schulze made classical discoveries of the following nitrogenous compounds and, by masterly methods, established their constitution:

Glutamin, an amide of glutamic acid;

Arginin, guanido-α-aminovalerianic acid;

Phenylalanin, β-phenyl-α-aminopropionic acid;

Vernin (identical with the guanosin subsequently obtained by Levene from nucleic acid);

Stachydrin, the dimethylbetain of α-prolin;

Lupinin, an alkaloid from lupins.

Schulze found the following nitrogenous substances in different plant materials and studied their role in plant metabolism: aminovalerianic acid, leucin, isoleucin, prolin, glutamin, asparagin, phenylalanin, tyrosin, arginin, histidin, lysin, vicin, convicin, xanthin, hypoxanthin, guanidin, vernin, allantoin, cholin, betain, trigonellin, and stachydrin. Schulze was working with the betains during his last illness but, unfortunately, he was unable to complete this research. Considerable interest was aroused by his discovery of the presence of allantoin in plants.

Schulze was the first to make a successful investigation of phytolecithins (phosphatids) and their cleavage products. He found that the lecithin in many seeds can be extracted only with *boiling* alcohol. For this reason he believed that lecithin exists in such

seeds in some sort of combination with proteins. In this connection he investigated the plant cholesterols, or phytosterols.

Schulze then began his thorough studies of the carbohydrates and nitrogen-free reserve materials in plants. A paper entitled: "Untersuchungen über die stickstofffreien Reservestoffe der Samen von *Lupinus luteus* und über die Umwandlung derselben während des Keimungsprozesses," was given a prize by the Königlichen Gesellschaft der Wissenschaften in Göttingen.

In this connection Schulze studied the constituents of cell membranes in plants. He showed that the walls of various plant-cells contain carbohydrates which resemble cellulose to a certain extent but differ from it by dissolving easily in warm dilute solutions of acids and alkalis. These cell-wall constituents proved to be xylans, arabans, galactans, and mannans. They play the part of food reserves in seeds. Schulze called them "hemi-celluloses." He showed, further, that ordinary cellulose on hydrolysis yields other glucoses besides dextrose. *Stachys* tubers were found to contain stachyose, a tetrasaccharid. All these researches yielded data and experience that proved useful to Schulze in his discussions and development of analytical methods for phytochemical research.

The role of asparagin and glutamin in the protein metabolism and synthesis in plants greatly interested Schulze to the end of his life. Although he was not able fully to explain the process of protein synthesis, he made fundamental contributions to the subject. He clarified our knowledge of protein metabolism in seedlings. What chemist or biologist has not heard of the investigations which were begun in 1876, and whose results were usually published in Prussian agricultural year books and also in the *Zeitschrift für physiologische Chemie?* Even in his second paper on the subject, in 1878, Schulze showed the importance of the characteristic composition of etiolated seedlings and their high asparagin content. He concluded from his observations that *the protein decomposition products do not persist, in seedlings, in the proportions in which they were originally produced from protein, but, that after such protein cleavage, these nitrogenous substances seem to be changed for the most part into asparagin.*

Schulze prepared a great many plant proteins and studied their

decomposition products. After his pupils, working outside our Institute, had taken an active part in the study of protein metabolism in seedlings, and after it had been shown in our laboratory that protein decomposition in seedlings is an enzymic process, Schulze came to the following general conclusions regarding the protein transformations in seedlings: Asparagin is formed in seedlings at the expense of proteins and arises from the same material in etiolated young green plants, and in young leaves and shoots; arginin also results in seedlings from *direct* decomposition of protein. Perhaps the individual *amids* arise in the leaf-buds *in the proportions of their production from protein by hydrolysis with acids and other agents outside the organism,* but probably with the difference that, in the leaf-buds, neither aspartic acid nor glutamic acid is produced, the amids of these amino acids, viz., asparagin and glutamin, resulting instead. *Amino acids,* however, do not occur in plants in such proportions, since they are consumed in the plant metabolism, some more rapidly, it seems, than others. The accumulation of asparagin in seedlings is caused by the formation of this amide from other products (amino acids) of the transformation of protein. One of the best arguments for this conception is the observation, made by Schulze in his experiments and repeatedly emphasized in his papers, that *in many cases asparagin is produced abundantly even after the processes of protein decompositions in the plant have ceased.*

An admirable outcome of Schulze's investigations is his great compilation on the composition of cultivated plants, where he reviews briefly the methods of research and gives abundant data on the chemical constituents of these plants.

The later years of Schulze's life were spent in close retirement because of a serious and long standing eye-disease that prevented him from appearing in public. He lived, at the end, only for his science and for his family. His colleagues often wondered how, with his weak eyes, he was able to do any experimental work whatever. It was pathetic to see with what extreme care and patience he had to tax himself in order to proceed with his work.

When Schulze celebrated his seventieth birthday, two years ago, we all hoped that the twilight of his life might be long and happy,

but in vain, for pitiless death took him from us. His pupils mourn a beloved friend and guide; and science, a distinguished investigator.[1]

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[1] The foregoing biographical communication was translated from Prof. E. Winterstein's manuscript, in German, by Dr. Ernest D. Clark. Prof. Winterstein's manuscript of the appended bibliography is reproduced verbatim. [Ed.]

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A RESUMÉ OF THE LITERATURE ON INOSITE-PHOSPHORIC ACID, WITH SPECIAL REFERENCE TO THE RELATION OF THAT SUBSTANCE TO PLANTS

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Contents.—Discovery: by the microscopist, 21; by the chemist, 22. Occurrence, 23; preparation, 25; properties, 26; constitution, 31; terminology and classification, 35; analytical methods, 37; role in plants, 39. Bibliography, 46.

DISCOVERY OF INOSITE-PHOSPHORIC ACID SALTS BY THE MICROSCOPIST

In 1854 Hartig,[1] engaged in a microscopic study of the seeds of various plants, noted in all his sections small particles similar to the starch grains of the potato, which at that time were absorbing the interest of plant physiologists.[2] These grains were obviously not starch, as they did not give the characteristic blue color with iodine in potassium iodid solution. They are more commonly present in seeds than starch, the latter being frequently replaced by fat. Hartig considered them an essential reserve product designed to play an important part in the germination of the seed and the growth of the plant. He first called them "Klebermehl" but within a year renamed them "aleurone grains" from the Greek ἄλευρον (wheat flour), a term still in use. Not only did he consider them significant for the plants in which they are found but also for the animals which eat them. The method which he employed for separating them from the other parts of the seed is the one usually followed by the investigators who have since worked with these

[1] The papers referred to in the text and not accompanied by footnote references are those which pertain specially to the literature of inosite-phosphoric acid and are given at the end of this review in the alphabetical order of the names of the authors.

[2] The starch grains were minutely studied by Nägeli and his coworkers. Their work (collected in Die Stärkekörner, 1858) probably afforded the stimulus for the efforts which led to the discovery of inosite-phosphoric acid so early.

grains, namely, extraction of the macerated seeds with ether and removal of the aleuron grains from the cellular debris by sedimentation in this medium. They are insoluble in both alcohol and ether, somewhat soluble in water, and quite soluble in dilute acids.

In the years following Hartig's discovery several other botanists turned their attention to the aleuron grains and came to conflicting conclusions as to their nature. Von Holle considered them protein carriers and referred to them as "Proteinkörner." Both Sachs and Gris looked upon the particles as fat concentrates. By far the most important study in this field was the comprehensive work of Pfeffer in 1872. He differentiated the grains described by Hartig into three groups: (1) crystals of calcium oxalate, (2) a protein substance, and (3) a compound giving no reactions for protein, fat, or inorganic salts. This last type was found in all of the one hundred different seeds which he examined. These particles he described as having rounded surfaces, assuming spheroidal shapes and frequently twinning, so as to present a convoluted appearance. Enough of the grains were obtained for a chemical examination, which was made for him by his colleague Brandon. The solubilities were the same as those reported by Hartig. Nitrogen could not be detected. Positive tests were obtained for calcium, magnesium, and phosphorus. Organic matter was noted and the suggestion made that the substance was a phosphate combined with a carbohydrate. These phosphorus-bearing spheroidal bodies occurring with or in the aleuron grains Pfeffer named "Globoid."

DISCOVERY OF INOSITE-PHOSPHORIC ACID BY THE CHEMIST

Palladin, while engaged on a study of the proteins of *Sinapis nigcr* in 1893, observed an unusual phenomenon. After extraction of the fat-free finely ground seeds with ten per cent. sodium chlorid solution and heating the extract, he obtained a voluminous precipitate which partly dissolved on standing. A few trials showed that he had a substance soluble in cold but insoluble in hot water. By filtering off the permanent coagulum, reheating the filtrate and filtering while hot, he obtained a fairly pure product rich in phosphorus, containing calcium and magnesium, but no nitrogen. It proved non-reducing when tested with Fehling solution, both before

and after hydrolysis with acids. It was soluble in water and acids, and precipitated by the alkali earths and the heavy metals.

Subsequently Schulze and Winterstein published a paper confirming the observations of Palladin, and also noting that the phosphorus was not precipitated by ammonium molybdate. These authors expressed the opinion that the compound thus discovered by chemical procedure is identical with Pfeffer's "globoid." The following year (1897), a more detailed paper was published by the junior author, in which the identity and properties of the substance were more fully revealed, and "inosite-phosphoric acid" was suggested as the proper name for the compound, inasmuch as it yielded inosite and phosphoric acid on hydrolysis.

The most complete study of this substance has been made by Posternak; his findings are embodied in eight papers and several applications for patents. He discarded the name suggested by Winterstein and proposed a structural formula which does not include the inosite ring. He gave to the substance the name "phytin" (from the Greek φύτην) and under this trade name it is now placed on the market by a chemical firm in Basel.

OCCURRENCE OF INOSITE-PHOSPHORIC ACID

As already noted, phytin was first thought to be a storage product in seeds; and this early impression has been confirmed by subsequent investigation, no case having been reported of a seed in which it is completely lacking. The accompanying table (1) lists the plants specially mentioned in the literature in connection with the study of inosite-phosphoric acid.

The relative data in the table are not in close accord, but no true comparison can as yet be drawn between the species, for most of the data were obtained at periods when adequate and uniform analytical methods were unavailable. The figures quoted in the table give an approximate idea of the quantitative significance of this important compound, in relation to other forms of phosphorus available for seedling growth and the phosphorus requirement of man and beast. Posternak makes the statement that seeds rich in fat carry the largest amount of phosphorus, which is in harmony with the microscopist's observations that the aleuron grains are particularly numer-

TABLE I

Recorded analytic data on the occurrence of inosite-phosphoric acid

Plant.	Total P Per Cent.	Ratio of the P in phyto-phosphate to Total P Per Cent.	Plant.	Total P Per Cent.	Ratio of the P in phyto-phosphate to Total P Per Cent.
Spruce fir	0.66	91.5	Rye flour		25.0
Pea (*Pisum sativum*)..	0.37	70.8	Rape (*Brassica napus olifera*)	0.98	80.0
Pea, yellow		19.0		1.19	44.5
Bean, white (*Phascolus vulgaris*)	0.51	81.6		0.54	38.0
Bean, brown		58.0	Rape cake		49.5
Hemp (*Cannabis sativa*)	1.46	91.4	Soy bean (*Soja hispida*)	0.57	58.0
	0.76	15.0	Lentil (*Lens esculenta*)	0.30	82.6
Rice (*Oryza sativa*)...	0.95	45.9		0.30	9.3
Rice flour		69.0	Cocoanut (*Cocus nucifera*)		88.4
Rice bran	2.22	74.1			69.5
Rice germ	6.20	89.2	Cottonseed (*Gossipium herbaccum*)		93.6
Wheat (*Triticum sativa*)	0.45	29.9	Pine: *Pinus cembra* ...	0.47	14.39
Wheat bran	1.11	84.0	*Pinus excelsa* ...	0.63	21.6
	1.11	52.0	Castor bean (*Ricinus communis*)	0.26	41.6
Graham flour		29.0	Millet (*Panicum milliaceum*)	0.77	44.97
Sesame (*Sesamum indicum*)	0.77	16.3	Vetch (*Vicia faba minor*)	0.47	4.4
Corn (*Zea mays*)	0.29	54.0	Red Clover (Hay)	0.24	70.0
	0.35	48.9	Radish: Root (*Raphanus vulgaris*)	0.02	15.0
Oats (*Avena sativa*) ..	0.46	48.0	Turnip: Root (*Brassica esculenta?*)	0.02	15.0
		87.4	Dahlia: Tuber (*Dahlia variabilis*) ...	"Spheroids of phytin"	
Barley (*Hordium sativum*)	0.50	38.0	Potato: Tuber (*Allium cepa*)	"Spheroids of phytin"	
	0.47	36.5			
	0.54	44.0			
Barley bran		60.4			
Sunflower (*Helianthus annuus*)	0.83	86.3			
Rye (*Secale cereale*) ..		90.3			
	0.43	28.9			

The analytic results in the above table are those for *seeds* of the plants, except in the last five cases. They are compiled from a number of sources. Among the plants studied for their phyto-phosphate content, in which the relative amount of this substance is not given, are the following: *Beta vulgaris, Brassica campestris, Cucurbita pepo, Ervum lens, Lupinus albus, L. angustifolius, L. luteus, Pinus laricio, P. maritima, Sinapis nigra, Solanum tuberosum,* and the tubers of *Allium cepa* and *Dahlia variabilis.* In only two materials reported, namely, rutabaga root and alfalfa hay, could no phyto-phosphate be found. In several instances the total phosphorus was not reported. Where there is a close agreement between two or more results, only one figure is given above.

ous in oily seeds. He also remarks that the smaller seeds such as cereals are the richest in "phytin." This compound is not entirely confined to seeds, its presence having also been noted in the potato near the eye and as characteristic spheroids in the tubers of *Allium* and *Dahlia*. Roots functioning as storage organs, such as those of the *Brassicae*, contain small amounts. None was found by Tottingham and Hart in the mature stems and leaves of the common fodder plants, but it occurs in clover leaves and in millet during the late flowering period, and also in tender shoots.

PREPARATION OF INOSITE-PHOSPHORIC ACID AND ITS SALTS

To prepare phytin or its closely related compounds from seeds, they should be finely ground and, if fat is present in large amounts, it should be removed by extraction with ether and alcohol. Most of the preparations reported in the literature have been obtained from cereals by leaching with 0.2 per cent. hydrochloric acid solution. Acetic acid has also been used in 1 per cent. solution, and in a few cases acid solutions of greater concentration have been employed. To remove the soluble proteins from the extract, Levene used picric acid; other investigators have coagulated them by heating and filtration after cooling; but when acidulated water is used the proteins do not seem to interfere appreciably with the preparation of pure phytin. The reserve proteins of the seeds are of the globulin type and are soluble only in the presence of salt in the extracting agent. Precipitation of inosite-phosphoric acid from its solutions can be accomplished by several methods, such as the use of the acetates of the heavy metals, barium chlorid in ammoniacal solution or magnesia mixture. In these cases the precipitated compound is obviously in a form different from that in which it occurs in the original material. To obtain the salt more nearly in the form in which it is found in the seed, it may be precipitated by heating the solution to almost boiling and filtering while hot; or better, by adding four volumes of ninety-five per cent. alcohol. In obtaining pure preparations of inosite-phosphoric acid or its salts, a number of reprecipitations are necessary. These have been made alternately with copper, lead, and barium. Salts of the first two are decomposed by suspending in distilled water and bubbling hydrogen sul-

phide through the liquid; the third is removed by adding dilute sulphuric acid. In all cases, the lead or copper salt is the last precipitated in this manner of purification; and when the product is carefully washed, and the metal removed by hydrogen sulphide, the filtrate from the lead or copper sulphide is evaporated at a low temperature, leaving the inosite-phosphoric acid.[3]

The various salts which have been studied were made from this acid. In obtaining the acid for the preparation of pure compounds, the greatest difficulty lies in removing the last traces of magnesium. Rising overcame this difficulty by taking up the syrupy acid with absolute alcohol, and adding ether until droplets of the acid formed. He then filtered off the acid magnesium inosite-phosphate and again evaporated. The commonest impurity in phytin is inorganic orthophosphate which, however, is easily removed. Starkenstein uses the calcium salts of the mixed acids and washes with glacial acetic acid, which dissolves the inorganic part but not the organic phosphorus compound. Forbes precipitates with magnesia mixture, removes the excess of this reagent by washing with ammonia water, washing again with alcohol, and extracting with 95 per cent. alcohol containing 0.2 per cent. mineral acid, which also dissolves all the inorganic phosphorus and none of the "phytin." Attempts to prepare these salts synthetically will be referred to in a later section.

PROPERTIES OF INOSITE-PHOSPHORIC ACID AND ITS SALTS

The substance widely known as "phytin," and described in the middle of the last century by the microscopists as "spheroid bodies," frequently assumes the globular shape when forced out of solution. In most cases, the precipitate comes down as a flocculent amorphous mass. Inosite-phosphoric acid has not as yet been obtained in crystalline form. At room temperature, it is a syrup of light straw color, which becomes very viscid on cooling to —20° C., and darkens on heating to 100° C. Vorbrodt found that this coloration could not be prevented by replacing the air with an inert gas during the heating, and from this concludes that the change is not due to oxidation. If the heat is allowed to reach 125° C., an insoluble dark char is produced (cf. Posternak). Inosite-phosphoric acid may

[3] For details of the method of preparing the acid see Hart and Patten (page 48).

form neutral salts, acid salts, double salts, or acid double salts. The acid, neutralized with alkali and evaporated to dryness, gives a brownish horny mass; but if an alkali earth is also present, double salts are formed, which crystallize in fine needles with eight molecules of water. The magnesium salts crystallize in small and uniform spherules, while the copper salts form large and irregular spherules. Twin forms are frequently produced in the copper precipitates, resembling the globoids of which drawings appear in Pfeffer's paper. These spheroid masses may be clusters of needles of approximately equal lengths, as is suggested by the regularly pitted surfaces sometimes seen, and the term spherocrystal can accordingly be applied to them. The copper compounds are green; the others, as far as reported, are white. Occasionally a faint pinkish cast has been noticed in pure preparations.

The acid is miscible in all proportions with water. It is soluble in alcohol but not in the other common lipoid solvents. Ether added to an alcoholic solution precipitates the acid in droplets. According to Posternak, the acid-alkali and acid-magnesium salts are soluble in alcohol and water. The double salts are soluble in water, forming opalescent solutions from which they are precipitated by chlorid and acetate of potassium, redissolving if these are added in excess. The decrease in solubility of the salts of inosite-phosphoric acid is in the following order: alkali, alkali earth and heavy metal. The magnesium compounds are more soluble than the calcium salts and the latter more soluble than those of barium or strontium. The same order of solubility also holds for acid salts, double salts and normal salts. These phytophosphates are more soluble in cold than in hot water, and heating frequently precipitates them, even in the presence of dilute acetic acid. This precipitation is largely influenced by other compounds in solution, halogens and sulphates inhibiting, and phosphates facilitating the reaction. Posternak noted that the precipitates thus formed by heating were not always completely dissolved on cooling; also that the phytophosphates not readily soluble in cold water were changed to more soluble forms by dissolving in dilute acid and precipitating with alcohol. Dilute mineral acids are solvents for all of these compounds. Acetic acid does not dissolve the salts of inosite-phosphoric acid with the heavy metals, barium,

calcium and strontium; but the magnesium and alkali salts, and the double salts, are very soluble in this reagent. Posternak says that the alkali salts of this acid are solvents for the compounds with alkali earths, and that on standing, crystals of double salts form in these solutions, tending to arrange themselves in rosettes—a further suggestion as to the mode of formation of the characteristic spherocrystals mentioned above. Inosite-phosphoric acid solutions do not polarize light, and pass through semi-permeable membranes comparatively slowly.

All the salts of inosite-phosphoric acid, except the alkali salts and the acid magnesium salts, are precipitated from aqueous solution by four volumes of alcohol. The acid and its salts in alcoholic solution are precipitated by ether. The addition of neutral solutions of silver, lead, copper, cadmium, iron, uranium, strontium, barium and calcium precipitates the acid from its solutions; so also do magnesia mixture and albumin. According to Posternak, precipitation with copper is prevented by the presence of fat. The copper salts are soluble in ammonium hydroxid solution. Ammonium molybdate in nitric acid does not cause precipitation in dilute solutions of inosite phosphates, but in concentrated solutions white needles are formed on long standing which are insoluble in nitric acid and soluble in water. Preparations from seeds retain persistently small amounts of magnesium, several reprecipitations being necessary to get a salt containing a single metal. It is equally difficult to get a preparation free from the hydrogen ion, and it may be said in general that the property of forming acid salts and double salts is very characteristic of inosite-phosphoric acid. The most important contribution to our knowledge of the nature and properties of its salts has been made recently by Anderson. From acid purified by means of barium precipitation and the method described by Hart and Patten, the following compounds have been prepared: tri-barium, penta-barium, penta-barium di-ammonium, penta-magnesium, penta-magnesium di-ammonium, tetra-cupric di-calcium, tetra-calcium, penta-calcium, hexa-cupric, octa-silver, and hepta-silver salts. Most of them are white amorphous powders, but the tri-barium and tetra-calcium salts can be reprecipitated in irregular crystalline form.

Pure preparations have been made and analyzed by several other investigators. The results of their work are given in Table 2.

TABLE 2

Analytic data pertaining to inosite-phosphoric acid
(Compiled from results reported in the literature of the subject)

Name of Author	Elements							Ratios[1]	
	Carbon	Hydrogen	Phosphorus	Barium	Magnesium	Calcium	Other Metals	C=6 P=x	P=6 M′=x
Anderson.	(Prepared from the purified commercial product.)[2]								
	6.42	1.44	16.87	37.21	——	——	——	6	6
	4.59	1.15	13.46	48.87	——	——	——	6	10
	——	——	14.07	46.99	——	——	——	6	12
	——	——	21.29	——	14.13	——	——	6	12
	10.56	3.21	26.37	——	——	——	——	6	—
	10.76	3.22	26.16	——	——	——	——	6	—
	——	——	19.07	——	42.9	13.03	6.42(K)	6	12
	——	——	20.62	——	——	22.46	——	6	10
	——	——	21.75	——	——	17.66	——	6	8
	——	——	22.53	——	14.69	——	——	6	10
	——	——	16.88	——	——	——	33.54(Cu)	6	12
	——	——	11.94	——	——	——	55.98(Ag)	6	8
	——	——	13.02	——	——	——	52.43(Ag)	6	7
	(Synthesized by means of inosite and ortho-phosphoric acid.)								
	14.24	3.45	24.09	——	——	——	——	4	—
	14.23	3.61	24.31	——	——	——	——	4	—
	9.16	1.64	16.34	35.57	——	——	——	4	6
	9.60	1.68	16.05	34.48	——	——	——	4	4
	(Synthesized by means of inosite and pyro-phosphoric acid.)								
	12.62	3.24	26.51	——	——	——	——	5	—
	8.17	1.58	16.55	35.90	——	——	——	5	6
Contardi.	(Prepared from rice bran.)								
	(acid)		26.08	——	——	——	——	6	—
	——	——	21.08	——	9.0	13.8	——	–	12
	(Synthesized by means of inosite and ortho-phosphoric acid.)								
	10.89	3.00	28.10	——	——	——	——	6	—
	——	——	12.50	56.2	——	——	——	–	12
	——	——	15.60	23.2	——	——	22.1 (Cu)	–	12
	——	——	21.40	——	8.9	13.5	——	–	12
Hart, Patten.	(Preparations from wheat bran.)								
	10.63	3.40	25.98	——	——	——	——	6	—
	17.30	3.63	16.38	——	5.8	1.13	2.6 (K)	2	6[3]
Hart, Tottingham.	(Similar preparations.)								
	——	——	26.08	——	——	——	——	–	–
Horner.	(The commercial preparation.)[2]								
	——	——	20.32	——	1.45	11.96	——	—	7
Levene.	(Preparations from hemp.)								
	9.84	——	23.00	——	——	——	——	5.5	—
	5.66	1.47	13.93	44.50	——	——	——	6	8.5
	17.90	3.57	13.16	——	——	——	——	1.5	—[4]
	7.70	——	11.95	40.14	——	——	——	2	9.5

[1] The empirical formulas have been calculated from the analytic results, and the relations between the carbon and phosphorus, and the phosphorus and base (M′), are given in these two columns. The carbon and the phosphorus are, in each case, assumed to be six atoms per molecule.

[2] Placed on the market by the Gesellschaft für Chemische Industrie in Basel.

[3] A 0.2 per cent. HCl extract of wheat bran several times reprecipitated from weakly acid solution by alcohol.

[4] The first substance; obtained by extracting with sodium chlorid solution and, after removing protein with picric acid, precipitating with copper and removing the copper.

TABLE 2 (*continued*)

Name of Author	Elements							Ratios[1]	
	Carbon	Hydrogen	Phosphorus	Barium	Magnesium	Calcium	Other Metals	C=6 P=x	P=6 M′=x
Posternak.	(A large number of preparations were made in which several kinds of seeds were used.)								
	——	——	26.08	——	——	——	——	–	—
	9.97	3.70	25.89	——	——	——	——	6	—
	4.79	1.00	12.70	50.45	——	——	——	6	11
	7.25	1.34	19.42	——	——	8.41	——	6	4
	7.44	1.49	19.73	——	——	——	19.02(Na)	6	12
Rising.	(Preparations from barley bran.)								
	5.37	1.03	13.08	——	——	——	52.65(Ag)	6	7
Suzuki, Yoshimura, Takaishi.	(Preparations from rice bran.)								
	——	——	23.48	——	5.81	17.48	——	6	10.6
Vorbrodt.	(Preparations from corn.)								
	6.51	1.21	15.18	42.62	——	——	——	5.5	8
Winterstein.	(Preparations from black mustard.)								
	——	——	18.42	——	7.8	——	——	—	6.5
Winterstein, Schulze.									
	9.65	2.83	15.20		——	——		3.7	—?[4]

The alkali salts are hygroscopic, but the others do not change in weight under ordinary conditions. Posternak assigns to the double alkali salts of the alkali earths eight molecules of water. The barium salt prepared by Vorbrodt lost 9.33 per cent. of its weight in the presence of phosphorus pentoxid, but regained it when exposed to a moist atmosphere. At 110° C., 11.5 per cent. was lost. Anderson reports for his tri-barium salt five molecules of water, for his tetra-calcium salt twelve, and for his penta-magnesium salt twenty-four.

Inosite-phosphoric acid is easily decomposed by heating with strong acids in sealed tubes but does not spontaneously break down into its cleavage products. Mendel and Underhill kept a solution of the acid for many months and found at the end of the time no apparent change. Posternak states that heating phytin in alkaline solution to 100° C. causes no decomposition, but Winterstein found that if a twenty per cent. solution of alkali were used (sodium hydroxid) and the temperature raised to 230° C., cleavage occurred. Contardi states that cleavage does not occur when these salts are

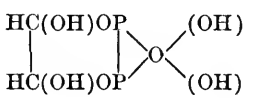
[4] Extracted with sodium chlorid solution, precipitated by heating, and filtered hot.

heated in water to 200° C. under pressure. According to Giacosa it is more readily hydrolyzed than lecithin. From the fact that the products of hydrolysis are inosite and phosphate, Winterstein came to the conclusion that the compound is inosite-phosphoric acid.

CONSTITUTION OF INOSITE-PHOSPHORIC ACID

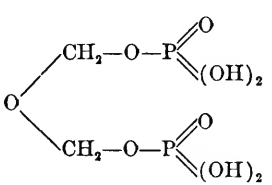
Posternak, who has done more work on this substance than any other one investigator, did not agree with Winterstein in the conclusion set forth above. From his analyses he first constructed the following formula:

(1)



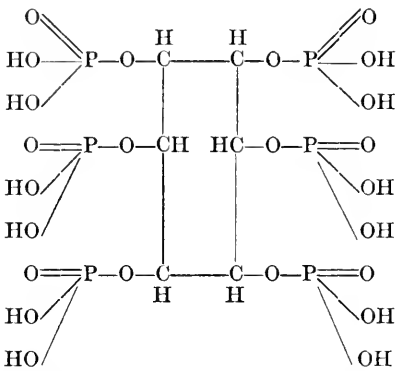
but, as benzoyl chlorid gave no positive test for the hydroxyl group, a second formula was proposed (anhydro-oxymethylene-diphosphoric acid):

(2)



He was of the opinion that inosite is synthesized from the products of hydrolysis when the "phytin" is heated under pressure with mineral acids. A number of chemists have expressed doubt concerning the probability of such a formation of inosite, either from this organic group or any part of it that might result from the action of the acid thereon. In 1907 Suzuki and his co-workers obtained inosite from "phytin" by the action of an enzyme, from which they concluded that inosite is an integral part of the "phytin" molecule and constructed the following formula to represent their view:

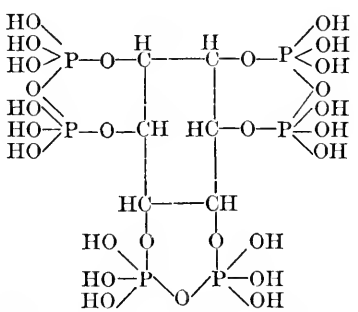
(3)



M. W., 660; C = 10.91%; P = 28.18%.

Neuberg came to similar conclusions the following year when he obtained inosite and *furfurol* on mixing "phytin" with phosphoric acid and distilling under reduced pressure, and also showed that furfurol can be obtained from inosite. He proposed the following formula:

(4)



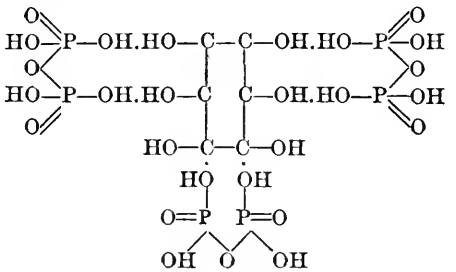
M. W., 714; C = 10.085%; P = 26.05%.

Levene, working with a preparation from hempseed, was led to believe that the "phytin" of this grain contained in its molecule phosphate, inosite and a carbohydrate of the pentose group. His work was criticized by Neuberg, who claimed that there were impurities in the preparation. In view of the known intimate association of the phytin with protein and carbohydrate in the aleuron grain, and the possible occurrence of a chemical combination of both phyto-phosphate and carbohydrate with protein, it is conceivable that Levene had a product holding pentose as an integral part

and not as an impurity, though in view of all the available evidence Neuberg's criticism seems at the present time somewhat justifiable.

Starkenstein also refused to accept the simple formula proposed by Posternak and offers the following:

(5)



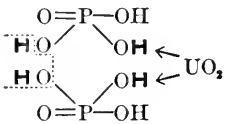
M. W., 714; C = 10.985%; P = 26.05

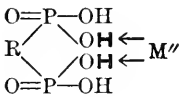
He argues that the phosphoric acid is in the pyro-form[4] from the fact that its silver salt is the same color as silver pyro-phosphate, and that its behavior when titrated with standard uranium acetate solution is also like that of pyro-phosphoric acid. That it is not combined in the usual form of an ester but held loosely in a complex "addition form," he maintains from the fact that an increase of inosite and inorganic phosphate resulted from heating some of the calcium salt for an hour at 100° C. These arguments are not altogether convincing. Anderson has prepared a silver salt of the ortho-tetra-phosphoric acid ester with inosite and reports it as being white like the pyro-phosphoric acid salt.

In the quantitative titration of pyrophosphoric acid with a uranium acetate solution, standardized by ortho-phosphate and using ferrocyanide as indicator, only one half of the phosphorus value is obtained. Starkenstein explains this phenomenon by the assumption that one half of the more reactive ions of the phosphoric acid have been removed in the dehydration. Now if two phosphoric-acid groups had formed esters with one polyalcohol, analogous conditions would have resulted as far as the ions are concerned, and bivalent ions would be expected to connect the two phosphoric acid

[4] That phosphorus occurs in plants in the pyro form may seem strange to many, but this is not the first time that such an occurrence has been suggested. In 1892 Hardin (*S. C. Exp. Sta. Bull.*, N. S., No. 8) reported his finding both pyro- and meta- phosphate in cottonseed meal, when he sought, in this feeding material, a substance toxic to cattle.

radicles. This may be illustrated by these two graphic representations:





The idea of this type of reaction for ortho- and pyro-forms is in harmony with the fact that when inosite-phosphoric acid is precipitated in acid solutions by divalent metals, the tri-metal salt is the more readily formed. The activity of the hydrogen ions is relatively greater in the inosite-phosphoric acid than the sum total of the ions of the ortho-forms would be, probably due to the influence of increased negative electric charges in the many phosphorus atoms held in one molecule, so that, altho six having been eliminated in the assumed ester formation, the very reactive ions are eight in number. The last column in Table 2 is interesting in this connection. Finally, the statement that the inosite-phosphoric acid is decomposed by dry heat has been shown to be erroneous by both Anderson and the writer.

That phytin is a salt of inosite-phosphoric acid seems to be conclusively demonstrated by the synthetic work of Contardi, whose preparations from rice bran gave analyses identical with a synthetic preparation obtained by heating anhydrous inosite with ortho-phosphoric acid (sp. g. 1.7). Other workers have attempted to substantiate this result, but so far without success. Carré could obtain only a mixture of the two chemicals; Anderson was able to produce tetra-ortho and di-pyro-phosphoric acid esters.

It does not follow that these preparations of inosite-phosphoric acid are identical in form with the organic phosphorus compound occurring in plants. The writer calls attention elsewhere to a difference of behavior between the phytophosphate in seeds and in preparations.[5] Certain data, not as yet published, as well as differences in the products described in the literature, fall in with those suspicions.[6]

What inosite-phosphoric acid is, in terms of a definite chemical

[5] Rose: *Technical Bulletin 20* of the New York Agricultural Experiment Station.

[6] Cf. Preparations analyzed by Patten and Hart, Winterstein and Schulze, and Levene, in table 2, p. 29.

structure, is an open question. It is probably an ester of phosphoric acid with inosite, in which six phosphoric acid groups are united with each inosite molecule. This ratio, $C_6 : P_6$, is indicated for the vast majority of the pure preparations analyzed; exceptions are the preparations by Levene, Vorbrodt and Rising (Table 2), these authors giving the ratio $C_6 : P_{5.5}$. Anderson has pointed out that his and Rising's silver salts are probably identical, Rising's analysis being equally well adapted to the formula $C_6H_{17}O_{27}P_6Ag_7$. It seems probable that the molecular weight when accurately determined will be reported as 714 or will differ from this by the molecular weight of three molecules of water. The molecule seems to contain twelve hydrogen atoms readily separated in ionization, six of which are exceedingly reactive; the remaining hydrogen atoms gradually diminish in reactivity by twos, the last four being slow to enter into an exchange with bases. The most readily formed salts are therefore those corresponding to an octavalent acid and the other common ones are in six and tenvalent combinations.

TERMINOLOGY AND CLASSIFICATION

The investigations of which this paper is a brief review have brought to the biological chemist and plant physiologist a type of phosphorus compound from the plant world which is relatively new and probably of prime importance. The phosphorus which occurs in acid and water extracts was formerly considered inorganic phosphate, and awakened no especial interest, and the mention of organic phosphorus immediately brought to mind nucleoproteins and lecithins. In the organic laboratories combinations of phosphorus with various organic radicals have been made and recently-prepared phosphoric acid esters[7] resemble "phytin" in some respects, and so may be considered of special concern to the biological chemist, as possibly bearing on problems in his field. A cleavage product of inosinic acid, *d*-arabinose phosphoric acid,[8] is significant as showing that carbohydrate esters are not confined to those produced by the synthetic operations of the laboratory. Even more

[7] v. Lebedew: *Biochem. Zeit.*, 1909, 20, 114; Neuberg and his coworkers: *Ber.*, 43, 2060; *Biochem. Zeit.*, 23, 515; 26, 115 and 529; 36, 5; Langheld, *Ber.*, 44, 2076.

[8] Levene and Jacobs, *Ber.*, 1911, 44, 746.

striking are Iwanow's experiments[9] in which, when yeast was allowed to ferment sugar in the presence of sodium phosphate, there was noted a disappearance of the inorganic phosphorus, amounting to from eighty to ninety-three per cent.; and in the liquors there was found an organic phosphorus compound optically active and giving reactions for aldehydes and ketones. Biochemical syntheses of this class have also been successfully made by other investigators.[10] Of special interest are the inosite esters with ortho- and pyrophosphoric acid prepared by Anderson. Mention may also be made of the spherocrystals discovered by Hansen[11] in the parenchyma cells of the *Euphorbia caput medusae,* which he describes as amorphous masses of calcium and magnesium phosphate, but which Belzug[12] later has shown to be salts of a new organic acid, phosphomalic acid. We may reasonably expect that additional phosphorus-bearing substances of this kind will be discovered in nature by the phytochemist for which a rational system of nomenclature will be required.

Rising, in his paper on inosite-phosphoric acid, refers to soluble phosphorus compounds obtained from grains, which he promises to discuss in later contributions. These substances he considers closely related to "phytin," and proposes classifying them as a single group with the generic name "phyto-phosphoric acid." This term we may profitably adopt to indicate the acid radicals of those organic phosphorus compounds which may be found in the water and dilute acid extracts of plant materials. In this group will be included the glycerophosphates, phosphomalates, such hexose and pentose phosphates as may be discovered in plants, and the phytin-like substances.

The term "phytin" as used at present seems to designate that substance which is extracted from seeds by leaching with dilute acids, reacting positively in the tests for calcium, magnesium, and, after hydrolysis, for phosphoric acid and inosite. The multiplication of trade names for definite chemical compounds is not desirable. There are many students and other workers who must of necessity

[9] Iwanow: *Zeit. für physiol. Chem.,* 1907, 50, 281–288.

[10] Young and Hardin: *Biochem. Zeit.,* 1911, 32, 173–188; *Proc. Chem. Soc.,* 21, 23, 24; *Proc. Roy. Soc.,* London, 77, 80, 81, 82; Euler and Ohlsén: *Biochem. Zeit.,* 1911, 37, 313.

[11] Hansen: *Arbeit. des bot. Inst.,* Würzburg, 1888, 92–122.

[12] Belzug: *Jour. de bot.,* 1893, 7, 211–229.

carry in memory more names of organic compounds than they can reasonably be expected to define in terms of chemical formulae, if the common names do not in themselves offer suggestions of chemical structure. Unsystematic naming is contrary to the modern spirit of chemical nomenclature. Winterstein's "inosite-phosphoric acid" has priority over Posternak's "phytin" and the further advantage of being a chemically descriptive term. The preference of several authors for this latter designation is evidenced by the fact that the name phytin is not adhered to or is given in parenthesis after the name "inosite-phosphoric acid." In this particular case the probability of confusion is very much increased by the fact that the term "phytine" is already applied to chlorophyll preparations whose chemical composition we cannot hope to know for some time and for convenience must perforce carry a non-chemically descriptive appellation. The word "phytin" seems to have all the psychologic requirements of a really good trade name and the substance which it designates in the market is widely advertised in the European medical journals for its therapeutic properties, which are more than likely of questionable character, and the term will undoubtedly persist.

It can be readily conceived that this may not be the only inosite-phosphoric acid in plants and we should look for other combinations in which the phosphorus may be in the ortho or pyro form—even the meta phosphate—and be present as the hexa, tetra, di, or mono phosphoric acid. Various incidents have suggested to the writer that some of these forms occur in preparations from seeds when certain treatment other than those described above is used.

ANALYTICAL METHODS[13]

The quantitative estimation of phytin phosphorus has been effected only by determining the difference between the total soluble phosphorus and the inorganic phosphorus. Phytin research in animal and plant metabolism is therefore very largely dependent upon the accuracy of the determination of inorganic phosphorus.

[13] The analytical methods are here treated very briefly, for their development is as yet imperfect and the literature conflicting. Many papers have not been mentioned and the reader is referred for these to the bibliography on page 46. A more complete statement with experimental data will be published later.

The term soluble phosphorus above and elsewhere means of course the phosphorus compounds which dissolve in cold acidulated water; the amount is obtained by evaporating the extract and destroying the organic matter with sulphuric and nitric acids according to the method of Neumann,[14] after which the phosphorus is determined by the usual ammonium molybdate and magnesia mixture method as described by Sonnenschein and later modified by Woy. As extracting agents both acetic acid and hydrochloric acid have been used.

The first method to approximate an accurate determination of inorganic phosphorus in the presence of soluble organic phosphorus was that used by Hart and Andrews in 1903. Their extracting agent was 0.2 per cent. hydrochloric acid solution, a solvent which has since been used by most investigators. Hart and Andrews noted that ammonium molybdate did not precipitate the phytin phosphorus, and used this fact to devise a method for separating the two kinds of phosphorus combination in solution. They had some apprehension lest the strong acid in the usual molybdate solutions would hydrolyze some of the organic phosphorus compounds and thus yield high results for the inorganic portion. They determined the minimum amount of nitric acid necessary to give a rapid, complete, and crystalline separation of the yellow precipitate (2 c.c. of nitric acid, specific gravity 1.2, in each 250 c.c. of solution) and added to the liquid of this acidity neutral ammonium molybdate solution.

Vorbrodt, in his excellent monograph on "phytin," developed a method which is based on a triple precipitation of the inorganic phosphorus, first precipitating in general by means of magnesia mixture and dissolving the precipitate in the least amount of nitric acid. This is diluted to 50 c.c., heated to 100° C., and treated with an equal volume of ammonium molybdate solution. The yellow precipitate is dissolved in ammonia water, 25 c.c. of 5 per cent. barium chlorid are added,[15] and the precipitate after being washed and dried is weighed; or the phosphorus may be precipitated with magnesia mixture and weighed as magnesium pyrophosphate.

[14] Neumann: *Zeit. für physiol. Chem.*, 1902, 37, 115.
[15] Riegler: *Zeit. Anal. Chem.*, 1902, 41, 675.

Stutzer in Germany and Forbes in this country, working independently, introduced a new idea in the determination of inorganic phosphorus, namely, the use of acid alcohol. Forbes and his associates make an acidulated water extract and precipitate with magnesia mixture; the precipitate is then washed successively with ammonia water and alcohol, and the inorganic phosphorus separated from the phytins by digesting in cold 95 per cent. alcohol containing 0.2 per cent. of nitric acid. This alcoholic solution is finally filtered, the filtrate evaporated, and phosphorus determined in the residue in the usual way.

Starkenstein has studied in some detail the application of titration methods to this problem, and his results point to the possibility of determining quantitatively these different forms of phosphorus in the same solution. He found that titration of a solution containing ortho-phosphate, pyro-phosphate and inosite-phosphate with uranyl acetate standardized by ortho-phosphate, using cochineal as an indicator, gave in each case true values for total phosphorus; that with ferrocyanide as an indicator, the total phosphorus was equivalent to all of the phosphorus as ortho-phosphate, one half of that as pyro-phosphate and inosite-phosphate, the glycero-phosphate not entering into the reaction at all. Anderson notes that pyro-phosphoric acid can be converted into the ortho form by heating with dilute acids, while the inosite-phosphoric acid is not affected by this treatment. With these facts in mind a volumetric process may readily be devised.

THE ROLE OF INOSITE-PHOSPHORIC ACID AND ITS SALTS IN PLANTS

The literature on phosphorus metabolism in animals has become voluminous, but the botanists have published comparatively little on the changes of these compounds and their probable significance in a plant's life history. That cell functioning is impossible in the absence of phosphorus is again emphasized in the recent work of Frouin,[16] which shows its absolute necessity in the growth of micro-organisms. The study of the role of phytin in plant life involves an investigation of the changes and distribution of all the

[16] Frouin: *Compt. Rend. Soc. Biol.*, 1910, **68**, 801–803.

phosphorus compounds and of inosite in the several stages of plant development. Since the methods of differentiating between the various combinations of phosphorus in plant substances are becoming highly perfected, we may expect rapid developments in our knowledge of their functions in plant processes. The universal presence of phytin in propagating and growing parts must be highly significant. This constant occurrence led Starkenstein to assume that "phytin" plays a specific role in the mechanism of growth of both plants and animals. If this be so, its biochemical reactions must be closely linked with carbohydrate and protein formation, and its occurrence with these substances in the aleuron grain must be more than a mere coincidence.

In this connection it may be well to review briefly the literature regarding the aleuron grains. The best summary was found in Vines's text book of plant physiology (1886) but this is too brief to be satisfactory. From Pfeffer's comprehensive description, it appears that these grains form in the vacuoles during the ripening and desiccation of the seed; that the forms assumed are globular, which are less distorted and attain a larger size in the more fatty seeds. They consist morphologically of three parts: the large protein particle, Pfeffer's globoid, and a membrane. Crystals of calcium oxalate are sometimes present. Weyl[17] isolated the grains from the "Paranuss" employing the method of all the previous investigators, and made an extensive study of their proteins. This was in the days of the vegetable vitellins (globulins), and the chief protein of the aleuron grain having been shown to belong to this group, Weyl thought that the membrane was a modified form of the same protein, an albuminate. Three or four years later, Vines[18] undertook a study of these proteins and from his observations on material from a large variety of seeds, grouped them into five classes: *vegetable peptone* (water soluble), *vegetable myosin, crystalloid, vitellin* (all three soluble in sodium chlorid solution), *albuminate* (soluble in sodium carbonate solution). These are described as plastic proteins, in part transported to the cells of the seed from other portions of the plant. According to Posternak, these pro-

[17] Weyl: *Zeit. für physiol. Chem.*, 1877, 1, 84–96.

[18] Vines: *Proc. Roy. Soc. London*, **28**, 218; **30**, 387; **31**, 59, 62; see also Lundtke: *Jahrb. wiss. Bot.*, 1890, **21**, 62.

teins constitute from fifty to seventy-five per cent. of the aleuron grain. It is doubtful whether they are simple proteins; more likely they contain both phosphorus and bases in their molecules. Besides protein, Posternak found carbohydrates which were not free, but combined with some other substances of the grain; also ash, to the extent of twenty-five to fifty per cent. The following analytic data were recorded:

	Per Cent.		Per Cent.
Phosphorus	0.11–3.83	Magnesium	0.28–1.27
Sulphur	0.64–0.81	Calcium	0.11–0.37
Silicon	0.01–0.36	Iron	0.03–0.09
Potassium	2.29–2.71	Manganese	Trace

These results were obtained from aleuron grains of sunflower, white lupin, hemp, and red fir. The author calls attention to the interesting fact that all the elements essential to plant growth are present in these bodies. These results are notable when compared with Bernardini's analyses of rice embryo: P_2O_5, 0.95; SO_3 (not given); SiO_2, 0.25; K_2O, 1.691; MgO, 1.389; CaO, 0.279; Fe_2O_3, 0.06; Mn, trace; Na_2O, trace. One would like to know whether the silicon in these two substances is present in organic combination.

The globoid or "phytin" is a calcium-magnesium salt of inosite-phosphoric acid. Phyto-phosphate is also combined in the protein granules, possibly in the form of the potassium salt, as Posternak believed, inasmuch as he could not separate the potassium salt from the globulin, although it is soluble in alcohol and globulin is not soluble in this liquor. He concluded that it is chemically attached to the protein. In germination, the aleuron grains swell up, forming a granular viscid mass; both globoid and crystalloid go into solution, enzymatic action sets in, and both phytin and protein are hydrolyzed.

The presence of an enzyme having the power to decompose phytin into inosite and an inorganic phosphate was first demonstrated by Suzuki and his associates in the bran of rice. It has also been found by Vorbrodt in other small grain, including wheat, rye, and barley; likewise in larger seeds, as vetch and lentils. An extract of the kernel of indian corn gave no evidence of the presence of a phytase, but it was shown to develop during the germination of the grain.

In the earlier analyses of seeds, the inorganic phosphorus was not given a very prominent place and was usually reported as that portion obtained by subtracting the sum of the protein and lecithin phosphorus from the total phosphorus; but as this element began to receive special attention its direct determination was attempted, and consequently the amount of inorganic phosphorus reported was lessened. Thus Umikoff[19] estimates the inorganic portion as fully half of the total phosphorus, but the more recent workers report it in very small percentages. With the germination of the seed, the inorganic phosphorus gradually increases at the expense of the organic form. This was at first attributed to the breaking up of the phosphorus-bearing proteins and lecithins. Tammann,[20] one of the earliest investigators to make direct determinations of inorganic phosphorus in seeds and their sprouts, found during germination an increase of this form which, in terms of P_2O_5, was from 0.324 per cent. to 0.443 per cent. in a period of only twelve days. According to the work of Prianischnikow[21] and Merlis,[22] lecithin decreased one half during fifteen days' germination of *Vicia sativa* and *Lupinus angustifolius*. Iwanow found that the inorganic phosphorus increased from a very small amount to 93.7 per cent. of the total phosphorus in germinating seeds of *Vicia faba*. He held that lecithin is the most stable of the organic forms and is altered very little. Phytin, owing to the presence of phytase, is practically all changed by this process.

Vorbrodt has shown that the phosphorus compounds, especially inosite-phosphoric acid, are particularly abundant in the germ. When the seed begins to sprout, this supply is increased by transportation of phosphorus from other parts of the grain, as is indicated by Zalesky's[23] observation that in the sprouts of *Lupinus angustifolius* the total phosphorus increased in twenty-five days from 0.302 gram to 0.514 gram; the inorganic phosphorus doubled in amount, while the protein and lecithin phosphorus remained prac-

[19] Umikoff: Russian *Dissertation*, 1895. (Cited by Zalesky: *Ber. bot. Ges.*, 1902, 20, 426–433.)

[20] Tammann: *Zeit. für physiol. Chem.*, 1885, 9, 416–418.

[21] Prianischnikow, 1895. (Cited by Zalesky. See footnote 19.)

[22] Merlis: *Landw. Vers. Stat.*, 1897, 18. (Cited by Zalesky. See footnote 19.)

[23] Zalesky: *Ber. bot. Ges.*, 1902, 20, 426–433.

tically unchanged. Bernardini found that the phytin decreased also in the germination of wheat, but the lecithins increased.

In the early stages of plant development subsequent to germination, inorganic exceeds organic phosphorus. Staniszkis could find no trace of inosite-phosphoric acid in millet during this period. The phosphorus is now drawn from the soil and its increase in the plant is proportional to the increase in dry matter. The organic forms of phosphorus are synthesized from this supply, according to Staniszkis, very slowly until the heads are formed. Hart and Tottingham found no phytin phosphorus in the dried forage plants. Balicka-Iwanowska, working with barley, found the phosphorus compounds at the end of the fourth week present in the same proportion as that in the seed; thereafter there was a constant decrease in the inorganic phosphorus. In the seventh week the protein phosphorus had doubled and the inosite-phosphoric acid had increased to seven times the amount present in the fourth week. As the barley seeds began to form, in the ninth week, the increase of organic phosphorus occurred mostly through the synthesis of nucleoproteins. The small increase which Staniszkis reports was more in the form of lecithins and protein phosphorus compounds than of inosite-phosphoric acid. At the period of flowering, the lecithins reach their maximum, which may be, according to Stoklasa, 71.6 per cent. of the total phosphorus. During the formation of millet seed, the synthesis of phytin goes on energetically at the expense of both inorganic and protein phosphorus. In the barley, Balika-Iwanowska found an increase of inosite-phosphoric acid and also one of nucleoprotein which was even greater than that of the phytophosphate. In the ripening of the seeds of millet the formation of inosite phosphates and nucleoprotein ran parallel, but in barley the inosite phosphate increased at the expense of some of the protein phosphorus. As the panicle grew and matured in both of these plants, there was a transportation of both the phosphorus and the protein to this part of the plant.

The mobilisation of phosphorus and changes in its form in *Vicia faba* and other plants were also studied by Iwanow. The most interesting part of his contribution is the relation between sunlight and changes in the form of phosphorus. The plants which remained in a dark room contained more inorganic phosphorus than

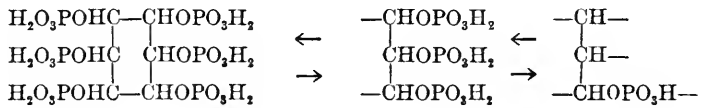
those which had sunlight. Opaque shields on the leaves produced the same results in the protected part of the leaf, hence the change of inorganic into organic phosphorus may be attributed in part if not altogether to photosynthesis. Stoklasa and his pupils[24] consider phosphorus an integral part of chlorophyll, existing in a form which does not give the HPO_4 ion. Schimper[25] in his rather comprehensive study of the assimilation of the ash constituents in plants also noted the decrease of the inorganic phosphorus through the action of light. Posternak, attempting to account for the formation of "phytin," which he then thought to be anhydro-oxymethylene-diphosphoric acid, assumed that it was formed simultaneously with the reduction of carbon dioxide by a direct combination of the products of the photo-chemical action and inorganic phosphates, an hypothesis suggested by Schimper's experiments. If Posternak's assumption is true, even in part, phosphorus may play a very significant rôle in carbohydrate anabolism. Several authors have expressed doubt about this explanation of "phytin" synthesis, advancing the argument that inosite-phosphoric acid is not found in the early stages of growth, and when formed later is not uniformly produced, as would be expected if it were due entirely to the action of the chloroplasts. There is still the possibility that it is so synthesized and instantly broken down in the formation of other compounds.

Soave could find no inosite in dormant seeds unless they were first boiled in strong acid, but after they began to sprout its presence could be easily demonstrated until the reserve material of the cotyledons was almost exhausted. It was also present in unripe seeds, indicating that the inosite-phosphoric acid is formed in the seed by the combination of the inorganic phosphorus, abundantly present at this stage, with the inosite. The occurrence of inosite in the unripe seed and the green parts of the mature plant, and the later disappearance of this substance as inosite-phosphoric-acid forms, indicate that phytin is probably produced by the reversible action of an intracellular phytase, and that Posternak's explanation is incorrect.

Rising suggests that inosite-phosphoric acid may be an interme-

[24] Stoklasa, Brdlika and Ernest: *Ber. d. deut. bot. Ges.*, 1910, **27**, 1.
[25] Schimper: *Flora*, 1890, **23**, 207–261; *Bot. Zeitg.*, 1888, **46**, 81.

diary product in the formation and destruction of the lecithins, and in this way may play an exceedingly important part in the life of the plant. The possible relation between the two compounds is shown in the following graphic representation:



The most striking suggestion as to the functions of inosite-phosphoric acid is contributed by Starkenstein, who thinks that the inosite is in itself inert and incidental and functions only in its combination with phosphorus. He has demonstrated that, in the body, inosite yields lactic acid, an interesting fact in view of its possible significance in carbohydrate formation. He assigns to inosite-phosphoric acid, as its special function, some part in the process of growth, basing his view on his experiments with animals. In harmony with this view is the distribution of phosphorus in the seed, the greater part being localized in the germ; according to Bernardini over eighty per cent. of the total phosphorus in the rice germ is in the form of inosite-phosphoric acid. It is interesting to note in this connection the observation of Iwanow that there is a tendency to concentration of phosphorus in the parts of the plant where growth is most active, and also that when the phosphorus supply of the substrate is insufficient, the phosphorus of the other parts of the plant is rapidly transported to the growing shoots. As previously stated the phosphorus of the seed is in the form of the calcium-magnesium salt of inosite-phosphoric acid, but, according to Posternak, the phosphorus, in transportation, is in the potassium salt of this acid.

Phyto-phosphoric acids, whether they are inosite esters or other compounds, undoubtedly play very significant roles in all higher plants, but as their specific functions have not as yet been ascertained, even the chemical structures being as yet uncertain, nearly all statements on the subject must be pure conjectures. The chief suggestions from experimental work are that these acids are concerned in the process of photo-synthesis or in the changes of the photo-synthetic products, for example, the formation of carbohydrates and fats; that it is an intermediary step in the synthesis of phospho-proteins and lipoids; and that it acts as a specific controlling factor in

growth. The various functions as thus outlined are probably overestimated, but those who have worked in this field seem to be strongly of the opinion that inosite-phosphoric acid is more than a reserve material. It is attracting considerable attention and as the necessary analytical methods are perfected, we may expect to see, in increasing number, valuable contributions that will elucidate in detail the part which this interesting compound plays in nature.

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A NEW TYPE OF ARTIFICIAL CELL SUITABLE FOR PERMEABILITY AND OTHER BIOCHEMICAL STUDIES

E. NEWTON HARVEY

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Research on the permeability of membranes has been largely confined to a study of the properties of what may be termed macroscopic membranes; composed of parchment, collodion, rubber, or silk impregnated with various substances. The best example of membranes of a type and size comparable to the surface film of cells and yet available for permeability studies are the precipitation membranes of Traube, investigated by Walden, Tamann, and Meerburg.

Protein membranes of exceeding fineness are formed at the surface of various non-miscible fluids shaken with protein solutions, such as the surface film of oil globules in protein-oil emulsions, or the films formed on chloroform or benzol when shaken with albumen solutions.[1] Such membranes are useless for permeability studies so long as they surround fluids that do not mix with water. However, it is an easy matter to replace the fluid within the membrane by a watery solution, provided the former fluid is readily volatile and slightly soluble in water. Chloroform conforms to these conditions. When chloroform is shaken with egg albumen solutions, the globules, in the course of 10–15 minutes, shrink in size and their membranes become crumpled, due to the passage of chloroform from water to air and from globule to water. Lecithin, if previously dissolved in the chloroform, will take up water as the chloroform passes out. In the course of one to two hours, in an open vessel, all the chloroform disappears and we obtain, instead of a chloroform solution of lecithin, a water solution of lecithin enclosed in a fine protein membrane, the whole of a size comparable with cell sizes. The diameter of the droplets may be varied at will

[1] Robertson: *Journal of Biological Chemistry*, 4, p. 1, 1908.

according to the degree of shaking. The rôle of the lecithin is to hold the water as the water replaces the chloroform. The protein membrane is impermeable to lecithin.

These artificial lecithin cells are stable, persisting until destroyed by bacteria. In many ways—in shape, in general appearance and in consistency—they resemble, to a very remarkable degree, sea-urchin or star-fish eggs. Some of their properties have been described in *Science* (n. s.), Vol. 36, p. 564, 1912.

The point I wish to emphasize here, however, is not that we can prepare artificial cells closely resembling real cells, but that a solution of lecithin may be obtained within a protein membrane, the whole of known composition and of a size comparable with cell sizes. Much can be inferred concerning the living cell from a knowledge of the properties of such artificial cells where composition is definitely known.

As chloroform is exchanged for water, some of the lecithin separates in the form of granules, most of which agglutinate in a dense clump. The cell as a whole, but more particularly these granules, take up neutral red from dilute solution, becoming *red* in color. (Chloroform alone takes up only the yellow base of neutral red. When lecithin is dissolved in chloroform it unites with the yellow base, forming a red salt.)

If the permeability for alkalies of such red-stained cells is studied, a marked difference from that of living cells is noted. Both ammonium hydroxid and sodium hydroxid in $n/2000$ concentration enter rapidly and at the *same* rate. It will be remembered that all living cells are very easily permeable to ammonium hydroxid, but very slightly so to sodium hydroxid.[2] The surface membrane of living cells is evidently of quite different composition from the protein film which condenses on chloroform droplets.

Living cells behave *toward alkalies* as though they were surrounded by a layer of a fat solvent, as postulated by Overton. Lipoid-soluble alkalies (ammonium hydroxid) penetrate readily, lipoid-insoluble alkalies (sodium hydroxid) do not. The lipoid solubility of ammonium hydroxid can be readily demonstrated by means of a benzol-lecithin solution shaken with egg albumen solu-

[2] Harvey, E. N.: *Journal of Experimental Zoology*, 10, p. 507, 1911 and Biochemical Bulletin, 1, p. 227, 1911.

tion. The same type of protein-film is formed on these globules but they differ from chloroform-lecithin globules in that the benzol is *not* replaced by water. If the benzol-lecithin globule is stained in neutral red solution and placed in $n/1000$ ammonium hydroxid solution, the color change from red to yellow takes place almost instantly. But it is only after 15–20 minutes that sodium hydroxid in relatively high ($n/10$) concentrations can enter. Ammonium hydroxid is readily soluble in the benzol droplet, while sodium hydroxid is not; and in this fact lies the explanation of the difference in penetrability. When stained in neutral red solution, practically all living cells behave as though they were protected from alkali by a benzol-lecithin surface layer.

It is a simple matter to introduce various substances into these cells by dissolving or suspending the material in the chloroform-lecithin solution before it is shaken with the protein solution. Thus, oil may be dissolved by chloroform and will separate in the cell in several large droplets much like those in a *Nereis* egg. Or cholesterol, starch grains and finely divided protein particles can be likewise included; or substances to be used as indicators in studying the permeability of the protein film.

Such cells, regarded as complex systems of biological substances, offer exceptional advantages for interpreting phenomena observed in living cells under special conditions; for example, during the passage of an electric current. Movements and disintegrations take place which I have as yet only partially investigated. In the near future I intend to describe these phenomena and shall give more complete data upon the permeability of the film which surrounds the cells.

ON A NEW FUNCTION OF THE CATALYZER CALLED "PEROXIDASE" AND ON THE BIOCHEMICAL TRANSFORMATION OF ORCIN TO ORCEIN[1]

JULES WOLFF

In a recent publication I have described the influence which peroxidase exerts on certain phenols in the presence of various salts and alkalies.[2] When dissolved in a weak sodium carbonate solution freely exposed to the air, orcin, for example, fixes from four to five times more oxygen in the presence of peroxidase than in its absence. In this note I wish to call attention to the fact that peroxidase has other powers than the fixation of atmospheric oxygen.

In determining the combined influence of ammonia and peroxidase upon aqueous solutions of orcin, I have studied conditions which favor the transformation of orcin[3] into orcein, the beautiful coloring matter which is one of the principal constituents of commercial orseille.

My observations are interesting from many points of view. They show that (1) if a 2 per cent. solution of orcin is exposed to the air in a thin layer and subjected to the influence of different proportions of ammonia, orcein is not formed even after a month under such conditions, but, instead, there is produced a substance which imparts a brownish-red color to the liquid. (2) If, however, a portion of the same solution is put in a narrow tube, so that the reaction takes place in a deeper layer, *and the surface of contact with air is limited* (other conditions being equal), one observes *a very slow but regular formation of orcein.* (3) If (everything else being equal) one repeats the *first* experiment (1), but adds to the ammoniacal solution of orcin a suitable quantity of peroxidase, orcein fails to appear, just as in the first experiment. (4) If, how-

[1] Translated from the author's manuscript, in French, by Dr. J. J. Bronfenbrenner. [Ed.]

[2] Wolff: *Comptes rendus,* 1912, clv, p. 618.

[3] Orcin has been the subject of interesting work by Robiquet, Dumas, Liebig, and Laurent and Gerhardt.

ever (all other conditions being equal), one repeats the *second* experiment (2), but adds to the ammoniacal solution of orcin a suitable amount of peroxidase, *the transformation into orcein takes place very rapidly and is quite advanced in four or five days.*[4] Comparing the coloration intensities of products 2 and 4, one sees that in five days 4 contains more than twice as much orcein as 2. This gain is due to the action of the peroxidase. By boiling the peroxidase solution for from 5 to 6 minutes, before adding it to the ammoniacal solution of orcin in experiment 4, there is no acceleration in the formation of orcein, evidently because the peroxidase, as the active agent in the transformation, is thus destroyed.

In order to determine the rate of oxidation in the different experiments, I measured the volumes of absorbed oxygen. In experiment 2, eight to nine times more oxygen was absorbed than in 1, in the course of 48 hours. In experiments 3 and 4 there was a similar difference but, because of the presence of peroxidase, the proportions of absorbed oxygen were larger.

Without discussing the nature of the combined action of ammonia, oxygen, and peroxidase upon orcin, we may conclude that in dilute solutions of orcin, *slow* oxidation by ammonia is the primary condition for the formation of orcein. If, however, to this condition is added the accelerating influence of peroxidase, the action is directed toward formation of coloring matter rather than toward increased absorption of oxygen. These facts may possibly be helpful in the commercial preparation of orseille.

Paris, France.

[4] My first and third experiments could easily be performed in flasks with flat bottoms. A small test tube would be satisfactory for experiments 2 and 4. The proportions of materials indicated below are well adapted for the purposes of the experiments:

For 1 and 2 { 2 c.c. of 2.8 per cent. solution of orcin and 50 mg. of NH_3 } Diluted with water to 3.5 c.c.

For 3 and 4 { 2 c.c. of 2.8 per cent. solution of orcin, 50 mg. of NH_3 and 1 c.c. of active peroxidase solution } Diluted with water to 3.5 c.c.

STUDIES OF DIFFUSION THROUGH RUBBER MEMBRANES

1. Preliminary observations on the diffusibility of lipins and lipin-soluble substances

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CONTENTS

I. INTRODUCTION

When I proposed to my biochemical associates in cancer research, in 1909, that we undertake a study of intracellular chemistry,[1] I realized that new analytic methods and unconventional experimental procedures were prerequisites for material progress in this as in any other chemical relation. The greatest obstacle in the path of progress in intracellular chemistry is the evident lability of the essential intracellular constituents. Our best chemical methods increase this predicament because each is essentially anti-biological in character. Biochemical discoördinations are enforced whenever any of our present chemical processes is effectively applied to protoplasmic material.

In reflecting on the properties and possible coördinations of intracellular lipins, it seemed probable that such lipins might be separated from protoplasmic material with the least chemical vio-

[1] Gies: *Studies in cancer and allied subjects, conducted under the auspices of the George Crocker Special Research Fund; Volume III, Department of Biological Chemistry, Introduction* (in press).

lence, and isolated with the least possible alteration of their qualities, if they could be removed by dialysis.[2] When this idea first came to mind, however, execution of its essential feature appeared to be impossible. I believed that the diffusion of a solute depends very largely on chemical affinity between the separating membrane and the solvents on both sides of the partition. In that view, it seemed highly improbable that any of the ordinary membranes, except possibly collodion, could be of service in the dialysis of lipins under any circumstances. Collodion appeared to possess favorable qualities because of its solubility in common lipin solvents and its possible affinity for the latter under conditions of dialysis.[3]

Collodion is the only one of the available membranes which, while soluble in ether-alcohol solutions, freely permits the passage of salins, extractives, carbohydrates, and proteins from aqueous solutions to water, or to aqueous solutions, outside, and *vice versa*. At first thought this suggested special availability of collodion for the work in mind. On the other hand lipins could not be expected to dialyze through collodion in the presence of much water and, as preliminary dehydration seemed an inevitable necessity for the dialysis of lipins from cellular matter, the permeability of collodion membranes to *water-soluble* substances did not appear, after all, to imply any practical advantages for the diffusion of lipins. I also recalled the fact that, in some experiments in another relation, we found that collodion was occasionally rendered defective by ether when the latter was used as a preservative of aqueous solutions undergoing dialysis.[4]

Continuing actively to consider these matters from one viewpoint and then another, I thought of rubber as a possible choice of membrane. Recalling the well-known fact that rubber swells very markedly in ether and even in ether vapor, I assumed that the rubber expands in ether under such conditions because ether dissolves in the rubber or combines with it. This was but the prelude to the

[2] After preliminary desiccation by treatment with anhydrous sodium sulfate or other suitable process.

[3] Collodion is a serviceable membrane for such purposes. See page 70.

[4] In some experiments which Professor Welker has conducted at my request, we have found that the disintegrative effect of ether on collodion membranes may be due to contained alcohol and other impurities. (See page 70.)

deduction that if ether dissolves in or combines with rubber, ether would also carry dissolved lipins with it into a rubber membrane; and if ether were on the opposite side of such a membrane, to work inwardly under such conditions, ether currents would develop; and lipins would pass from the solution of higher concentration to that of the lower, and there accumulate until an equilibrium was established.

This conception was so attractive that I proceeded at once to state it to Dr. Rosenbloom and, with his coöperation, immediately tested it. The solid residue from an evaporated ether extract of egg yolk offered the greatest advantages for a preliminary test. We accordingly made an ether solution of such a yellow residue, transferred the deep yellow solution to a rubber condom, immersed and supported the latter in ether in a stoppered bottle, and almost immediately observed diffusion currents as well as the rapid egress of lipochrome. Fat and cholesterol were easily detected in the diffusate.

Assuming that this prompt positive result might be due to defects in the rubber, we made many tests to satisfy ourselves that the observations were or were not what they appeared to be. Dr. Rosenbloom gave very earnest attention to this phase of the matter for some time and established the fact that we were dealing, except in a few cases of obviously imperfect membranes, with true diffusion phenomena.

The original experimental observations were made on March 1, 1910. At that time I was ignorant of similar results of previous work with rubber membranes, although I recalled rather vaguely the fact that Kahlenberg had made use of such membranes in another connection. The references to Kahlenberg's work which are given in the *Chemisches Zentralblatt* [1906 (2), pp. 1391 and 1772], the only ones we could find on this subject at that time, satisfied us that if we extended these experiments, the observations of a previous observer would not be repeated.[5] A month or two after the work

[5] The references to which I allude gave the substance of a paper in the *Transactions of the Wisconsin Academy of Sciences, Arts, and Letters,* 1905, xv (1), pp. 209–272, entitled: "On the nature of the process of osmosis and osmotic pressure, with observations concerning dialysis." The results with which our own could be directly compared were the following ones: Copper oleate was

was inaugurated we also saw a late reference to the well-known fact, regarding the swelling of rubber in lipin solvents, on which our work was based.[6] Ten months later we demonstrated these findings at a meeting of the American Society of Biological Chemists (see page 64).

The succeeding sections of this paper present reprinted preliminary reports on various portions of the studies which thus far have developed from the observations described above. It is my intention to discuss in detail each section of the work, and additional experiments, at the earliest opportunity, when I hope to dwell more particularly on the significance of such results for the student of the functions of cell membranes, and for the investigator of the coordinations and equilibria in intracellular affairs.

II. ON THE DIFFUSIBILITY OF BIOLOGICAL SUBSTANCES THROUGH RUBBER[7]

The writer and his associates have found that many ether-soluble substances of biological origin, such as fat and cholesterol, pass readily from ether solutions through rubber membranes into ether when the mechanical conditions for such diffusions are favorable. Lecithans appear to be wholly indiffusible.

Many substances which are soluble in fatty oils, chloroform, alcohol, acetone, ethyl acetate, and other solvents of similar powers, or in mixtures of such solvents, promptly diffuse through rubber under suitable conditions. Collodion is one of the products which appears to be indiffusible under such circumstances. When an ordinary ethereal solution of collodion (containing 24 per cent. of alcohol) is dialyzed in a rubber condom against ether in a closed vessel, the alcohol rapidly passes to the exterior and the collodion gradually *gelatinizes*. Liquid accumulates in the bag under these conditions.

Various inorganic substances diffuse through rubber under the

found to diffuse from benzene through a rubber membrane into benzene; and camphor diffused from pyridin, alcohol, and toluol through rubber membranes into the same solvents, respectively. A recent study of Kahlenberg's paper in the original makes it evident that our results may be explained on the theory of diffusion which Kahlenberg has done much to render convincing.

[6] Flack and Hill: *Journal of Physiology*, 1910, xl, p. xxxiii.

[7] Gies: *Proceedings of the Biological Section of the American Chemical Society: Science*, 1911, xxxiv, p. 223; BIOCHEMICAL BULLETIN, 1911, i, p. 125.

conditions mentioned above. Ferric sulphocyanate readily passes from ether solution through rubber into ether.

The writer inaugurated these studies, with Dr. Rosenbloom's coöperation,[8] in the hope of devising improvements in the methods for the isolation of lipins. The work is progressing along several lines, especially with reference to methods of isolation and purification, and to osmosis (see page 64).

III. A DEMONSTRATION OF OSMOTIC PRESSURE EXERTED BY FAT[9]

In the first of two demonstrations, a cylindrical *rubber* bag (condom), 1½ inches in diameter and 8 inches long, was lowered into an oiled *muslin* bag of about the same dimensions. The rubber bag was then filled to overflowing with olive oil. The rubber bag expanded, as the oil filled it, to the full length and width of the muslin sheath. The sheath prevented further extension of the rubber bag and imparted rigidity to the osmometer that was ultimately constructed. The double bag, full of oil and with its mouth wide open, was then raised so as to inclose about an inch of the lower end of a long glass tube which was firmly supported vertically above the demonstration table. The glass tube was 5 feet long and its bore was 4 mm. in diameter. Ligatures were tightly secured around the neck of the double bag against the immersed lower end of the vertical tube. The bag then hung directly from the end of the tube. The bag and its sheath were in a tightly distended condition and a stationary column of oil an inch high in the tube was visible above the protruding edge of the sheath. The tube and bag were then lowered into a salt-mouth liter bottle on the table until the bag almost touched the bottom of the bottle. The height of the bottle and the length of the bag were nearly equal. The tube was then marked with a label on the plane of the oil meniscus just above the neck of the bag, and enough ether was poured into the bottle to provide immersion for the bag to the depth of an inch. For a moment no change in the volume of oil was apparent, and the lateral

[8] Rosenbloom and Gies: *Proceedings of the American Society of Biological Chemists,* 1911, ii, p. 8; *Journal of Biological Chemistry,* 1911, ix, p. xiv.

[9] Rosenbloom and Gies: *Proceedings of the Society for Experimental Biology and Medicine,* 1911, viii, p. 71.

pressure of the ether was obviously without mechanical effect. But in a minute or two downward diffusion currents were visible along the surface of the bag and oil rose rapidly in the tube.

After the initial effects of the ether had been shown, the bottle was filled with ether containing Sudan III, and a 5-foot vertical extension of the same bore was added to the upright glass tube. In a moment the upward movement of the liquid was markedly accelerated.

The demonstration was started at about 9 P. M. At 10 P. M. the osmotic pressure had carried the column of oily fluid to the top of the 10-foot tube, and liquid continued to run rapidly from the upper orifice until the apparatus was dismantled after the adjournment of the meeting, at about 11.30 P. M.

During the progress of the demonstration, Sudan III diffused rapidly from the exterior, through the rubber, to the very top of the rising column of fluid, before any of the liquid passed out of the upper opening. Oil diffused rapidly through the rubber into the ether.

The *second demonstration* was essentially the same in principle and technic as the first. Instead of a 10-foot upright tube, however, the authors substituted an L tube with an inside diameter of 6 mm. The vertical extension of the tube was 17 inches, the horizontal extension was only 3 inches. The latter extension was drawn out to a narrow bore in an inclined plane, to facilitate direct delivery of any liquid that might pass through that end of the tube.

When partial immersion of the bag first occurred there was no visible response, but, in a minute or two, oil began to rise in the tube. The bag was then completely covered with ether. The upward movement proceeded rapidly; and in about an hour nearly 200 c.c. of liquid passed through the upper orifice into a graduated cylinder which was supported underneath the outlet to catch the overflow.

IV. A DEMONSTRATION OF THE DIFFUSION OF PIGMENTS FROM FAT THROUGH RUBBER INTO FAT[10]

The writer has found that many fat-soluble pigments, such as Sudan III and Scarlet R, diffuse readily from solid and liquid fats

[10] Gies: *Proceedings of the Society for Experimental Biology and Medicine,* 1911, viii, p. 73.

through rubber into various solid and liquid media, among them both solid fat and oil. Thus, when Sudan III is dissolved in melted lard, the red liquid poured into a rubber bag, the bag supported in melted lard in a bottle, and the apparatus promptly immersed in ice water—the fatty matter will congeal before any sign of pigmentary diffusion occurs but, in a few hours, a reddish tinge will develop outside of the bag, and on each successive day for several weeks further extension of the pigmented matter may be witnessed, until the whole of the external lard is deeply suffused with red. This process takes place quite rapidly when the lard and apparatus are kept in a thermostat at 40° C.

The demonstrations were intended to exhibit a few instances of such pigmentary diffusions as occur speedily enough at room tem-

No.	Contents of the Rubber Bag		Nature of the Liquid in which the Bag was Suspended	Result
	Oil	Pigment		
1	Olive oil	Scarlet R	Olive oil	Visible diffusion of the pigment occurred promptly
2	Cocoanut oil	Scarlet R	Cocoanut oil	Visible diffusion of the pigment occurred promptly
3	Lard oil	Sudan III	Lard oil	Visible diffusion of the pigment occurred promptly
4	Paraffin oil	Sudan III	Paraffin oil	Visible diffusion of the pigment occurred promptly
5	Olive oil	Sudan III	Ether	Visible diffusion of the pigment occurred *almost immediately*

perature to yield positive results within an hour. The accompanying summary indicates briefly the precise nature and results of the demonstrations (including two control tests—4 and 5), which were made with thin rubber bags in ordinary glass bottles.

The bags were securely supported in the bottles, and the mixtures were shaken occasionally during the demonstration. The bags were found, after the adjournment of the meeting, to be without defects.

V. COMPARATIVE DIALYSIS EXPERIMENTS, WITH DEMONSTRATIONS[11]

When dry bags of rubber, gold-beater's skin, parchment, and collodion, each containing olive oil and Sudan III, are separately

[11] Goodridge and Gies: *Proceedings of the Society for Experimental Biology and Medicine*, 1911, viii, p. 74.

immersed in olive oil, and the remaining conditions of the environment are uniform, diffusion of the pigment promptly occurs through rubber, but does not take place at all through any of the other three membranes. When the bags are lifted from the oil, washed externally with ether, and then immersed in ether,[12] the pigment quickly passes through the rubber, but diffuses very slowly if at all through the remaining membranes.

Successive immersions of *moist* impermeable membranes (gold-beater's skin and parchment) in alcohol and ether, for different periods of time, fail to render the treated membranes more permeable to Sudan III than before.

The authors demonstrated the general facts in this connection pertaining to rubber and gold-beater's skin.

VI. EXPERIMENTS ON THE DIFFUSIBILITY OF ALKALOIDS THROUGH RUBBER[13]

Various ether-soluble substances, when dissolved in ether and placed in rubber bags immersed in ether, readily pass through the rubber membranes thus imposed (I–V). We have found that various alkaloids and some related substances readily diffuse through rubber under such conditions.

Our experiments were conducted as follows: A moderate quantity of the pure ether-soluble substance was mixed with 15 to 25 c.c. of ether.[14] This mixture was poured through a funnel into a new air-tight rubber condom in such a way as to preclude the possibility of overflow upon the external surface. The bag was then immersed in about 50 c.c. of ether in a narrow salt-mouth bottle 7 inches high. With the bag suspended at full extension in this position, its mouth was about an inch above the opening in the bottle. The protrud-

[12] In experiments which the senior author has been conducting with Prof. Welker's coöperation, it has been found that collodion bags are disintegrated by *ether containing more than about 1.5 per cent. of alcohol. Pure ether* does not dissolve or in any way disorganize collodion membranes. A collodion bag containing *pure* ether may be immersed for a week or more in *pure* ether without undergoing any appreciable deterioration. (See page 70.)

[13] Sidbury and Gies: *Proceedings of the Society for Experimental Biology and Medicine,* 1911, viii, p. 104.

[14] Substances which did not dissolve readily were triturated with ether in a mortar.

ing condom was supported in the neck of the bottle by a tightly fitting cork stopper, which also served to keep the bag closed. After a diffusion period of convenient length (sometimes 2 to 5 days),[15] the condom was cautiously removed from the bottle, the ether diffusate was poured into a porcelain dish, and the ether completely removed by evaporation on a steam bath. At least one appropriate test was then applied to the residue.[16]

Meanwhile, the ether solution in the condom was removed. A large volume of water was then poured into the suspended bag, which, during its distention by the water, was carefully examined for signs of leakage. In a few instances defective membranes temporarily rendered the outcome doubtful. All results with such bags were ignored, of course. Each of the tests, even after reliable positive responses, was repeated at least once with a *new* rubber bag.

The substances named below (the complete list of those already tested in this connection) are readily diffusible under the conditions of these experiments:—

A. Apomorphin, atropin, brucin, caffein, cocain, codein, colchicin, coniin, morphin, narcein, narcotin, nicotin, physostigmin, quinin, strychnin, veratrin.

B. Acetanilid, antipyrin, phenacetin, picric acid, picrotoxin, pyramidon, salicylic acid.

Experiments with other solvents, and with additional substances of alkaloidal type, will be added to this series.

[15] Some of the alkaloids pass through rubber almost immediately under the conditions of these experiments.

[16] In the experiments with nicotin, the "tobacco odor" of the concentrated liquids was very pronounced.

STUDIES OF DIFFUSION THROUGH RUBBER MEMBRANES

2. Diffusibility of lipins from ether through rubber membranes into ether

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I. INTRODUCTION

Many experiments, in completion of the diffusion work I have been doing in collaboration with Dr. Gies,[1] have been performed to determine the diffusibility or non-diffusibility of lipins and similar substances (page 57). Such data must obviously be obtained in detail, if any attempt to devise methods for the isolation and purification of lipins by dialysis through rubber can be successful.

I present here briefly the essential results of the work already completed in this connection.

II. DIFFUSION EXPERIMENTS

Methods. In the experiments described below, ordinary rubber condoms were used as diffusion membranes.[2] Various kinds of "sheet rubber," known as "pure Mexican plantation rubber," and fumed with carbon-disulfid, were found to be good membranes for this kind of work, but besides allowing fats, fatty acids, soaps, cholesterol and lipochrome to diffuse through it, this sheet rubber also permits the passage of lecithans under the conditions to be described, although the lecithans pass through the sheet rubber very slowly compared with other lipins such as fat and cholesterol. Condoms

[1] Rosenbloom and Gies: *Proceedings of the American Society of Biological Chemists,* 1911, ii, p. 8; *Journal of Biological Chemistry,* 1911, ix, p. xiv.

[2] Before the condoms were used for this purpose, they were placed in fresh portions of ether daily for several days, to free them from the powder adherent to them. This is especially important when one proposes to test the dialysates for phosphorus, since the adherent powder has been found to contain phosphorus.

do not permit the diffusion of lecithans under the conditions of the tests to be described, and they were preferred for this work for that reason. The cause of the observed difference in permeability is unknown to us, but will soon be made the subject of special inquiry.

The substances to be tested in the diffusion experiments were dissolved in 100 to 200 c.c. of ether (anhydrous and distilled over sodium), the concentrations of the solutions varying from 0.5 to 5 per cent. The solution or suspension was carefully poured through a funnel into a new air-tight rubber condom in such a way as to preclude the possibility of overflow upon the external surface. The bag was then immersed in from 100 to 200 c.c. of pure ether in a wide-mouthed bottle of convenient size, and suspended loosely by a thin cord held securely between the stopper and neck of the tightly stoppered bottle. The bottle was kept well stoppered throughout the whole of each test to prevent egress of ether and ingress of dust and other extraneous matter.

1. Ether extract of egg yolk. Within five minutes after ether extract of egg yolk is subjected to the diffusion treatment described above, the lipochrome appears in the dialysate, diffusion currents being visible about the same time. The following substances can be detected in the dialysate after short periods of dialysis: fat, fatty acid, cholesterol, and lipochrome. The lecithans do not pass through the condoms, even during prolonged periods of dialysis.

We tested for lecithans in the dialysate by analyzing the evaporation residue for phosphorus by the fusion and Neumann methods, and by seeking an "acetone precipitate" in the concentrated ether solution after the addition of electrolyte (sodium chlorid). Sometimes a positive phosphorus test was obtained from a dialysate which did not yield an "acetone precipitate." In such cases, it was found that this result was due to the presence of glycerophosphoric acid in the dialysate. If to a solution of lecithans, which, after dialysis in a condom, does not yield a phosphorus compound to the dialysate, one adds some glycerophosphoric acid, and then dialyzes this solution through the same rubber condom, glycerophosphoric acid appears in the diffusate.

2. Ether extract of brain. The dialysate from ether extracts of brain contained fat, fatty acid, and cholesterol. Lecithans failed to dialyze.

3. Ether extract of heart muscle (ox). Fat, fatty acid, lipochrome, and cholesterol were detected in the dialysate from ether extracts of the heart muscle of oxen. Lecithans did not diffuse.

4. Ether extract of kidney and liver (dog). Fat, fatty acid, lipochrome, and cholesterol appeared in the diffusates from ether extracts of dog kidneys and livers. Lecithans did not dialyze.

5. Ether extract of blood (dog). Fat, fatty acid, lipochrome, and cholesterol occurred in the dialysates from ether extracts of dog blood. No lecithans dialyzed.

6. Ether extract of carrots. The coloring matter dialyzed very rapidly from ether extracts of carrots. A small amount of fat was also present in the dialysate.

7. Ether extract of xanthoma (skin). The yellow coloring matter dialyzed very quickly from an ether extract of xanthomatous skin, but it faded in twelve hours.

8. Ether extract of cerumen. Cholesterol, fat, and fatty acid were present in the dialysates from ether extracts of cerumen. Neither the coloring matter nor the lecithans diffused.

9. Ether extract of yeast. The dialysates from ether extracts of yeast exhibited a peculiar opalescence, even at the end of six weeks' dialysis. A small amount of fat dialyzed, but lecithans did not diffuse.

10. Ether solutions of individual substances or special products. The following substances or special products, when subjected to diffusion by the method described above, were found to be diffusible:

Acetic acid	Ethyl butyrate	Palmitic acid
Acetone	Formic acid	Potassium palmitate[4]
Beta-hydroxy-butyric acid	Glycerol[3]	Potassium stearate[4]
Butter (fresh and rancid)	Lactic acid	Propionic acid
Butyric acid	Lead oleate	Sodium palmitate[4]
Cholesterol-acetate	Mutton tallow	Sodium stearate[4]

[3] When ether-alcohol solutions of glycerol are dialyzed against ether-alcohol, and alcohol solutions of glycerol are dialyzed against ether, the dialysates contain glycerol.

[4] Treated with water, then with alcohol to the point of precipitation, then with ether until a precipitate was produced. The filtrate was dialyzed against water, alcohol, and ether in identical proportions.

Cholesterol-benzoate	Oleic acid	Stearic acid
Cholesterol (from brain, egg yolk, and gall-stones)	Olive oil	Sudan III
	Olive oil stained with Sudan III	Urochrome[5]
Ethyl acetate		Valerianic acid

In some special experiments we found[6] that cholesterol benzoate, cholesterol stearate, cholesterol oleate and cholesterol palmitate, when dissolved in ether, readily diffuse through rubber into ether.

Cholesterol stearate with a molecular weight of 652.61 diffuses, whereas the various lecithans, with molecular weights considered to be 770 to 785, do not. If we assume that the diffusion of a substance depends on the size of its molecules, the above facts strengthen Hiestand's conclusion that the molecular weight of egg-yolk lecithin is 1446, which figure he obtained by a molecular weight determination.

11. **Indiffusible substances.** The following substances, when subjected to diffusion by the method described above, were found to be indiffusible.[7]

Sodium chlorid	Lecithans from yeast
Lecithans from brain	Lecithans from wheat embryo
Lecithans from egg yolk	Kephalin from brain
Lecithans from heart muscle	Cuorin from heart muscle
Lecithans from pig testicle	Compound of lecithin with platinic chlorid
Lecithans from liver and kidney	

Koch[8] has lately described the preparation of various compounds with lecithans, but it is uncertain whether these compounds are colloidal adsorptions, mechanical mixtures, or true chemical compounds. It seemed of interest to study the behavior of these substances in ether solution, when subjected to dialysis in rubber bags suspended in ether.

The preparations used in these experiments were made accord-

[5] Ether-alcohol solution (equal amounts) dialyzed against ether-alcohol.

[6] Boas and Rosenbloom: *Proceedings of the Society for Experimental Biology and Medicine,* 1911, viii, p. 132.

[7] We have found that lecithans prepared by the Zuelzer, Bergell, or Roaf and Edie method, when dialyzed, always yield traces of cholesterol to the dialysate; and often fat.

[8] Koch and collaborators: *Journal of Pharmacology and Experimental Therapeutics,* 1910, xii, 239–269.

ing to the method described by Koch. For the dialysis tests the solutions of the lecithan compounds were evaporated to dryness at 38° and the residues triturated with ether. The extracts were filtered, and the filtrates placed inside of rubber bags and dialyzed against ether for thirty-seven days. The dialysates were tested weekly to see if the substance combined with the lecithan had diffused.

Compounds of lecithin with glucose, lactic acid, strychnin, digitonin, salicin, urea, creatin, creatinin, and caffein were prepared. It was found that the glucose and lactic acid dialyzed completely, the strychnin, digitonin, and salicin dialyzed partially, while urea, creatin, creatinin, and caffein did not dialyze at all.[9]

It was thought that some of the various substances which did not diffuse might do so in the presence of a considerable amount of diffusible material, but on dialyzing various mixtures of the above-named indiffusible substances with varying amounts (up to 15 grams) of neutral fat, fatty acid, cholesterol, or olive oil, no diffusion of lecithan occurred.

When solutions of lecithans are subjected to dialysis by the method described above, they take up a great deal of ether, and the volume of liquid in the bag is greatly increased. We have demonstrated that lipins exert strong osmotic pressure. (See page 59.)

We have also placed ether solutions of lecithans with cholesterol and fat in closed rubber bags suspended in Soxhlet extractors. Soxhlet extraction in the usual way failed to remove lecithan from the bag under these conditions. These findings favor the development of a method for the thorough removal of impurities from lecithan solutions.

It is perhaps superfluous to add that the results already mentioned may be obtained by placing the solution to be tested outside the rubber bag and allowing dialysis to take place into pure ether contained in the bag.

III. SUMMARY OF GENERAL CONCLUSIONS

1. Most lipins, chief among them, fat, fatty acid, soaps, cholesterol, cholesterol-esters, lipochrome, and various other ether-sol-

[9] Boas and Rosenbloom: *Loc. cit.*

uble substances, diffuse from ether solution through rubber membranes into ether.

2. Sodium chlorid, lecithans prepared from various sources, kephalin, cuorin, and the compound of platinum with lecithin, do not diffuse under such conditions.

3. One or more of the diffusible substances in these experiments may be dialyzed from solutions containing them, together with one or more of the indiffusible ones, without inducing any of the latter to pass through the membrane.

STUDIES OF DIFFUSION THROUGH RUBBER MEMBRANES

3. Diffusibility of protein through rubber membranes, with a note on the disintegration of collodion membranes by common ethyl ether and other solvents

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I. INTRODUCTION

In the course of our studies of proteins, under the auspices of the George Crocker Special Research Fund, we obtained a protein product which is soluble in a mixture of equal parts of absolute alcohol and absolute ether, and which responds to the biuret, xanthoproteic, Millon and Hopkins-Cole tests. The material was prepared by the following method: 25 grams of Witte peptone were dissolved in 500 c.c. of 0.2 per cent. hydrochloric acid solution. The liquid was evaporated to a thick syrup on a water bath. This syrup was thoroughly extracted with absolute alcohol and the resultant yellow liquid filtered. The filtrate was treated with an equal volume of absolute ether, which produced a white flocculent precipitate. After the sedimentation of the precipitate, the supernatant liquid was decanted and filtered. When *five volumes of absolute ether* were added to this filtrate, a white flocculent precipitate was produced. This product was isolated by filtration, washed with absolute ether, and exposed to the air in a thin layer on a watch glass, where it solidified as yellowish and somewhat hygroscopic granular material, which could easily be pulverized. The product dissolved promptly in absolute alcohol. From *concentrated* alcoholic solution the product may be precipitated by the addition of an *equal volume* of absolute ether. Whether the product is peptone or a much simpler polypeptid has not yet been determined.

Dr. Gies and his collaborators have lately given much attention to

the diffusibility of lipins and other substances through rubber membranes. The solubility of the above mentioned product in alcohol-ether solution led Dr. Gies to propose a study of the comparative diffusibility of the protein through membranes of rubber, parchment and collodion. Such an investigation was accordingly conducted by the diffusion process described on page 55. The data in the accompanying tables indicate the conditions and the results of the tests in this connection.[1]

II. COMPARATIVE DIFFUSION EXPERIMENTS

Experiments with rubber membranes. The results of the tests in the first four series show collectively [Table I (1–15)] that *biuret-reacting matter* appeared in the diffusates; that the rubber itself did not yield such substance; and that the occurrence of biuret-

TABLE I

Results of experiments with rubber membranes

A. Data showing the diffusibility of biuret-reacting material

FIRST SERIES. WITH RUBBER CONDOMS.

Exp. No.	Duration of the experiment, days	Contents of the bag	Liquid outside of the bag	Results of the *biuret* test in the diffusate	Results of the test for *leaks* at the end of the experiment
1	4	30 c.c. ether-alc. sol. 10 c.c. abs. ether	Abs. ether	+++	Small hole in the bag
2	4	20 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	++	No leak
3	4	10 c.c. ether-alc. sol. 30 c.c. abs. ether	Abs. ether	+	No leak

SECOND SERIES. WITH RUBBER CONDOMS.

4	5	30 c.c. ether-alc. sol. 10 c.c. abs. ether	Abs. ether	+++	Small hole very high up in the bag
5	5	20 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	++	No leak
6	5	10 c.c. ether-alc. sol. 30 c.c. abs. ether	Abs. ether	+	No leak

[1] In the tables, "ether-alc. sol." indicates the protein solution as it was made available by the above mentioned process *before final precipitation with five volumes of ether.* Such precipitation was effected only when the solid was desired for special reasons. See the data pertaining to the eighth series of tests, Table I.

TABLE I (*continued*)

THIRD SERIES. WITH RUBBER CONDOMS.

Exp. No.	Duration of the experiment, days	Contents of the bag	Liquid outside of the bag	Results of the *biuret* test in the diffusate	Results of the test for *leaks* at the end of the experiment
7	5	30 c.c. ether-alc. sol. 10 c.c. abs. ether	Abs. ether	+++	No leak
8	5	20 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	++	No leak
9	5	10 c.c. ether-alc. sol. 30 c.c. abs. ether	Abs. ether	+	No leak
10[2]	5	Abs. ether ("control")	Abs. ether	−	No leak
11[3]	5	Abs. ether ("control")	Abs. ether	−	

FOURTH SERIES. WITH BAGS OF SHEET RUBBER.[4]

12	30	60 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	+++	No leak
13	30	40 c.c. ether-alc. sol. 40 c.c. abs. ether	Abs. ether	++	No leak
14	30	20 c.c. ether-alc. sol. 60 c.c. abs. ether	Abs. ether	−	No leak
15	30	Abs. ether ("control")	Abs. ether	−	No leak

B. Data showing that the diffusible biuret-reacting material (1–15) was true protein

FIFTH SERIES. WITH RUBBER CONDOMS.

Exp. No.	Duration of the experiment, days	Contents of the bag	Liquid outside of the bag	Results of the tests for protein in the diffusate			Results of the test for *leaks* at the end of the experiment
				Biuret	*Hopkins-Cole*	*Xanthoproteic*	
16	3	30 c.c. ether-alc. sol. 10 c.c. abs. ether	Abs. ether	+	+	+	No leak
17	3	30 c.c. ether-alc. sol.	Abs. ether	+	+	+	No leak

SIXTH SERIES. WITH BAGS OF SHEET RUBBER.[4]

18	3	60 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	+	+	+	No leak
19	3	60 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	+	+	+	No leak

SEVENTH SERIES. WITH RUBBER CONDOMS.

20	3	40 c.c. ether-alc. sol.	Abs. ether	+	+	+	No leak
21	3	40 c.c. ether-alc. sol.	Abs. ether	+	+	+	No leak

[2] This control experiment (10) was carried out in duplicate, with negative results in each case.

[3] In this experiment (11) a new, clean, empty condom, with the bottom removed, was suspended in absolute ether, for "control" purposes.

[4] "Pure Mexican plantation rubber."

TABLE I (*continued*)

C. Data showing the effect of water on the diffusion phenomena (1–21)

EIGHTH SERIES. WITH RUBBER CONDOMS.[5]

Exp. No.	Duration of the experiment, days	Contents of the bag	Liquid outside of the bag	Results of the *biuret* test in the diffusate	Results of the test for *leaks* at the end of the experiment
22	5	3 c.c. abs. alc. sol. 9 c.c. abs. ether 6 c.c. H_2O 3 c.c. abs. alc.	6 c.c. abs. alc. 18 c.c. abs. ether 1.2 c.c. H_2O	–	No leak
23	5	3 c.c. abs. alc. sol. 21 c.c. abs. ether 1 c.c. H_2O 6 c.c. abs alc.	9 c.c. abs. alc. 21 c.c. abs. ether 1 c.c. H_2O	–	No leak
24	5	3 c.c. abs. alc. sol. 3 c.c. abs. ether	Equal volumes of abs. ether and abs. alcohol	+	No leak

D. Data showing the effect of fat on the diffusion phenomena (1–24)

NINTH SERIES. WITH RUBBER CONDOMS.

25	10	Olive oil and Witte peptone (solid)	Olive oil	–[6]	——
26	10	Olive oil, Witte peptone (solid) and H_2O[7]	Olive oil	–	——

TENTH SERIES. WITH RUBBER CONDOMS.

27	30	Olive oil and Witte peptone (solid)	Olive oil	–	——
28	30	Olive oil, Witte peptone and H_2O[7]	Olive oil	–	——

ELEVENTH SERIES. WITH RUBBER CONDOMS.

29	10	Ether-alc. sol. Lard	Abs. ether	–	——
30	10	Ether-alc. sol. Lard	Abs. ether	–	——

TWELFTH SERIES. WITH RUBBER CONDOMS.

31	1	Ether-alc. sol. Lard	Abs. ether	+	No leak
32	1	Ether-alc. sol. Lard	Abs. ether	+	No leak

THIRTEENTH SERIES. WITH RUBBER CONDOMS.

33	2	30 c.c. ether-alc. sol. Lard	30 c.c. abs. ether	+	No leak
34	2	30 c.c. ether-alc. sol. Lard	30 c.c. abs. ether	+	No leak

[5] For this series of tests, we used 0.2 gram of the protein material dissolved in 10 c.c. of absolute alcohol.

[6] On the water-soluble extract of the oil.

[7] Water sufficient to make a paste of the Witte peptone was used. The paste was triturated into the oil.

reacting material in the diffusate was not due to perforations of the bags. The diffusion of biuret-reacting material was always greatest in degree through the bags containing the largest proportion of protein.

The results of tests 1–15 show that biuret-reacting material *diffused* through the rubber membranes under the conditions imposed. In order to determine more definitely, however, whether *protein* diffused through the rubber, we repeated the essential features of tests 1–15, but applied additional tests to the diffusates [Table 1 (16–21)].

The results of tests 16–21 (Table 1) confirm the findings of tests 1–15, and also show definitely that the diffusible biuret-reacting material was *true protein.*

The data of tests 1–21 suggest that osmosis depends upon affinities between the membrane, and the solvent or solute, or both. We made a direct test of this mattter in a preliminary way by adding water to the solvent and thus disturbing its affinities with the membrane without decreasing the solubility of the solute. The findings are given in the summary pertaining to the eighth series (Table 1).

The results of tests 22–24 show that water exerted a disturbing osmotic influence and that diffusion of the protein was entirely prevented by the water. We extended these experiments to determinations of the influence of associated, readily diffusible lipins, in the presence or absence of water. The results are given in tests 25–34.

In the tenth series the oil in the *diffusates* was emulsified with a little soap solution and then repeatedly extracted with ether until all the fat was removed. The water containing the soap, and the aqueous extract of the oil, were evaporated to dryness and the biuret test applied to a concentrated solution of the residue.

Experiments with parchment-paper bags. The foregoing results with rubber membranes naturally increased our desire to make comparative observations with bags of parchment and collodion. The results of the tests with parchment are given in tests 35–42, Table 2. That osmosis depends upon accord between the solvent and the membrane is obvious from these results also, for the protein substance, which is readily diffusible through parchment from *aqueous* solution, does not dialyze through such a membrane from an *alcohol-ether* solution.

TABLE 2

Results of experiments with bags of parchment paper

Exp. No.	Duration of the experiment, days	Contents of the bag	Liquid outside of the bag	Results of the *biuret* test in the diffusate
		FOURTEENTH SERIES.		
35	1	10 c.c. ether-alc. sol. 30 c.c. abs. ether	Abs. ether	—
36	2	3 c.c. ether-alc. sol. 3 c.c. abs. ether	Abs. ether	—
		FIFTEENTH SERIES.[8]		
37	10	30 c.c. ether-alc. sol. 10 c.c. abs. ether	Abs. ether	—
38	10	20 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	—
39	10	10 c.c. ether-alc. sol. 30 c.c. abs. ether	Abs. ether	—
		SIXTEENTH SERIES.[9]		
40	10	30 c.c. ether-alc. sol. 10 c.c. abs. ether	Abs. ether	—
41	10	20 c.c. ether-alc. sol. 20 c.c. abs. ether	Abs. ether	—
42	10	10 c.c. ether-alc. sol. 30 c.c. abs. ether	Abs. ether	—

III. ON THE UTILITY OF COLLODION BAGS IN EXPERIMENTS OF THE KIND DESCRIBED IN THE FOREGOING SECTIONS

Experiments like those in the sixteenth series (Table 2) were attempted with bags made of collodion, but in each case the bags were perforated and in part dissolved, by the contents, before the experiment could be fairly started.

Several years ago, Dr. Gies observed, in some dialysis experiments with collodion membranes, that ethyl ether could be kept on the aqueous contents of collodion bags, for preservative purposes in such tests, without inducing distintegration of the bags. In repetitions of the experiments a year or two later, however, it was found that ether under such circumstances often caused deterioration of

[8] The results in this series, while apparently negative in each case, were somewhat doubtful owing to the fact that the paper contained some soluble material, which rendered the biuret test more or less uncertain. See the results of the tests in the sixteenth series.

[9] The parchment paper was washed free from soluble matter before the beginning of the tests.

the membrane, but that occasionally it did not. The reason for such variations in the action of the ether could not be conveniently ascertained at the time.

The prompt perforation of the collodion bags in our several attempts, as stated above, to determine the diffusibility through collodion of the alcohol-ether soluble protein, recalled Dr. Gies' previous experiences and led him to suspect that the alcohol, in the solutions employed by us, was responsible for the observed destructive effects on the collodion membrane in these experiments. He believed, also, that the previous variations in the action of ether on collodion in dialysis experiments, as already related, were due to differences in the degrees of purity of the ether employed. At Dr. Gies' request, therefore, I made direct tests of the solvent action of ether containing alcohol, and various other substances related in one way or another to alcohol and ether.

Collodion bags were made, in test tubes, from U. S. P. collodion.[10] It was found that such bags were not perforated by absolute ether when it was poured into them 10 minutes after their removal from the tubes, *i. e.*, after fairly complete evaporation of the residual alcohol. The time required for the evaporation of the residual alcohol is dependent on the prevailing temperature. At low temperatures the alcohol disappears from the collodion membrane very slowly. Common ether (Merck's 0.720 sp. gr.), however, when poured into such bags, passed through them almost immediately, with general solution of the collodion, even after 2 hours of preliminary exposure of the bag outside the mould. In the first tests of the effects of alcohol it was found that absolute ether containing 1.5 per cent. or more of added absolute alcohol promptly penetrated the bags.

In a series of more careful tests of absolute ether containing various percentages of added absolute alcohol, it was found that the bags were penetrated promptly by ether containing more than 1.25 per cent. of alcohol, but that the mixture containing 1.25 per cent. of alcohol acted more slowly. Ether containing less than 1.25 per cent. of alcohol exhibited no destructive action.

Qualitative tests showed that acetone, acetaldehyde, ethyl acetate, methyl alcohol and glacial acetic acid attack and penetrate collodion

[10] An ether solution containing alcohol.

bags[11] immediately, toluol slowly, whereas formic acid, formaldehyde (40 per cent.), chloroform, petroleum ether, carbon tetrachlorid, carbon bisulfid and paraffin oil were without distinguishable solvent action, even after long periods of contact. Acetone (5 per cent.) *in absolute ether* attacks collodion bags slowly, while a 10 per cent. solution acts rapidly. Acetaldehyde (4 per cent.) in absolute ether attacks the bags slowly, but a 5 per cent. solution acts rapidly. Methyl alcohol (3 per cent.) *in absolute ether* dissolves the bags, but a 2 per cent. solution does not. Glacial acetic acid (2 per cent.) *in absolute ether* attacks the bags slowly, a 3 per cent. solution acts rapidly but a 1 per cent. solution appears to be inert. *Five per cent. solutions* of chloroform, toluol, petroleum ether, carbon tetrachlorid, carbon bisulfid, benzol, ethyl acetate, and paraffin oil, *in absolute ether,* were without visible effect on collodion bags. Five per cent. solutions of formic acid (sp. gr. 1.2) and formaldehyde (40 per cent.), *in absolute ether,* IMMEDIATELY attacked and penetrated collodion bags.

Further work along these lines is in progress.

My cordial thanks are due Dr. Gies for his kind direction and assistance in these experiments.

[11] Bags practically free from residual alcohol were used.

STUDIES OF DIFFUSION THROUGH RUBBER MEMBRANES

4. The comparative diffusibility of various pigments in different solvents

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I. INTRODUCTION

Dr. Gies and his associates have shown that many biological substances diffuse through rubber under suitable conditions (page 55). Inorganic as well as organic substances exhibit this capacity and various colloids share it with crystalloids. Lipochrome and ferric sulfocyanate are among the colored substances which, in the early experiments, were found to be diffusible from ether solution through rubber membranes into ether.

At Dr. Gies' suggestion we undertook a similar study of the diffusibility of common pigments, especially "food colors." Following his advice we also sought data which might be of service in devising methods for the purification of pigments, and for their separation and detection under various circumstances.

Our diffusion tests were conducted by the following general method: A moderate quantity of the pigment was mixed with 15–25 c.c. of the solvent. The solution, or suspension, was carefully poured into a rubber condom in such a way as to preclude the possibility of overflow upon the external surface. The bag was then immersed in about 50 c.c. of solvent in a narrow salt-mouth bottle 7 inches high. With the bag suspended at full extension in this position, its mouth was about an inch above the opening in the bottle. The protruding condom was supported in the neck of the bottle by a tightly fitting cork stopper, which served to keep both the bag and the bottle closed. The diffusion periods varied from a few minutes to a week or more, according to the obvious requirements in each case for a definite conclusion regarding diffusibility.

Most of the original tests were made with new condoms. Many tests were repeated with condoms which had previously been employed by us in pigment-diffusion experiments but which, prior to being used again, had been thoroughly washed with portions of the solvent to which they were soon to be subjected in the new diffusion tests. Defects in the rubber could easily be detected. All doubtful results were ignored. Numerous repetitions prevented erroneous deductions.

In the accompanying summary we present an outline of the various tests and the main results of each. For the sake of convenience we use in the summary the following abbreviations:

D, diffusion; **D1**, pigment appears in the diffusate within 10 minutes; **D2**, pigment does not diffuse within 10 minutes, but appears in the diffusate within 30 minutes; **D3**, pigment does not diffuse within 30 minutes, but appears in the diffusate within 1 hour; **D4**, pigment does not diffuse within 1 hour, but appears in the diffusate before the lapse of 2 hours; **D5**, pigment cannot be seen in the diffusate before the third hour of diffusion, but appears before the fourth hour; **D6**, pigment cannot be seen in the diffusate before the sixth hour of diffusion, but appears before the eighth hour; **D7**, pigment cannot be seen in the diffusate before the tenth hour of diffusion, but appears before the twelfth hour; **D8**, pigment appears in diffusate in about 24 hours; **O**, no visible diffusion at any time within a week.

II. SUMMARY OF DIFFUSION DATA

Inside		Outside solvent	Result	Remarks
Solvent	Pigment			
1 Ether...	Sudan III.....	Ether.....	D1	D very rapid.
2 Ether...	Sudan III.....	Ether + alcohol (25%).	D1	D rapid though slower than 1.
3 Ether...	Sudan III.....	Ether + alcohol (50%).	D1	D slower than 2.
4 Ether...	Sudan III.....	Ether + alcohol (75%).	D1	D slower than 3.
5 Ether...	Sudan III.....	Alcohol (100%)...	D1	Ether was withdrawn, leaving a concentrated solution of Sudan III. Currents very distinct. Collection of color near top very marked.
6 Ether...	Sudan III.....	Chloroform..	D1	Color zone on top. No diffusion currents downward.

II. SUMMARY OF DIFFUSION DATA (*continued*)

Inside		Outside solvent	Result	Remarks
Solvent	Pigment			
7 Ether...	Sudan III.....	Methyl alcohol.....	D1	Ether was withdrawn, leaving layer of dye inside.
8 Ether...	Sudan III.....	Acetone.....	D1	Ether withdrawn. Solution of pigment in bag concentrated.
9 Ether...	Sudan III.....	Petroleum ether.....	D1	
10 Ether...	Sudan III.....	Gl. acetic acid	D1	Moderate diffusion.
11 Alcohol..	Sudan III.....	Ether.....	D1	Diffusion slow.
12 Chloroform...	Sudan III.....	Chloroform..	D1	Rapid diffusion.
13 Alcohol..	Sudan III.....	Alcohol.....	D1	Diffusion slow.
14 Ethyl acetate..	Sudan III.....	Ethyl acetate	D1	Slow diffusion.
15 Acetone..	Sudan III.....	Acetone.....	D1	Moderate diffusion.
16 Gl. acetic acid....	Sudan III.....	Gl. acetic acid	D1	Slow diffusion.
17 Ether...	Picric acid.....	Ether.....	D1	Rubber yellow; color not removed by ether.
18 Ether...	Hematoxylin	Ether.....	D3	
19 Ethyl acetate..	Methyl violet.....	Ethyl acetate	D5	Denser solution in bag than outside. (See 36.)
20 Acetone..	Methyl violet.....	Acetone.....	D6	
21 Alcohol..	Methyl violet.....	Alcohol.....	O	
22 Ether...	Methyl violet.....	Ether.....	D5	
23 Ether...	Magenta.....	Ether.....	D5	
24 Ether...	Naphthol yellow ...	Ether.....	O	
25 Ether...	Methyl violet and Sudan III.....	Ether.....	D1	Rapid diffusion of the Sudan III. Ether changed three times in 2 hours after which practically all Sudan III had been removed, leaving the methyl violet in the bag.
26 Ether...	Chlorophyl[1]	Ether.....	D1	Very slight diffusion.
27 Ether...	Annatto.....	Ether.....	D1	Very slight diffusion.
28 Ether...	Alcannin.....	Ether.....	D1	Very rapid diffusion.
29 Ether...	Metanil yellow.....	Ether.....	O	
30 Ether...	Martius yellow.....	Ether.....	D1	Rapid diffusion.
31 Ether...	Scarlet R.....	Ether.....	D1	Very rapid diffusion.
32 Ether...	Malachite green....	Ether.....	D2	Bag colored green.
33 Ether...	Brazil wood.....	Ether.....	D1	Slow diffusion.
34 Ether...	Chrysoïdin.....	Ether.....	D2	Very slow diffusion.
35 Ether...	Turmeric.....	Ether.....	D1	Rapid diffusion.
36 Ethyl acetate..	Annatto.....	Ethyl acetate	D2	Pigment solution inside concentrated.[2]
37 Ethyl acetate..	Chlorophyl.....	Ethyl acetate	D1	Pigment solution inside concentrated.

[1] "Fat-soluble" chlorophyl was used in all the chlorophyl tests.

[2] In these cases (36–44) the solvent diffused more rapidly than the solute.

II. SUMMARY OF DIFFUSION DATA (*continued*)

Inside		Outside solvent	Result	Remarks
Solvent	Pigment			
38 Ethyl acetate..	Alcannin.....	Ethyl acetate	D1	Pigment solution inside concentrated.
39 Ethyl acetate..	Martius yellow	Ethyl acetate	D1	Pigment solution inside concentrated.
40 Ethyl acetate..	Scarlet R.....	Ethyl acetate	D1	Pigment solution inside concentrated.
41 Ethyl acetate..	Brazil wood.....	Ethyl acetate	D1	Pigment solution inside concentrated.
42 Ethyl acetate..	Chrysoïdin.....	Ethyl acetate	D3	Pigment solution inside concentrated.
43 Ethyl acetate..	Turmeric.....	Ethyl acetate	D3	Pigment solution inside concentrated.
44 Ethyl acetate..	Metanil yellow.....	Ethyl acetate	D3	Pigment solution inside concentrated.
45 Ethyl acetate..	Malachite green....	Ethyl acetate	D6	
46 Ethyl acetate..	Naphthol yellow ...	Ethyl acetate	O	
47 Ethyl acetate..	Sudan I.....	Ethyl acetate	D1	Rapid diffusion.
48 Ethyl acetate..	Sudan G.....	Ethyl acetate	D1	Moderate diffusion.
49 Ethyl acetate..	Rhodamin.....	Ethyl acetate		D in about 2 days.
50 Ethyl acetate..	Fast red A.....	Ethyl acetate	D3	Diffusion very slight in each of tests 50–56 inclusive.
51 Ethyl acetate..	Rose bengal.....	Ethyl acetate	D2	
52 Ethyl acetate..	Erythrosin.....	Ethyl acetate	D2	
53 Ethyl acetate..	Methylene violet...	Ethyl acetate	D5	
54 Ethyl acetate..	Phloxin red.....	Ethyl acetate	D3	
55 Ethyl acetate..	Auramine.....	Ethyl acetate	D5	
56 Ethyl acetate..	Orange G.....	Ethyl acetate		D in about 5 hours.
57 Methyl alcohol..	Gold orange.....	Methyl alcohol....	D8	Color of diffusate very slight 1 week later.
58 Methyl alcohol..	Naphthol yellow ...	Methyl alcohol....		No appearance of color in 8 hours.
59 Meth alcohol..	Carmosin B	Methyl alcohol....	D8	Color of diffusate very slight 1 week later.

II. SUMMARY OF DIFFUSION DATA (*continued*)

Inside		Outside solvent	Result	Remarks
Solvent	Pigment			
60 Methyl alcohol..	Ponceau, G. A.....	Methyl alcohol....	O	
61 Methyl alcohol..	Ponceau, 2 R.....	Methyl alcohol....	O	
62 Methyl alcohol..	Naphthol red S....	Methyl alcohol....	O	
63 Methyl alcohol..	Curcumin S.....	Methyl alcohol....	O	
64 Amyl alcohol..	Fast red A.....	Amyl alcohol	D7	
65 Amyl alcohol..	Safranin.....	Amyl alcohol	D7	Color of diffusate very slight 1 week later.
66 Amyl alcohol..	Eosin A.....	Amyl alcohol	O	
67 Amyl alcohol..	Phloxin.....	Amyl alcohol		D in about 3 days. Color of diffusate very slight 1 week later.
68 Amyl alcohol..	Rose bengal.....	Amyl alcohol	O	
69 Amyl alcohol..	Rhodamin.....	Amyl alcohol	D7	
70 Amyl alcohol..	Erythrosin.....	Amyl alcohol	D8	Color of diffusate very slight 1 week later.
71 Amyl alcohol..	Chrysoïdin	Amyl alcohol	D3	
72 Amyl alcohol..	Sudan I.....	Amyl alcohol	D2	
73 Amyl alcohol..	Sudan G.....	Amyl alcohol	D2	
74 Amyl alcohol..	Sudan III.....	Amyl alcohol	D2	
75 Amyl alcohol..	Alcannin.....	Amyl alcohol	D3	
76 Amyl alcohol..	Chlorophyl.....	Amyl alcohol	D3	
77 Acetone..	Alcannin.....	Acetone.....	D1	Very rapid diffusion.
78 Acetone..	Auramine.....	Acetone.....	D1	
79 Acetone..	Barwood.....	Acetone.....	D1	
80 Acetone..	Chlorophyl.....	Acetone.....	D3	
81 Acetone..	Chrysoïdin.....	Acetone.....	D5	
82 Acetone..	Fast red A.....	Acetone.....	D4	Color of diffusate not very strong 1 week later.
83 Acetone..	Methylene violet...	Acetone.....	D4	
84 Acetone..	Malachite green....	Acetone.....	D5	
85 Acetone..	Martius yellow.....	Acetone.....	D3	
86 Acetone..	Metanil yellow.....	Acetone.....	D8	
87 Acetone..	Naphthol yellow S..	Acetone.....	O	
88 Acetone..	Picric acid.....	Acetone.....	D1	
89 Acetone..	Rhodamin.....	Acetone.....	D7	

II. SUMMARY OF DIFFUSION DATA (*continued*)

Inside		Outside solvent	Result	Remarks
Solvent	Pigment			
90 Acetone..	Sudan I.....	Acetone.....	D1	
91 Acetone..	Sudan G.....	Acetone.....	D1	
92 Acetone..	Fustic.....	Acetone.....	O	
93 Acetone..	Rose bengal.....	Acetone.....	D8	Color of diffusate very slight 1 week later.
94 Acetone..	Phloxin.....	Acetone.....	D4	Color of diffusate very slight 1 week later.
95 Acetone..	Eosin W. gelblich...	Acetone.....	D7	Color of diffusate very slight 1 week later.
96 Acetone..	Eosin A.	Acetone.....	O	
97 Acetone..	Cape aloes.....	Acetone.....	D4	
98 Gl. acetic acid....	Sudan G.....	Gl. acetic acid	D1	Slow diffusion.
99 Gl. acetic acid....	Sudan III.....	Gl. acetic acid	D1	Rapid diffusion.
100 Gl. acetic acid....	Sudan I.....	Gl. acetic acid	D1	Slow diffusion.
101 Gl. acetic acid....	Alcannin.....	Gl. acetic acid	D3	
102 Gl. acetic acid....	Chlorophyl.....	Gl. acetic acid	D6	
103 Gl. acetic acid....	Rose bengal.....	Gl. acetic acid	D8	
104 Gl. acetic acid....	Phloxin.....	Gl. acetic acid		D in about 2 days.
105 Gl. acetic acid....	Malachite green....	Gl. acetic acid	O	
106 Gl. acetic acid....	Methyl violet.....	Gl. acetic acid	D8	
107 Gl. acetic acid....	Scarlet R.....	Gl. acetic acid	D2	
108 Gl. acetic acid....	Methylene violet...	Gl. acetic acid		D in about 10 days.
109 Gl. acetic acid....	Martius yellow.....	Gl. acetic acid	D8	
110 Gl. acetic acid....	Biebrich Scarlet....	Gl. acetic acid	D8	
111 Gl. acetic acid....	Erythrosin.....	Gl. acetic acid		D in about 4 days.
112 Gl. acetic acid....	Orange G.....	Gl. acetic acid	O	
113 Gl. acetic acid....	Tropeolin OO.....	Gl. acetic acid		Very slight color after 2 days, which did not increase after standing about four days.
114 Gl. acetic acid....	Auramine.....	Gl. acetic acid	O	
115 Gl. acetic acid....	Rhodamin.....	Gl. acetic acid	D8	
116 Gl. acetic acid....	Eosin A. gelblich...	Gl. acetic acid	D8	
117 Gl. acetic acid....	Chrysoïdin.....	Gl. acetic acid		D in about 5 days.
118 Gl. acetic acid....	Eosin W.	Gl. acetic acid		D in about 4 days, which did not increase during the succeeding 3 days.

II. SUMMARY OF DIFFUSION DATA (*continued*)

Inside		Outside solvent	Result	Remarks
Solvent	Pigment			
119 Gl. acetic acid	Fast Red A	Gl. acetic acid	D3	
120 Gl. acetic acid	Safranin	Gl. acetic acid		D in about 4 days, which did not increase during the succeeding 3 days.
121 Gl. acetic acid	Turmeric	Gl. acetic acid	O	
122 Gl. acetic acid	Metanil yellow	Gl. acetic acid	D7	
123 Gl. acetic acid	Barwood	Gl. acetic acid		D in about 2 days.
124 Gl. acetic acid	Annatto	Gl. acetic acid	O	
125 Gl. acetic acid	Picric acid	Gl. acetic acid		D in about 6 days.
126 Alcohol	Auramine	Alcohol		D in about 3 days.
127 Alcohol	Chrysoïdin	Alcohol	D7	
128 Alcohol	Eosin A	Alcohol	D7	
129 Alcohol	Eosin W	Alcohol	O	
130 Alcohol	Fast Red	Alcohol	D7	
131 Alcohol	Methyl violet	Alcohol	D8	Color of diffusate very slight 1 week later.
132 Alcohol	Methylene violet	Alcohol	D7	Color of diffusate very slight 1 week later.
133 Alcohol	Malachite green	Alcohol	D8	
134 Alcohol	Martius yellow	Alcohol	D7	
135 Alcohol	Metanil yellow	Alcohol	D7	Color of diffusate very slight 1 week later.
136 Alcohol	Rose bengal	Alcohol	O	
137 Alcohol	Rhodamin	Alcohol	O	
138 Alcohol	Sudan G	Alcohol	D2	
139 Alcohol	Sudan I	Alcohol	D2	
140 Alcohol	Bismarck brown	Alcohol	O	
141 Alcohol	Benzopurpurin	Alcohol	O	
142 Alcohol	Tropeolin OO	Alcohol	D7	Color of diffusate very slight 1 week later.
143 Alcohol	Phloxin	Alcohol		D in about 3 days.
144 Alcohol	Safranin	Alcohol		D in about 5 days. Slight 1 week later.
145 Alcohol	Naphthol yellow	Alcohol	O	
146 Alcohol	Alcannin	Alcohol	D5	
147 Alcohol	Chlorophyl	Alcohol	D7	Color of diffusate very slight 1 week later.
148 Alcohol	Barwood	Alcohol	O	
149 Alcohol	Fustic	Alcohol	O	
150 Alcohol	Turmeric	Alcohol	O	
151 Alcohol	Cape aloes	Alcohol		D in about 2 days.
152 Alcohol	Curcumin S	Alcohol	O	
153 Alcohol	Naphthol green	Alcohol	O	
154 Alcohol	Orange G	Alcohol		D in about 2 days. Very slight 1 week later.
155 Alcohol	Carmosin B	Alcohol		D in about 2 days. Very slight 1 week later.

III. ATTEMPTS TO SEPARATE PIGMENTS BY DIALYSIS

The outcome of Test 25 encouraged us to ascertain whether two dissimilar pigments like scarlet R and malachite green might be wholly separated from each other by dialysis thru rubber in a suitable solvent, *e. g.*, ethyl acetate (see tests 40 and 45). A mixture of the two pigments dissolved in ethyl acetate was accordingly subjected to the usual mechanical treatment, but the diffusate was repeatedly replaced with fresh solvent. The results are indicated in the following summary:

Continuous differential diffusion of scarlet R and malachite green.

March 28—1st diffusate 11–1 p.m. Bright red.
March 28—2nd diffusate 4 p.m. Bright red.
March 28—3rd diffusate 11 p.m. Deep red.
March 29—4th diffusate 12.30 a.m. Deep purplish red.
March 29—5th diffusate 1 a.m. Light purplish red.
March 29—6th diffusate 10.45 a.m. Deep red with decidedly bluish tinge.
March 29—7th diffusate 12.30 p.m. Light blue.
March 29—8th diffusate 9.15 p.m. Blue green.
March 30—9th diffusate 11.50 a.m. Blue green.
March 31—10th diffusate 9.15 a.m. Blue green.
April 1—11th diffusate 1 p.m. Blue green.
April 2—12th diffusate 11.50 p.m. Light green.
April 3—13th diffusate 11.50 p.m. Light green.

Altho scarlet R and malachite green showed widely different rates of diffusion when they were treated separately, the results detailed above made it evident that it would be difficult if not impossible to obtain *all* the scarlet R from mixtures like the one employed without removing some of the malachite green with the red pigment.

By subjecting solutions of scarlet R and malachite green of similar concentrations independently to diffusion in the usual way, we duplicated the blue and green effects with malachite green and the red effects with scarlet R, *but the purplish colorations could not be obtained under such circumstances.* That these purplish effects were due to early diffusion of the malachite green with scarlet R, and that the red pigment facilitated the passage of the green one, are clearly indicated by the results.

Repeated removals of the diffusate in the independent scarlet R experiment, and replacements with fresh solvent for a period of about a week, led to the separation from the original pigment-product of all its red *diffusible* matter. The bag, at that stage of the treatment, contained considerable brownish-red, indiffusible material, which evidently was not scarlet R. This result, and similar observations with other pigments, emphasized Dr. Gies' opinion that it might be possible to purify pigment preparations in this way and that their value as coloration agents, for histological staining especially, might thus be considerably enhanced.

It will be noted that those pigments which diffused most rapidly were the so-called "fat colors," *i. e.*, those soluble in, or staining, the common fats and oils. Again, with these pigments the diffusion is the most rapid, and therefore the most satisfactory, when the solvents are those which, in the state of vapor, soften rubber. It will thus be seen that apparently the membrane, as well as the solvent, exert selective action. This is true to a far greater extent in experiments of this kind than in the ordinary dialyses in aqueous media.

When we arrived at this point in these experiments, to which we could give but a few hours weekly, our period of residence at Columbia University was about to close and, after completing some repetitions of previous observations in this connection, we were obliged to discontinue the work. It is Dr. Gies' intention to proceed along lines suggested by the results already obtained in this preliminary investigation.

THE COLLOIDAL NITROGEN IN THE URINE FROM A DOG WITH A TUMOR OF THE BREAST

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In 1892 Töpfer[1] found that the urine of patients suffering from cancer contained a very large amount of "extractive substance." This "extractive substance" was calculated by first determining the total nitrogen, and then subtracting from this amount the sum of the nitrogen values for the urea, uric acid, and ammonia contained in the same urine. Bondzynski and Gottlieb,[2] five years later, reported that the nitrogen in oxyproteic acid was 2 to 3 per cent. of the total urinary nitrogen. Salkowski,[3] and Hess and Saxl,[4] using different procedures in their researches, came to the conclusion that the oxyproteic acid or the alcohol-precipitable substances are increased in the urine of human beings suffering from carcinoma.

Salkowski and Kojo,[5] in a preliminary communication, recently suggested several methods for the determination of colloidal nitrogen in the urine. A year later Kojo published the results of a comparative study of the various procedures suggested in this connection.[6] Einhorn, Kahn and Rosenbloom[7] studied the zinc sulfate-precipitable, colloidal, nitrogenous material from the urine of normal subjects as well as from the urine of carcinomatous patients, and came to the conclusion that the amount of colloidal nitrogen was invariably increased in subjects with carcinomatous growths.

[1] Töpfer: *Wiener klin. Wochenschrift,* 1892, v, p. 49.

[2] Bondzynski and Gottlieb: *Zentralbl. f. d. med. Wissenschaften,* 1897, xxxv, p. 577.

[3] Salkowski: *Berliner klin. Woch.,* 1910, xlvii, p. 1746.

[4] Hess and Saxl: *Beiträge zur Carcinomforschung,* 1910, Part II.

[5] Salkowski and Kojo: *Berl. klin. Woch.,* 1910, xlvii, p. 2297.

[6] Kojo: *Zeitschr. f. physiol. Chem.,* 1911, lxxiii, p. 416.

[7] Einhorn, Kahn and Rosenbloom: *Amer. Journ. of Gastro-enterology,* 1911, i, p. 2; and *Archiv f. Verdauungs-Krankheiten,* 1911, xvii, p. 557.

The writers lately embraced an opportunity to study the colloidal nitrogen output in the urine of a dog with a large tumor.

The dog upon which this study was made had a hard calcified growth about the size of an orange in one of the breasts. The tumor involved the nipple and the breast tissue for some distance around the nipple. Several metastatic deposits were present along the "breast lines." Microscopic examination of sections of the original growth and of the metastatic infiltrations, according to several pathologists who examined them, indicated that the tumor was a chondroma which had undergone carcinomatous degeneration. Other pathologists, on the contrary, believed the growth to be of a benign nature, with the histological structure of a chondroma.

For the determination of colloidal nitrogen the alcoholic precipitation method of Salkowski was used, with modifications, as follows:[8]

The total nitrogen was determined in 5 c.c. of the urine by the Kjeldahl process. Two portions of 100 c.c. each of the urine were evaporated in a porcelain dish over a gently steaming water bath till they were of the consistency of thin syrup. The residues were then taken up in 100 c.c. of alcohol (98.5 per cent.) and thoroughly stirred. The alcoholic extracts were then filtered through ashless filter papers, and the precipitates washed with alcohol.

We determined the effect of dialysis upon this alcohol-precipitable, so-called "colloidal," nitrogenous material. Most colloidal substances fail to dialyze through the very best grade of parchment paper. Only that fraction of the alcoholic precipitate which would remain indiffusible under suitable conditions of dialysis could be called "colloidal," at the present stage of our knowledge of the subject. Accordingly, the two precipitates on the ashless filter paper were treated as follows:

The precipitate on one filter paper, together with the filter, was placed in a Kjeldahl flask, digested with sulfuric acid, and the nitrogen determined in the usual way. The second precipitate and

[8] Before subjection to analysis the urine was first tested for protein, which, if found, was removed by means of heat coagulation aided by the addition of a few drops of dilute acetic acid solution.

filter paper were placed with water in a bag of the finest grade of parchment paper and dialyzed for forty-eight hours. The liquid in the bag was then analyzed quantitatively for nitrogen.

The appended summaries present the results obtained for urine from the dog with the breast tumor and also for urine from several normal dogs.

In the Salkowski method for the determination of "colloidal" nitrogen (as the results in the summary show), diffusible nitrogenous substance is precipitated as "colloidal" nitrogen. It has not yet been shown that such diffusible nitrogenous matter in the colloidal precipitate is true colloidal material.

A. Data pertaining to the urine of normal dogs

Specimen No.	Total Nitrogen in 100 c.c. of Urine	Colloidal Nitrogen in 100 c.c. of Urine	Percentage of Total Nitrogen as Colloidal Nitrogen	Indiffusible Colloidal Nitrogen	Percentage of Total Nitrogen as Indiffusible Colloidal Nitrogen
	Grams	Gram		Gram	
1	2.3045	0.0437	1.85	0.02775	1.2
2	3.2051	0.0314	0.98	0.01634	0.5
3	0.8590	0.0172	2.00	0.01202	1.4
4	1.6436	0.0214	1.28	0.01841	1.1

B. Date pertaining to the urine of the dog with a tumor of the breast

5 *a.*	4.0088	0.3392	8.40	0.0939	2.3
5 *b.*	6.3034	0.3897	6.10	0.2293	3.6
5 *c.*	4.4591	0.3210	7.10	0.0767	1.7
5 *d.*	3.6862	0.3294	8.10	0.0817	2.2
5 *e.*	3.1414	0.0958	3.04	0.0867	2.7
5 *f.*	3.9642	0.3566	8.90	0.1175	2.8
5 *g.*	2.5139	0.4617	13.10	0.1342	3.6

The results demonstrate that the "colloidal" nitrogen, both before and after dialysis, was greater in amount in the urine of the dog with the tumor than that in the urines from normal dogs.

It is desirable to study the effect of dialysis upon the "colloidal" nitrogenous substances in the urine of cancer patients.

GENERAL ASPECTS OF FASTING[1]

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Fasting (starvation or inanition) is a state in which the dietary elements are withheld, either wholly or in part, so that the organism is compelled to draw upon its own resources to maintain its existence. In discussing this subject it is my purpose to make a rather general survey of the changes which take place as the result of fasting; to show briefly how such results have been used to elucidate other scientific problems; and, also, to touch upon the therapeutic value of fasting, with relation to man.

A distinction is made between physiological and experimental fasting. The first form is illustrated by the hibernation of mammalia (hedge hog and bear), and cold blooded animals (frog), by the normal condition of the salmon during the spawning season and by the period of metamorphosis of the insects, these being natural phenomena for which the organism has made suitable preparation. In experimental fasting the animal is forced to live without sustenance, of one kind or another. Under this last state we may consider pathological fasting as a special case in which the organism is forced to fast as a result of impairment of some organ or of a general diseased condition. These forms of inanition present certain differences as evidenced in the effect upon the organism; yet it is quite probable that they are chiefly phylogenetic and we can conceive that any of the animals which do not experience these periodical physiological fasts might do so under the proper adverse circumstances.

In our discussion we will consider only the phenomena which take place as the result of experimental fasting. Here, too, we must distinguish between a number of forms of fast; such as the com-

[1] A lecture delivered at the College of Physicians and Surgeons, New York, May 1, 1912, under the auspices of the Columbia University Biochemical Association.

plete fast in which there is total abstinence from both food and water; a modification of this, in which the subject is permitted to take water "ad libitum" or caused to ingest a uniform quantity from day to day; and the incomplete fast, in which one or more of the food principles or chemical elements contained therein is withheld, such as a diet lacking in protein, fat, carbohydrate, water, salt or certain amino acids.

There is not a marked distinction between complete fasting and fasting with water taken "ad libitum," for under the latter conditions the quantity of water taken decreases as the fast progresses until finally there is a natural abstinence from water. Some hold that the desire for water returns just before death. The ingestion of water causes a lengthening of the life of the animal and the severity of the fast is lessened. If at any time the quantity of water given is increased there will be for a time an increase in the metabolism (14). This condition also holds for the well nourished animal (8), *i. e.,* under all conditions when the water ingestion of the animal is sufficiently increased the general metabolic processes of the organism are stimulated.

The length of a fast which would result in death depends upon the size, the species, the age, the nutritive condition, the external surroundings (*e. g.,* temperature, humidity, etc.) and the intrinsic rate of metabolism. In general, we may say that the smaller the subject the shorter will be the time it can live without food; but this does not hold in all cases, for certain of the lower animals can fast much longer than the higher forms, *e. g.,* the salamander, which is about 3–4 inches long, has been fasted for more than 125 days (19). Adult organisms can fast longer than the young of the same species. Thus, a young pup can fast but a few days, while a full grown dog will fast from 20 to 60 days. Of the fasts on man and other warm blooded mammals, the longest on record is one of 117 days (15). This experiment was conducted in our laboratory, a Scotch collie dog being the subject. Subsequent to this long fasting interval the dog was fed, and it returned to its normal condition.

A comparison of the results obtained by various investigators shows that death does not ensue until there is a loss of between 40–50 per cent. of the original body weight. The real cause of death

from fasting has not been determined. The probable reason is the failure of some organ or life process (27) and not the depletion of all possible nutritive material. From our experiments (10) it would appear that a certain definite minimal proportion of nitrogen-holding substance must be present in the body for life to exist.

Fasts have been reported upon men covering periods of from 2–50 days, upon dogs as long as 117 days and salamanders for 125 days. In each of the extreme cases, the subjects were subsequently fed and they returned to normal. The influence of repeated fasting upon the resistance of the animal to subsequent fasts is a phenomenon which appears to be intimately associated with hibernation. As has been shown by Russian investigators (20), and more recently in our laboratory (10), repeated fasting decreases the rate of metabolism in each succeeding fast. A French investigator (21) has shown that repeated fasting, in which the subjects were alternately fasted and fed during equal periods of about a week each, resulted in the ultimate death of the animals. From the experimental data at hand it seems that where the animal is permitted to recover *completely* from a fast before it is subjected to another, there will be an increased resistance to the ravages of the succeeding fast.

The number of men who have made a study of the changes which take place as the result of fasting is so great that it is difficult to name those who have made the most important contributions upon this subject without doing an injustice to others. The investigations of Cathcart (4) in England and of Benedict (2) in this country, upon men, are the most extensive that have been conducted with the more refined methods of analysis which we possess today. The names of Succi, Cetti and Breithaupt stand out in the literature as the subjects of important experimental fasts.

What changes take place in an organism as the result of a fast? Outwardly the subject becomes emaciated, his body weight decreases, he becomes weak and apathetic and, should the fast proceed long enough, he would probably die in a state of coma. In man it has been demonstrated that the brain retains its activities unimpaired during a fast and that hunger is evident only at the beginning of the ordeal. These facts are substantiated in the popular writings upon fasting and also by an experiment made by us (11) in which the

subject prepared for the preliminary examination for the degree of Doctor of Philosophy during a seven-day fast.

The fasting state is indicated in the body by certain changes; such as a general decrease in the body metabolism, represented by variations in the nitrogen excretion and the respiratory exchange, a decrease in the fat and glycogen stores, a decrease in the volume of muscle and in the size and weight of certain organs. The temperature remains normal, for a time at least, but shows a tendency to decrease toward the end of the fast.

The decrease in the general metabolism is well illustrated by the data obtained from the respiration calorimeter experiments. It has been shown by the earlier investigators and more recently by Benedict (2) that, in a well fed man, the quantities of protein and fat which were utilized, and the energy change (calories) per day, decreased very gradually and tended toward a constant minimum. In addition to the excreted carbon dioxide, Benedict determined the amount of oxygen consumed. From these data it was shown that the glycogen consumption, which is most rapid on the first day, decreases as the fast progresses. It is probable that the glycogen store is never depleted and that even in fasting there is a resynthesis of glycogen from the protein material present in the body.

Decreased metabolism in fasting is also shown by the quantities of nitrogen-containing substances eliminated in the excreta. We are particularly concerned with the losses of nitrogen, for it is the protein material which is the most fundamental nutritive substance and which the body strives to protect. In fasting, the nitrogen-containing substances in the urine or feces arise from the tissues and hence the total nitrogen excretion is a measure of the quantity of muscular or organ tissue catabolized. The excretion of total nitrogen in the urine decreases rapidly at the beginning of the fast and soon reaches a minimum, which is maintained for some time. This minimum of nitrogen excretion, representing a minimum protein disintegration, is held to represent the "maintenance" metabolism of the individual, *i. e.*, that amount of protein substance which if supplied, with sufficient fats or carbohydrates, in the form of food would sustain life. This minimum has variously been shown to be greater or less than the metabolism as represented by fasting experiments.

The muscular disintegration is influenced by the factors already mentioned; the nutritive condition and the experience of previous fasts, or repeated fasting. The diet just before the fast influences the nitrogen excretion for a number of days. This has been demonstrated in the classical experiments of Voit (26), in which he fed varying amounts of meat and bread to a dog and showed that, when fasted the rate of nitrogen excretion varied, but that in each case the animal came to the same level of catabolism on about the seventh day of fasting.

The fat available in the body exerts a marked effect upon the protein metabolism and the life of an animal. So long as there is sufficient fat in the organism to supply the energy requirements, the protein metabolism will remain at a minimum. When, however, the fat deposits are depleted, the body is forced to use protein to furnish the necessary energy. The result is a more rapid protein consumption and an earlier death. This increased protein consumption, is, of course, accompanied by an increased nitrogen excretion, which has been designated as the "premortal rise." The feeding of carbohydrate or fat sufficient to supply the energy requirement of the body would prevent this increased consumption of protein and thus lengthen the life.

Repeated fasting will also modify the rate of metabolism. This point is well illustrated by the results obtained on a subject in a repeated fast (10), in which there was a rapid and increasing consumption of the protein reserves of the body during the first fast, but a more gradual and uniform consumption during the second fast. The total body weight and nitrogen losses were practically identical in the two fasts and the data from the intermediate feeding period would indicate that an increased fat store was not the cause of the more gradual utilization of the body resources.

A study of the differential distribution of the nitrogen in the urine serves to bring out certain points with regard to the protein metabolism of fasting animals. The percentage of total nitrogen occurring as urea-nitrogen decreases in man and is accompanied by an increased ammonia-nitrogen excretion. This has been explained as due to the condition of acidosis, which may result, at least in part, from the accelerated utilization of the fat deposits and the decreased oxidative powers of the animal.

In the case of dogs there is a difference of opinion as to the relation between the urea-nitrogen and the total nitrogen. Schöndorf (22) and others hold that the percentage of urea-nitrogen decreases, while in all of our experiments it has remained nearly constant, which fact, coupled with the failure to find marked quantities of organic acids in the urine, would show that dogs are better able to utilize their body stores. This may be due to the fact that the dog is naturally a "high-protein" animal.

The daily creatinine excretion, which is a constant for any individual under normal conditions of feeding and is generally believed to be a function of the muscular metabolism, decreases gradually as the fast progresses and in correspondence with the decreasing amount of protoplasm.

Creatine, which does not occur in normal urines, or is found only in cases associated with muscular disintegration, appears during fasting and ordinarily becomes a constant constituent. It has recently been shown that the feeding of carbohydrate causes the excretion of creatine to stop (5, 17); while ingested fats may even cause an increase in the excretion of this form of nitrogen. In one of our dog experiments (15) there was a disappearance of urinary creatine from the 19th to the 59th fasting days. This phenomenon might be explained on the above basis. It is improbable, however, that the body could synthesize sufficient glycogen at this stage of the fast to cause the disappearance of the creatine. The real explanation is therefore not apparent.

In connection with the repeated fast, previously mentioned, it is interesting to note that the excretions of creatine as well as of total nitrogen were practically the same during each of the two fasts, notwithstanding the fact that the second fast was twice as long as the first. This would indicate an intimate relation between the total-nitrogen excretion and the quantity of creatine excreted.

When the data representing the creatine and creatinine excretions of a fasting animal are examined, it is seen that there is generally a progressive increase in the creatine output and an accompanying decrease in the creatinine elimination, until the output of creatine exceeds that of the creatinine. In other words, when expressed graphically, the curve representing the course of the creatine excre-

tion *crosses* that representing the creatinine output. This phenomenon has been termed by us the "creatine crossing" and is believed to be very significant. It occurs with great uniformity a few days previous to the decrease in the total nitrogen excretion that precedes the pre-mortal rise of excreted nitrogen. By means of the "*creatine crossing*" the length of the subsequent life tenure of the animal may be quite closely estimated.

Certain pathological constituents, other than creatine, may appear in the urine as the result of fasting, such as acetone, diacetic acid, lactic acid, bile pigments, albumin, etc.

The processes in the large intestine during fasting have received but little attention. Various authorities contend that it is difficult to make a separation (2, 18) of fasting feces. Müller (19a) has shown that indican, which is now considered as an index of intestinal putrefaction, disappeared upon the third day of fasting. We have been able to make an undoubted separation of fasting feces and have found indican present during the whole of a seven day fast on man (23). Fasting feces are distinct from those of the normal individual in that they are of a peculiar brown color and are pasty in consistency. The percentage of nitrogen present is higher than in normal feces. The bacterial content of feces has received but little attention and only recently have results upon the bacterial content of fasting feces been determined. The results indicate a lower percentage content of bacteria (3).

There is not an equal wasting of all of the organs and tissues of the body, those organs most necessary for the maintenance of life show only a slight decrease in size and weight, while others are reduced to but a fraction of their original proportions. Thus the heart, lungs, and nervous system exhibit but little change while the muscles and fatty tissues exhibit a marked reduction of both volume and weight. The organs of regeneration are also resistant to the ravages of a fast. This fact is of especial significance for it demonstrates the tendency of nature to preserve the species.

A histological examination of the tissues and organs of fasting animals shows a decrease in the volume of the cells as a whole and of the nuclei. Morgulis (19) has shown that the decrease in the volume of the cells of the salamander is greater than that of the

nuclei and further that the nuclei become elongated. In the case of the liver, the cell walls finally begin to disappear and the small masses of pigment to clump together. Such a condition does not necessarily result in death, for salamanders of the same size have been caused to fast for even a longer time than those whose tissues demonstrated these changes; and, after feeding, it was found that the cell walls again appeared and the liver returned to a normal condition.

We will not take up the question of the localization of the degenerative changes, *i. e.*, as to whether they occur in an organ as a whole or in localized portions. It is an interesting fact, however, that even when the organism is undergoing the degenerative effects of a fast, there are still evidences of mitotic division of the nuclei.

What changes take place which enable one organ to waste away while another retains its normal condition? The explanation most generally accepted is that of the nourishment of the more vital organs by transference of the nutritive material from the less important tissues. Thus the less resistant tissues gradually give up their stores of fat and protein to the blood stream which in turn furnishes them to the actively functioning organs.

This idea has received further proof from the researches of Hottes.[1] This botanist (9) worked with beans and has shown that upon removing the cotyledons, and thus the food supply, from seedlings, the meristematic tissue which would normally go to produce lateral roots is transferred to the tip of the root (meristem) and there used for growth; and that at the end of from three to four weeks all the cells in the upper part of the root have lost the major portion of their protoplasm and the only actively functioning cells are those at the tip. Hottes has also shown that the decrease in size of the root is due rather to a reduction in the number of the cells than to a mere decrease in size. This is in opposition to the findings of certain zoölogists who hold that the reduction in the weight and volume of the organs is due more to reduction in size than in number, altho they admit a small decrease in the number of contained cells.

[1] I am indebted to Professor Hottes of the University of Illinois for this information which was taken from some of his unpublished work.

The blood of fasting subjects which are ingesting water shows in general a decrease in the number of erythrocytes and leucocytes, and of the percentage of hemoglobin. The differential distribution of leucocytes varies with the species. In the dog (12) there is a decrease in the percentage of polymorphonuclear leucocytes and a corresponding increase in the small lymphocytes. The changes in the other forms of cells are but nominal.

Fasting studies have been of great importance in the study of the minimum of food necessary to maintain life and upon which to base the calculation of dietary standards. Such studies have also been utilized in the explanation of phenomena occurring in pathological states and of metabolism in general.

Underhill and Rand (25) have explained certain anomalies in the urinary changes which occur in pernicious vomiting of pregnancy from their knowledge of fasting metabolism.

Two agriculturalists have recently made use of the results of fasting studies to elucidate problems of importance to both the scientist and the farmer. McCollum (16) fed a nitrogen-free diet to pigs and studied the efficiency of individual grains as feeding stuffs, as well as the nature of the repair processes in protein metabolism. He shows that the difference in the nutritive values of the wheat, oat and corn kernels is not so great as would be expected from the difference in the chemical composition; and further, that the repair processes of the cell are of a different character from those of growth, and that the cellular catabolism and repair do not involve the destruction and resynthesis of entire protein molecules. This last statement is not in entire accord with the most widely accepted theories of metabolism. Certain zoölogists have also shown that the changes of regeneration are unlike those which occur in growth.

Dietrich[2] shows (7) that fasting so reduces the plane of metabolism that the quantity of food which was insufficient for maintenance before fasting was afterward sufficient, not only for maintenance, but to produce a positive nitrogen balance; in other words, the animals were more efficient machines after fasting.

[2] I wish to thank Professor Dietrich of the University of Illinois for permission to refer to his unpublished data.

Aron (1), in his studies upon nutrition and growth, subjected dogs to incomplete fasts. The results showed that a growing animal, receiving only enough food to provide for little or no increase in weight, "is in a condition of severe starvation." Under such conditions the skeleton grows at the expense of the flesh, the organs retain their weight and the brain reaches its normal size. The fat and protein of the muscles are largely used up, altho this loss of material is balanced by gain of water and by the growth of the skeleton.

The biologists have made use of the fasting subject in the study of the problems of degeneration, of regeneration, and of growth. The work of Morgulis and of Hottes already considered was of this nature.

The therapeutic value of fasting is realized in the preliminary treatment of some digestive disorders and in the partial fasts of obesity cures. These latter cures consist in supplying only the protein requirements of the body and thus forcing the individual to utilize the surplus fat deposits to make good the energy requirements.

The increasing popular literature upon fasting and the tendency to fast on the part of certain people, especially the pronounced physical culturists, and their general good health, would seem to indicate that there are some beneficial results to be obtained from fasting. The various books upon fasting, of which the superficial, yet interesting book of some six hundred pages by Carrington (6) upon "Vitality, Fasting and Nutrition" is the most complete, lend strength to the idea that fasting as a therapeutic measure is important. The chief contention of this fasting cult is that by depriving the body of food the digestive organs are given a chance to recuperate and the body is enabled to rid itself more effectively of the waste products and toxic substances.

Fasting for short and widely-separated periods may be a beneficial procedure in some individuals. This conclusion is supported by the observed effects on dogs, which acquire increased resistance from repeated fasting. This view is strengthened, also, by the foregoing data pertaining to pigs as well as by Seeland's (24) results on pigeons and chickens, which show that repeated fasts,

for periods of from one to two days, were followed by better growth and greater strength.

It is probable, then, that fasting under proper conditions may be advantageous. Long fasts, however, seem to be devoid of benefit and may endanger health.

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THE PHYSICO-CHEMICAL BASIS OF STRIATED MUSCLE CONTRACTION[1]

2. Surface tension

WILLIAM N. BERG

(WITH PLATE 1)

If the physico-chemical basis of muscle contraction is ever to be understood or explained, it is almost certain that it will be brought about thru speculation and experiment of a **quantitative,** rather than of a **qualitative** nature. The mere statement that muscle contraction is caused by surface tension, or thru osmotic action, etc., unless accompanied by quantitative data of an experimental or theoretical nature, can add little toward the solution of the problem of the transformation of energy by muscle. It is, perhaps, regretable that so many of the "theories of muscle contraction" which have appeared in the recent literature belong to the qualitative class. Occasionally someone attempts to treat the subject quantitatively. From this point of view the works of Bernstein,[2] and of Zuntz[3] are particularly meritorious, even if the problem has not yet been solved by them.

Among the latest qualitative contributions to the theory of muscle contraction, is that of Strietman and Fischer.[4] They studied the contraction and relaxation of catgut strings immersed in various solutions. By attaching the strings to the usual arrangement of lever and recording drum, they found that when a catgut string is immersed in water or physiological salt solution, even for some time, no changes in length take place (p. 66). But if the string be immersed in solutions of hydrochloric or lactic acids ($n/80$ to

[1] Berg, W. N.: BIOCHEMICAL BULLETIN, 1912, 1, 535.

[2] Bernstein, J.: *Arch. f. d. ges. Physiol.,* 1901, **85,** 271–312.

[3] Zuntz, N.: *Die Kraftleistung des Tierkörpers.* Festrede; Berlin, 1908.

[4] Strietman, W. H., and Fischer, M. H.: *Ztschr. f. Chemie und Industrie der Kolloide,* 1912, 10, 65–77.

n/20) it contracts. On replacing the acid solution by water, the string relaxes. The relaxation is faster, however, when the acid is replaced, not by water, but by a solution of some salt such as sodium bicarbonate, which can neutralize the acid. From their diagrams it would seem that a minute or more may be required for a single contraction or relaxation, depending upon the strength of the acid, etc., etc.

These observations are, no doubt, interesting in themselves. But before connecting them with muscle contraction, might it not be well to consider whether the conditions under which a catgut string can contract and relax are at all similar to those existing in muscle?

Strietman and Fischer state that because lactic acid is formed in a working muscle and because a catgut string will contract when immersed in a lactic acid solution and will relax when the acid is removed, therefore, in the working muscle, the contraction is brought about by the formation of lactic acid. They quote several other investigators who have stated their belief in the same idea of the connection between lactic acid formation and contractility without, however, making any of the simple calculations that would naturally suggest themselves.

Their theory is open to the following objections: (1) It is not likely that there is any free lactic acid in the working muscle, it is probably neutralized at once by the phosphates present in lymph. At least this would be inferred from the work of Henderson[5] who showed that the mixture of phosphates and other substances in blood and various tissue fluids was such as to enable them to maintain an absolute neutrality in spite of the formation of even considerable quantities of acid or of alkali. This point was not overlooked by Zuntz (p. 20, l. c.) when calculating the amount of energy made available by the transformation of dextrose into lactic acid. The heat of neutralization of the lactic acid by sodium, as well as the heat required to separate the sodium from its presumable combination with protein, are given due consideration by Zuntz, who calculated that the heat liberated in the formation of lactic acid from dextrose is equivalent to 3.4 per cent. of the heat of combustion of dextrose.

[5] Henderson, L. J.: *Ergebnisse d. Physiologie,* 1909, **8**, 254–325.

A repetition of the experiments of Strietman and Fischer, in which catgut strings would be immersed in solutions comparable with lymph containing lactic acid (not exceeding the maximal amount possible if all of the muscle glycogen were changed at once to lactic acid), would probably give results more decisive than those in which free acids were used.

(2) And even if free lactic acid existed in muscle, or if combined lactic acid could induce proteins to swell, one such observation is only one of very many that are needed for a rational theory of muscle contraction. The statement that lactic acid swells protein adds very little to our knowledge of the mechanism in muscle by which the potential energy of the food is transformed into the kinetic energy of the moving muscle and its load.

It is to be regretted that the work of Brod[6] on the swelling of fibrin in acid solutions has received practically no attention in the recent literature. The paper can be profitably studied by those contemplating studies on protein swelling. A brief resumé of Brod's results is given by Berg.[7]

A good example of a quantitative theory of muscle contraction is the calculation of Bernstein[8] on the possible changes in the surface energy resident on the muscle fibrils. The method of making the calculations is, perhaps, unnecessarily complicated and, in one or two instances, the mathematical equations are of doubtful correctness. Bernstein finds that in order that a muscle may lift an ordinary load, the surface tension between fibril and sarkoplasm must have an improbably great magnitude. He nevertheless concludes that the principle, that energy is transformed in muscle thru changes in surface energy, is correct.

There are several reasons why, to the student at least, a proper understanding of some of the recent applications of physical chemistry to biology should be so difficult, if not altogether impossible. First: The indefiniteness of certain statements that the writer has

[6] Brod: Beiträge zu der Lehre von der Eiweissverdauung. *Dissertation,* Würzburg, 1892.

[7] Berg, W. N.: *Amer. Jour. Physiol.,* 1909, **23**, 427. Brod's method has recently been used by Tracy and Gies, BIOCHEMICAL BULLETIN, 1912, **1**, 468.

[8] Bernstein, J.: *Arch. f. d. ges. Physiol.,* 1901, **85**, 271–312.

frequently seen in the literature. This is an example taken from Freundlich's Kapillarchemie, p. 4:

Surface energy = surface tension × area of surface.

A similar statement is made by Michaelis,[9] and others. Nothing further was stated that would enable the reader to use such a formula in making calculations were it desired. Expressing the surface tension in dynes per centimeter and the area of the surface in cm.^{2}, what is the surface energy? The answer is very simple after one has taken the time to look the matter up. After a formula such as the above, a numerical example ought to be given, so that it means more than so many words to the average reader. Suppose it is desired to calculate the amount of energy required to form a water-surface (in contact with air) of 1 sq. cm. area? Or, what is the same thing, how much energy is liberated or is available for external work when the above water-surface diminishes by 1 sq. cm.? According to Michaelis (l. c., p. 14), this will require (or liberate) 70 ergs or 7×10^{-7} kilogram-meters. The method of using the formula to obtain this result, simple as it is, was not given by Michaelis, altho at least one example of the use of a formula is desirable because it will enable the reader to make many other calculations.

Following is a numerical example of the kind mentioned above. How much energy is required to form a water-surface (against air) of 1 sq. cm.? In the formula it is assumed that the surface tension remains constant during the change in area:

surface energy required = surface tension × increase in area,

or

surface energy liberated = surface tension × decrease in area,

(ergs) = (dynes per cm.) × (cm.^{2}).

Since the surface tension of water-air is about 70 dynes per cm., it is evident that $\frac{70 \text{ dynes}}{\text{cm.}} \times 1 \text{ cm.}^2 = 70 \text{ ergs} =$ the amount of energy required. The erg is a unit of work (or energy) and is the work done when a mass is moved 1 cm. by a force of 1 dyne.

[9] Michaelis: *Dynamik der Oberflächen*, p. 13. Dresden, 1909.

The element of time does not enter into the definition of the erg. The work done (ergs) is equal to the product of the force (dynes) times the distance (cm.) thru which the force acts. Of course, other units may be used. The surface tension may be expressed in grams per cm., and the area in square cm. The work then is expressed in gram cm. But on account of the unfortunate use of the word 'gram' to designate a certain mass or quantity of matter and also to designate a force, it is better, for the present, to use the erg and the dyne, and later to convert ergs into kilogram-meters, or any other of the customary units for expressing muscular work.

It is, of course, absolutely necessary that the terms used in such calculations be consistent. Here is the second reason why some so-called applications of physical chemistry to biology are not easily followed. An equation will sometimes be given that is not correct in its dimensions. To state that 2 sq. cm. = 2 cubic cm. is obviously incorrect. Such an inconsistency is to be found in one of Bernstein's[10] equations: 'Wir werden daher in dem Falle des isometrischen Tetanus, in welchem alle chemische Energie als Wärme erscheint, $\alpha_p - \alpha_r = c.\, w_p$ setzen können, wenn w_p die in einer Zeiteinheit erzeugte Wärmemenge, c eine Constante und α_p und α_r die Oberflächenspannung im Tetanus und in der Ruhe bedeuten. Da wir nun oben (S. 296) gesehen haben, dass α_r gegen α_p verhältnissmässig sehr klein ist, so können wir annähernd $\alpha_p = c.\, w_p$ annehmen.'

Here are two equations in which surface tension is equated with work (or heat). It makes no difference what units are used, on one side there is a *force* (surface tension expressible in dynes per cm.) and on the other a quantity of *energy* or work (ergs, or dynes × cm.). The constant above referred to is probably meant to be the mechanical equivalent of heat.

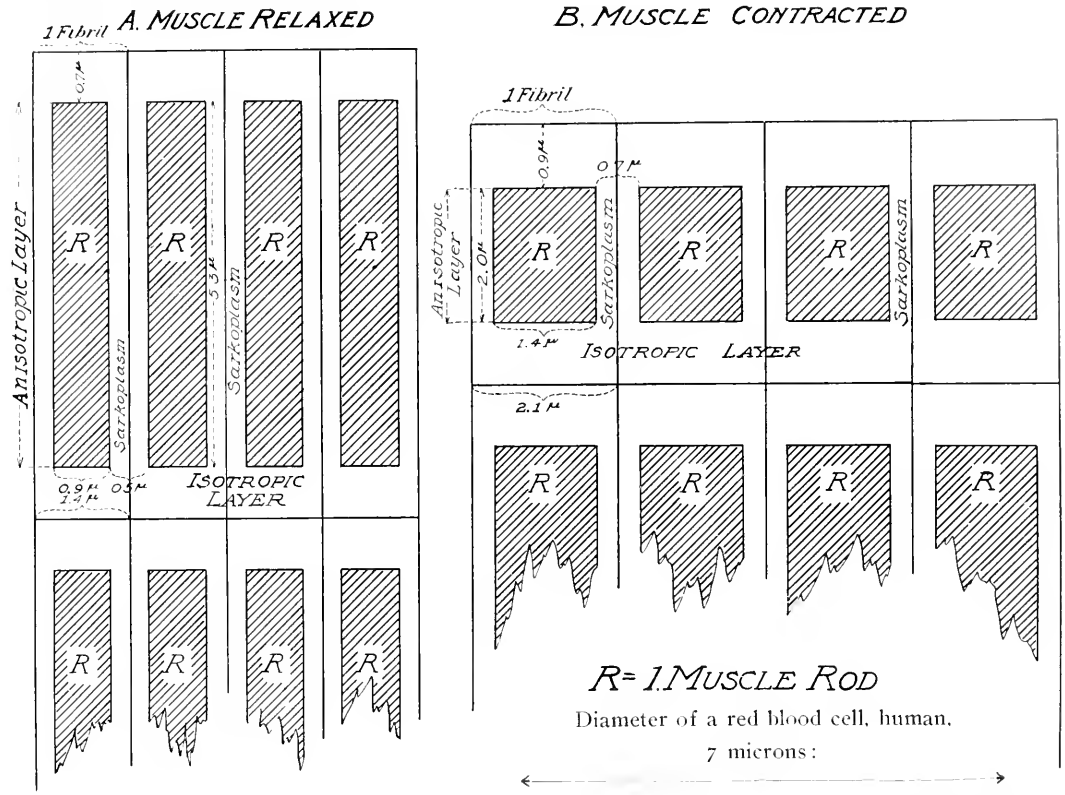
These equations are interesting for another reason. It is true that in isometric tetanus, a muscle does **work** in the **physiological** sense of the word. But **not** in the **physical** sense. In physics (or mechanics) work is defined as a product of force times distance thru which the force has acted. If either factor is zero, the product, work, is zero. The columns that support

[10] Bernstein, J.: *Loc. cit.*, p. 307.

a building do no work in the physical sense, nor would a man who took the place of one of them; altho *physiologically* he would do a great deal of work. In this case the distance thru which the force acts (the weight supported or the upward thrust of the man's shoulders) is zero and hence the work is zero. The above equations of Bernstein could be made consistent if there were two factors on the left-hand side; one, the surface tension or change in surface tension (expressed in dynes per cm.), the other, the area or change in area (expressed in cm.^{2}). The product (ergs) could be calculated to calories (gram-degrees C.) by dividing by 4.2×10^7, since 1 small calorie (gram-degree C.) is equivalent to 4.2×10^7 ergs. It is difficult to see what the other factor (omitted by Bernstein) can be. In an isometric tetanus the muscle does not change its length. In what way can an internal diminution in area take place? If the contractil units—whatever their shape may be—do not change in length, how do their areas diminish? This difficulty does not arise in the case of the ordinary (isotonic) contraction. Here one can assume a decrease in the areas of contact between contractil unit and sarkoplasm caused by an increase in the surface tension between the same surfaces. The product of these two quantities, according to the theory, should be an amount of work sufficient to account for the external work done and perhaps also for the heat liberated at the same time.

We have stated before that Bernstein's calculations on the magnitude of the surface energy changes in muscle are probably unnecessarily complex, involving, as they do, several pages of calculus. The same result is obtained in the following calculations, in which two simple quantities are calculated and then compared: (1) the amount of energy liberated in a working muscle thru increase of surface tension times diminution of area of contractil units; and (2) the external work done in lifting a weight a known distance.

Assume that in 1 c.c. of muscle a right section contains (as Zuntz assumes, l. c., p. 24) 62 million rods, and that there are 800 such layers, making a total of very nearly 5×10^{10} rods in 1 c.c. of muscle. Assume the general structure of muscle to be that described by Hürthle (see diagram), and that the muscle rod is THE



BERG: PHYSICO-CHEMICAL BASIS FOR THE CONTRACTION OF STRIATED MUSCLE.

Diagrammatic representation of the structure of the muscle fibrils of *Hydrophilus piceus;* from Hürthle. *Arch. f. d. ges. Physiol.*, **126**, pp. 1–164, 1909.

CONTRACTIL UNIT (Hürthle, p. 157). From the dimensions on the accompanying diagram, it is evident that the lateral area of a rod diminishes from 4.8 μ^2 when relaxed, to 2.8 μ^2 when contracted. (We omit the simple geometrical calculation.) The base areas are increased, but the energy apparently required for this work will for the present be disregarded—it is an additional load on a probably overloaded theory. Since the total area of the relaxed rod (5.2 μ^2) is greater than that of the contracted rod (3.8 μ^2), it follows that there is an increase in the surface tension immediately preceding the contraction, according to the requirements of the theory. To calculate the energy liberated as being equivalent to the diminution in area times the surface tension of water is probably incorrect, for without an increase in surface tension there seems to be no reason why the rods should contract against the external resistance—the downward pull of the weight lifted. The rod contracts presumably, because in the relaxed state the surface tension on the rod surface is low. How low can it be? It may be as low, perhaps, as 23 dynes/cm. if we assume the rod to be covered with a layer of pure acetic acid, and that the acid has the same surface tension in contact with the water that it has when in contact with air. Other fatty acids also give low values for the surface tension of their solutions, and they have still lower surface tensions in the pure state. Presently, the surface tension is raised, presumably by the removal of the fatty acid or other agent causing low surface tension, by instantaneous combustion, let us say. How high can the surface tension be raised? It might be raised[11] to 85 dynes/cm. if we imagine the rod now to be covered with a layer of saturated sodium chlorid solution. The surface tensions of aqueous solutions of salts cannot be raised[12] very much beyond that of pure water, which varies between 72 to 76 dynes/cm. (at 18° C.), according to the method of measurement. The upper limit for any solution that possibly could exist in the muscle might be assumed, then, to be the value for saturated sodium chlorid solution, or any other concentrated salt solution that might probably occur in living muscle. Of course, the existence of the films of pure acetic acid and of strong salt solution over the

[11] Freundlich, H.: *Kapillarchemie,* p. 27 and 62. Leipzig, 1909.
[12] Heydweiler, A.: *Ann. Physik.,* 1910, 33, 145–185.

muscle rods is purely hypothetical. The surface tension theory requires that changes in surface energy take place, and from what follows it is apparent that these changes must be great—greater, in fact, than the probable actual change on the rod surface. For it seems hardly possible that such great changes in concentration and in surface tension could take place. The values for the surface tensions of pure acetic acid and concentrated salt solution have been taken from the literature; whether such limiting values are ever reached in living muscle is, for the present, purely hypothetical.

If it be assumed that, during the chemical changes taking place in a working muscle, the inorganic ions in the rod-surface film rapidly change their concentrations, the film might be regarded as an electrical double layer or Helmholtz double layer. Without a doubt, changes in surface tension would result from the changes in ion concentration. The more ions in one of these layers covering a rod, the more they repel one another and the lower is the surface tension, and vice-versa. But as has been pointed out before,[13] it is not certain that such a double layer really exists between living particles and their surrounding medium. And even if there were such a layer, the total change in surface tension in such a layer is hardly significant for the present purpose. The small variation in surface tension when the variation is caused only by ions was probably overlooked by Robertson[14] and others who advocated a capillary electric theory of muscle contraction. It is really a special case of surface tension in which the variations in surface tension are caused by the mutual repulsion of the ions in each of the layers. But insofar as small amounts of certain organic substances, such as fatty acids, can affect (depress) the surface tension of water very much more than even improbably large amounts of inorganic salts, the surface tension theory is given the benefit of the greatest possibilities by assuming the changes in concentration from pure acetic acid (23 dynes/cm.) to saturated sodium chlorid solution (85 dynes/cm.). This is as large a difference as can be assumed from the experimental data on the surface tensions of solutions.

[13] Berg, W. N.: *New York Med. Journal,* 1907, July 20 and 27; and *Ion,* 1910, 2, 161–188.

[14] Robertson, T. Brailsford: *Trans. Royal Soc. South Australia,* 1905, **29**; and *Quarterly Jour. Exper. Physiol.,* 1909, 2, 303–316.

If in 1 c.c. of muscle there are 5×10^{10} rods, the lateral area of each of which diminishes from 4.8 μ^2 to 2.8 μ^2 when the muscle contracts, the total reduction in area is $5 \times 10^{10} \times 2\ \mu^2 = 10^{11}\ \mu^2 = 10^3$ cm.^{2} (1 μ = 0.001 mm.). The calculations will be simplified if it be assumed that the increase in surface tension is instantaneous, giving the contracting muscle the largest surface tension during the entire contraction phase. Then since

surface energy liberated = diminution in area $\times$ surface tension,

$$\text{(ergs)} \qquad \text{(cm.}^2\text{)} \qquad \left(\frac{\text{dynes}}{\text{cm.}}\right)$$

the energy liberated is 1000×85 ergs. Let it be assumed that *all* of this is transformed into external work—lifting a weight—and that the resultant heat arises from the activity of a different mechanism; in short, that the muscle is an engine having an efficiency of 100 per cent. How great a weight will this 1 c.c. of muscle lift? Since there are 800 layers of rods, and each layer shortens by 3 μ during the contraction (see Plate 1 herewith), the muscle shortens by 2400 μ or 2.4 mm., lifting a mass of W grams 2.4 mm. The energy (ergs) expended in lifting a mass of W grams thru the distance D (cm.) is $W \times D \times 981$ ergs, since gravity = 981 dynes. Therefore the 85,000 ergs will lift $\frac{85{,}000}{0.24 \times 981} = 361$ grams.

According to Zuntz (l. c., p. 23) 1 gram of muscle substance can do 0.002 kilogram-meter of work in one contraction under favorable conditions. If this muscle shortened 0.24 cm. as the above muscle did, it would lift a trifle more than 800 grams. Bernstein[15] mentions 600 grams at least, as the pull of 1 cm.^{2} of frog muscle in an isometric contraction. Insofar as 1 cm.^{2} of many kinds of muscle can support without lengthening (but not lift) several kilograms—about 6 kilograms for human, and probably more for certain types of insect muscle—the above figure of 361 grams, as the weight a muscle could lift, is small, especially when it is borne in mind that it is an improbable maximum.

The foregoing discussion may be summarized as follows:

1. Too often there is a general lack of definiteness in the mathe-

[15] Bernstein, J.: *Arch. f. d. ges. Physiol.*, 1905, 109, 326.

matical treatment of a biological problem. Formulae are stated with no information as to their use or application to the problem under discussion.

2. Bernstein's calculations on the surface energy changes in working muscle are criticized. A much simpler method of calculation is used with a result similar to Bernstein's, namely, the energy expended by a working muscle is much greater than the probable changes in surface energy can furnish. Of course, future investigations may bring to light sources of surface energy within muscle as yet unknown.

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A STUDY OF SOME PROTEIN COMPOUNDS

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I. INTRODUCTION

When I began Ph.D. work in this laboratory, six years ago, Dr. Gies was actively engaged in studies of the properties of various protein compounds which he had prepared as early as 1904.[1] He inaugurated that work from the standpoint of his interest in the chemical composition of protoplasm, and the nature of the structural and dynamic relationships of cell constituents and products. He believed that the knowledge gained from studies of *artificial* protein compounds would pave the way for successful inquiry into the nature of the protein correlations in the cells—relationships of the most fundamental biological character. At his suggestion, and in furtherance of this object, I have conducted the experiments described in this paper.[2]

The general plan of the research was: (*A*) The production of protein salts by combining *organic bases,* such as strychnin, morphin, conin, piperidin, etc., with *acid-reacting proteins,* such as tendo-mucoid, ovo-mucoid, yeast nucleoprotein, etc.; and (*B*) the production of protein salts by combining *basic proteins,* such as

[1] Gies: *Proc. Sec. Path. and Physiol., Amer. Med. Assn.,* 1906, p. 121.

[2] The detailed results of this work have been described in the writer's dissertation, On the synthesis of some protein salts, Columbia University, 1909 (pp. 61). A preliminary report was published by Eddy and Gies in the *Proceedings of the Society for Experimental Biology and Medicine,* 1907, iv, pp. 145–6.

histons and protamins, with the *acid-reacting proteins* enumerated above.

In developing the latter part of this plan certain anomalies arose in connection with the preparation of thymus histon, which led to a collateral investigation of histons. The results of the latter studies will be embodied in a future paper. (See page 169.)

II. EXPERIMENTAL

1. **Salts of various proteins with organic bases.** *A.* MORPHIN MUCOID. *Purification of the materials.* The first step in the preparation of a typical product was the removal of *free* alkali from the base—and *free* acid from the protein. Chemically pure, pulverized, morphin was washed with distilled water until the washings were entirely neutral to litmus. Tendo-mucoid was prepared after the manner of Cutter and Gies[3] but dehydration with alcohol and ether was omitted. The dry scales were soaked in distilled water until they softened. The protein was then washed with distilled water until the washings were entirely neutral to litmus.

Union of base and protein. The base and the protein were then triturated together in a mortar, a very little water being added to ensure an intimate mixture. A mechanical excess of the base was used in every case. Evidence of chemical action was seen in the peculiarly viscid, smeary character of the mixture. Mucoid and water give a thick, milky mixture, but it is not viscid or smeary. The mixture was finally treated with sufficient water in excess to dissolve the product. The viscid liquid was filtered through a wet, fluted, hardened, filter paper, but the first portions of filtrate were returned to the paper until a clear opalescent liquid appeared. This filtrate was neutral to litmus.

Purification of the product. The filtrate, preserved with toluene, was subjected to continuous dialysis in a parchment bag, immersed in frequently renewed distilled water, until the dialysate, even when concentrated to a very small volume at 40° C., gave no test for the base (morphin). The contents of the bag were then evaporated to dryness at 40° C., toluene being used and frequently renewed during the process. The resultant dry product was then

[3] Cutter and Gies: *Amer. Journ. Physiol.*, 1902, vi, pp. 155–6.

pulverized in a mortar and extracted three times with a large excess of ether for the removal of traces of admixed *free* base (morphin).

Isolation of the product. The powder was next dissolved in a small amount of water and this solution poured into a mixture of ⅓ ether and ⅔ alcohol. A copious precipitate resulted. The precipitate was gelatinous and dissolved easily in water. After dissolving the precipitate in water and filtering the solution, the filtrate was precipitated with alcohol-ether. This process was repeated several times. The final product was dehydrated in the usual manner with alcohol and ether.

Special difficulties in the preparation of morphin mucoid. The first solutions of the compound filtered very slowly. It was found that this was due to excess of mucoid. When a large excess of morphin was used there was less insoluble mucoid residue and filtration became correspondingly more rapid.

Precipitation of the purified product with alcohol became increasingly difficult with the increasing purity of the product. Ammonium sulfate, in excess, precipitated the product from its aqueous solution, but long dialysis was required to remove the salt.

The purified product failed to respond to the iodic acid test for morphin.[4] This fact was carefully investigated. The results showed that the failure was not due to the quality of the iodic acid used nor to interference with the test by the mucoid. In the purification of the product there seemed to be continuous loss of morphin. This was presumably due to hydrolytic dissociation.

Evidence of the compound-nature of the product. The product was water-soluble, demonstrating that it was neither mucoid nor morphin, nor a mechanical mixture of the two. The aqueous solution of the product frothed strongly on shaking and gave a good biuret test, indicating its protein character. Addition of a few drops of 0.2 per cent. hydrochloric acid solution yielded a flocculent precipitate of mucoid.

Conclusions regarding morphin mucoid. Morphin and mucoid

[4] For the detection of morphin, the iodic acid test was applied as follows: 1 c.c. of the solution to be tested was added to an equal volume of dilute sulfuric acid solution. To this was added a few c.c. of iodic acid solution and finally a little chloroform. After vigorous shaking, the presence or absence of a violet coloration served to indicate the presence or absence of morphin.

react to form a water-soluble protein compound which, in aqueous solution, yields mucoid on treatment with 0.2 per cent. hydrochloric acid. The morphin enters into combination in a proportion so small as to be incapable of responding to the iodic test, or is united in such a way as to fail to respond to the test.

B. STRYCHNIN MUCOID. In view of the extreme delicacy of the dichromate test for strychnin, this base was selected for the second series of preparations. Care was taken to insure purity of the original materials as in the preparation of the morphin-mucoid products.

Preparation. The method of preparation was identical with that for morphin mucoid (page 112) except in the following details: The final water-solution of the product was again evaporated to dryness at 40° C. and the dry matter, after pulverization, was extracted with chloroform. Twenty-six voluminous washings were necessary to free the powder from admixed strychnin and to obtain a strychnin-free washing. In view of the insolubility of strychnin in water and its ready solubility in ether this result seemed difficult to explain on any other basis than partial dissociation by the chloroform.

Evidence of chemical combination. The protein character of the compound was established by the following results: The water-solution gave a strong biuret test; was precipitable by saturation with ammonium sulfate or magnesium sulfate; gave a flocculent precipitate with 0.2 per cent. hydrochloric acid and 4 per cent. acetic acid solutions; and frothed strongly on shaking. The aqueous solution of the product was neutral to litmus.

The presence of strychnin was shown by the intense bitter taste and by strong "dichromate tests." Filtrates from precipitates formed by addition of 0.2 per cent. hydrochloric acid solution yielded, in every case, strong "di-chromate tests" for strychnin. Four physiological tests were also made to establish the presence of the strychnin. The results and methods follow:

The lethal dose of strychnin sulfate is about 2.5 mg. per kilo of weight for frogs and 7.6 mg. per kilo of weight for dogs. Volumes of aqueous solution of strychnin mucoid (0.575 mg. per c.c.) containing quantities equal to the lethal dose of strychnin sulfate were injected subcutaneously in frogs and dogs.

In the first of two experiments on frogs, the initial dose failed to produce any strychnin effects. An effect followed the second injection of an equal dose, but it required three doses to produce opisthotonus. Recovery was complete. In the second frog, each of two doses injected successively produced opisthotonus. The frog recovered.

For the first dog a double dose was required to produce hyperesthesia and tetanus. The results with the second dog duplicated those with the first.

In all these physiological tests the strychnin appeared to be liberated slowly in the animal, the effects coming on gradually and extending over a period of 3–5 hours, with complete recovery.

Conclusions regarding strychnin mucoid. The results seemed to leave no doubt regarding the compound-nature of this product. Whether it is a true salt or an adsorption compound can not be decided from the available data, but its neutrality, its water-solubility, and its power to yield both strychnin and mucoid, strongly suggest the production of a salt by a process directly comparable to the neutralization of base by acid.

The physiological tests show that the compound evidently contains a much smaller proportion of strychnin than that in the common sulfate. The quantitative examinations have not yet been completed.

C. Conin mucoid. Conin combines with mucoid very rapidly and yields a solution which filters easily. The product, after purification by dialysis and alcohol precipitation, is water-soluble and biuret-reacting. As in the case of morphin mucoid, however, it was impossible to demonstrate the presence of the alkaloid. All tests were negative with the potassio-mercuric iodid and phospho-tungstic acid reagents.

D. Piperidin mucoid. The purified piperidin product gave the protein tests and also a test for piperidin with platinic chlorid.

E. Anilin mucoid(?). A water-soluble product of mucoid and anilin was obtained but the anilin disappeared early in the purification process.

F. Morphin nucleoprotein. Two attempts were made to produce a compound of morphin with yeast nucleoprotein. The

method of preparation was similar to that described on page 112. Neither attempt was successful in establishing the presence of morphin in the final product. A water-soluble protein of different character from the nucleoprotein resulted in each case.

G. MORPHIN CASEINOGEN, STRYCHNIN CASEINOGEN, AND CALCIUM CASEINOGEN. Studies were made of the effects of morphin, strychnin and calcium hydroxid on caseinogen. In each case water-soluble, biuret-reacting products were obtained. Rigorous purification was not attempted.

SALTS OF OVOMUCOID. Neumeister[5] investigated a glucoprotein in eggs which he named "pseudopeptone." This compound was studied by Salkowski,[6] Mörner,[7] and Eichholz,[8] and called by them "ovo-mucoid." As a "cell-protein," this substance seemed to offer good material for our experiments. A pure product was prepared by Mörner's[7] well-known process.

Preparation of ovo-mucoid. (*From eggs.*) With increasing purity, precipitation with alcohol became correspondingly difficult. Alcohol-ether did not remove this difficulty but the addition of a few drops of sodium chlorid solution brought about precipitation in every case. The final water-solution was freed from chlorid by dialysis in a parchment bag in the presence of toluene. The solution, which then was acid to litmus, was evaporated to dryness at 40° C., yielding yellow flakes which were ground to a white powder.

Properties of the ovo-mucoid product. This ovo-mucoid was readily soluble in water and gave a good biuret test. The water-solution frothed on shaking, but was not viscid. Phosphotungstic acid, 0.2 per cent. hydrochloric acid, 4 per cent. acetic acid and tannic acid solutions precipitated the aqueous solution, which was acid to litmus.

(*From shad roe.*) The roe was ground in a mortar with sand and this mixture poured into boiling, slightly acidulated, water. The remaining steps were identical with those for the preparation of ovo-mucoid from eggs and the product responded to the same tests.

These two products were used in the following studies.

[5] Neumeister: *Zeitschrift für Biologie*, 1890, xxvii, p. 331.
[6] Salkowski: *Centralblatt f. d. med. Wissensch.*, 1893, xxxi, pp. 513 and 706.
[7] Mörner: *Zeitschr. f. physiol. Chem.*, 1893, xviii, p. 525.
[8] Eichholz: *Journ. Physiol.*, 1898, xxiii, p. 163.

H. STRYCHNIN OVO-MUCOID (EGG). The method of preparation followed the lines of the morphin-mucoid process (page 112) with the following abbreviation: After dialysis the solution was at once precipitated with absolute alcohol. No other methods of purification were used.

The filtrate from the original mixture of ovo-mucoid and strychnin was turbid and acid to litmus, but became neutral on standing, in the presence of toluene. On dialysis, and consequent dilution with water, the solution clarified. The dialysate on the other hand became turbid but failed to give a protein or strychnin test.

When the dialyzed liquid was treated with absolute alcohol, in excess, a mixed, cheesy and gelatinous precipitate was produced. The alcoholic filtrate from this precipitate was acid and gave a strychnin test, suggesting dissociation. The precipitate dissolved readily in water and the solution was then filtered. *It was now acid in reaction and gave no strychnin test.* Precipitated again with alcohol, the solid product failed to give the strychnin test, was acid and resembled in every way the original ovo-mucoid.

A portion of this precipitate was dissolved in water and the solution evaporated to dryness at 40° C. A new trituration with strychnin was made with this product. The results were the same as with the first preparation, viz., a turbin solution that cleared on dilution with water by dialysis and gave in this condition both strychnin and protein tests. Alcohol again dissociated it into strychnin and ovo-mucoid (?).

From the above results it was deemed desirable to make a careful study of the reactions of the product and a second preparation was conducted for this purpose. The turbid filtrate obtained from the initial mixture of strychnin and ovo-mucoid was found to be actually amphoteric to litmus, though acid to phenolphthalein. Its alkalinity to litmus was not increased by retriturating it with strychnin. Dilution with water resulted again in a clear solution, giving both strychnin and protein tests. On standing for a considerable time in a parchment bag, in the presence of toluene, the amphoteric reaction gradually disappeared and the solution became distinctly acid to litmus. It also finally yielded a precipitate in the bag and lost its power to respond to the strychnin test. The turbid dialysate grad-

ually acquired protein material, but the frequent renewals of water and large volume made it impossible to determine the presence of strychnin. Apparently complete dissociation resulted, but neither the character of the dissociation products nor the manner in which the strychnin separated was determined.

Strychnin ovo-mucoid (roe). The results with ovo-mucoid from shad roe were identical with those in the case of egg ovo-mucoid except that the disappearance of the strychnin on dialysis was much slower. It was ten days before the contents of the bag failed to give the strychnin test. Concentration of the dialysates in this case before applying the strychnin test failed to make its detection possible.

Evidence of the compound nature of the ovo-mucoid products. The clear amphoteric solution, with its response to strychnin and protein tests, indicates a chemical combination, especially in view of the water-insolubility of strychnin. The dissociability in alcohol of the shad roe product, and the results of dialysis, indicate that it is more stable than the strychnin product with egg ovo-mucoid. Again the question of whether we are here dealing with a true chemical compound or with an adsorption product remains open for further investigation.

2. **Protein-protein compounds.** The foregoing experiments were preliminary to attempts to bring about combinations between acid-reacting and basic-reacting *proteins,* such as protamins and histons.

1. Histon mucoid. *Preparation of histon hydrochlorid.* Histon was prepared by the method of Huiskamp.[9] Thymus glands from freshly killed calves were freed from fat with a knife and minced in a meat chopper. The hash was then placed in a large bottle and extracted in an ordinary ice box for 24–48 hours with distilled water. About 300 c.c. of water were used with each 100 grams of thymus. The extract was filtered through wet fluted filter papers. Nucleohiston was precipitated from the filtrate with 5 c.c. of 10 per cent. calcium chlorid solution per 100 c.c. of extract. The precipitate was then filtered off and redissolved in water to which a little ammonia had been added. This solution was filtered and reprecipitated with calcium chlorid solution in the usual way. The

[9] Huiskamp: *Zeitschr. f. physiol. Chemie,* 1901, xxxii, p. 145.

precipitate was then extracted with 0.8 per cent. hydrochloric acid for the production of the hydrochlorid. This extract of histon hydrochlorid was finally dialyzed in a parchment bag against distilled water until neutral to litmus. This solution of histon hydrochlorid was used for the preparation described below.

Preparation of potassium mucoid. Acid-free mucoid was dissolved in 0.3 per cent. potassium hydroxid solution and the liquid filtered. The filtrate was then dialyzed in a parchment bag against distilled water (in the presence of toluene) until neutral to litmus. The product in this neutral solution was presumably *potassium mucoid.*

Preparation of histon mucoid. Histon hydrochlorid solution was added drop by drop to the potassium mucoid solution. A precipitate formed immediately, and sedimented quickly beneath the clear supernatant liquid. Excess of the histon hydrochlorid solution dissolved the precipitate. The product was then filtered off and washed with water until the washings no longer gave precipitates with ten per cent. ammonium hydroxid or 0.2 per cent. hydrochloric acid solution.

Evidence of the compound nature of the histon mucoid product. A portion of the precipitate was triturated with 0.05 per cent. sodium carbonate solution. A colloidal solution was obtained. Its filtrate gave a heavy precipitate with 0.2 per cent. hydrochloric acid solution and a distinct precipitate with ammonium hydroxid solution.

These results did not determine whether the sodium carbonate merely dissolved the histon mucoid, or dissociated it into a histon solution and a sodium mucoid solution. To ascertain these points the following tests were made:

(*a*) A portion of the sodium carbonate solution was poured into 95 per cent. alcohol. It failed to precipitate at once or on standing.

(*b*) A portion of the sodium carbonate solution was poured into 95 per cent. alcohol, to which one drop of 10 per cent. sodium chlorid solution had been added. A precipitate appeared on standing.

(*c*) Alcohol-ether failed to precipitate the solution but with the addition of a drop of salt solution a precipitate appeared.

(*d*) The precipitates obtained in (*b*) and (*c*) failed, in this first set of tests, to dissolve in water and the washings gave no hydrochloric acid or ammonia precipitate. The precipitates dissolved in 0.05 per cent. sodium carbonate solution and the filtrates gave both the ammonia and hydrochloric acid tests.

(*c*) Histon hydrochlorid solution was not precipitated by alcohol even when sodium chlorid was present. Alcohol also failed to precipitate potassium mucoid solution but did so in the presence of a trace of sodium chlorid.

These results warrant the inference that the sodium carbonate acted as a solvent rather than as a dissociant. They also indicate that precipitation by alcohol in the presence of salt served to differentiate the histon mucoid from the histon hydrochlorid, and that our product was a compound and not a mixture.

A solution of histon hydrochlorid was tested with an excess of alcohol in the absence of salt. A similar solution of potassium mucoid was made. When these two clear solutions were mixed a *precipitate of histon mucoid formed at once.* This histon mucoid was then dissolved in 0.05 per cent. sodium carbonate solution and the filtered solution precipitated with alcohol in the presence of a little salt. This precipitate, unlike that above (*d*), *dissolved readily in water.* The water-solution gave both the ammonia precipitate and the hydrochloric acid precipitate. This and similar results indicated the formation of a soluble histon mucoid compound.

Finally a new histon mucoid product was made by the original method. This product was washed free from excesses of both histon hydrochlorid and potassium mucoid, as before, and then treated as follows:

A portion was macerated in a mortar with 0.1 per cent. hydrochloric acid solution and a second portion in another mortar with 0.1 per cent. potassium hydroxid solution. These liquids were filtered. The acid *filtrate* gave a precipitate with ammonia but not with hydrochloric acid. The alkali *filtrate* gave a heavy precipitate with hydrochloric acid but none with ammonia.

These results suggest that mixtures of (*a*) histon hydrochlorid and potassium mucoid solutions yield a precipitate of histon mucoid; (*b*) pure histon mucoid and 0.1 per cent. hydrochloric acid

solutions yield histon hydrochlorid and insoluble mucoid; (*c*) pure histon mucoid and 0.1 per cent. potassium hydroxid solutions yield a potassium mucoid histon complex.

The failure to get a histon precipitate with ammonia in the potassium hydroxid extract may have been due to the small amount of resultant histon mucoid or to the formation of an insoluble form of histon, such as ammonia produces. Whatever the explanation of this failure, there seemed to be no doubt of the power of histon to combine with mucoid to form a compound different in properties from either histon hydrochlorid or potassium mucoid.

J. Histon nucleoprotein (yeast). Neutral potassium nucleoprotein (obtained by dissolving yeast nucleoprotein in 0.1 per cent. potassium hydroxid solution and dialyzing free from hydroxyl ions) combines with histon hydrochlorid in the same way as potassium mucoid (page 119). Much more of the solution of histon is necessary for the production of the salt. The product was similar to histon mucoid in being insoluble in water; in dissolving readily in 0.05 per cent. sodium carbonate solution but incompletely in 0.5 per cent. sodium carbonate solution; and in forming, with sodium carbonate, a water-soluble sodium-histon nucleoprotein complex.

K. Histon ovo-mucoid. *Preparation.* The ovo-mucoid (egg) was purified to such a degree as to be practically soluble in salt-free alcohol (page 116). A similarly pure solution of histon hydrochlorid was used.

When the water solutions of these two substances were combined, a precipitate formed slowly. A slight excess of the histon solution dissolved the precipitate. The precipitate dissolved to a turbid solution in 0.05 per cent. sodium carbonate solution. This turbid fluid was filtered and divided into two portions. One portion was poured into 95 per cent. alcohol to which 3 drops of 10 per cent. sodium chlorid solution had been added. The other portion was saturated with ammonium sulfate. Both portions gave heavy precipitates, which were soluble in water; the solutions were precipitated in part by ammonia. When purification of the ammonium sulfate precipitate by dialysis was attempted, the compound broke down. The "alcohol precipitate" was hydrolyzed with hydrochloric acid. The resultant liquid, neutralized with potassium

hydroxid, reduced the Fehling-Benedict reagent. This result, with the precipitation by ammonia, seemed to show the presence of both glucoprotein and histon in the precipitate.

When alcohol solutions of ovo-mucoid and histon hydrochlorid were poured together, a precipitate formed at once that gave both the ammonia test and the reduction test. The latter process is the simplest and quickest method of obtaining this product.

The results of these researches have shown that the methods for the preparation of histon, as outlined in the literature, are in serious need of revision. In fact, the results suggest that so-called histon is a protein salt rather than a simple protein. In a future paper will be presented the findings in regard to histon preparation.

EFFECTS OF INTRAPERITONEAL INJECTIONS OF EPINEPHRIN ON THE PARTITION OF NITROGEN IN URINE FROM A DOG

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I. INTRODUCTION

The action of epinephrin on nitrogenous metabolism has been the object of investigation by several authors. The experimental results of Kraus and Hirsch,[1] and Quest,[2] indicate that intravenous or subcutaneous injections of epinephrin exert very little influence on the nitrogenous metabolism of healthy dogs, the insignificant increase of eliminated nitrogen being caused both by the glycosuria and (after subcutaneous injections) by skin necrosis. Fasting animals seem to be differently affected. Falta and Rudinger,[3] and Underhill and Closson[4] were able to show an accelerating influence, on protein metabolism, of subcutaneous and intravenous injections of epinephrin.

Underhill and Closson have shown that the subcutaneous injection of "adrenalin chlorid" solutions into dogs is not attended by any significant change in the proportions of the urea-, ammonia- and creatinin-nitrogen of the urine, in partial disagreement with Paton,[5] who also found that, although on a sufficient diet, the catabolism of proteins is not interfered with, there is a markedly increased production of ammonia.

In all the above mentioned experiments the epinephrin was injected into veins or into subcutaneous tissues. The intraperitoneal way has not been utilized by previous observers in this con-

[1] Kraus and Hirsch. Cited by Kraus and Friedenthal: *Berl. klin. Woch.*, 1908, xlv, p. 1709.

[2] Quest: *Zeit. f. exp. Path.*, 1908, v, p. 43.

[3] Falta and Rudinger: *Central. f. klin. Med.*, 1908, lxvi, p. 1.

[4] Underhill and Closson: *Amer. Journ. Physiol.*, 1906, xvii, p. 42.

[5] Paton: *English Journ. of Physiol.*, 1903, xxix, p. 286; 1904, xxxii, p. 59.

nection, although it is possible that this mode of administering epinephrin has a different effect on nitrogenous metabolism, as is the case in *carbohydrate* metabolism (Löwi).[6]

II. DESCRIPTION OF THE EXPERIMENTS

This investigation consisted of two metabolism experiments. One animal was used for both experiments. An intermediate period served to allow the animal to recuperate from the effects of the first experiment before the second one was begun. The metabolism work was conducted by the general methods in use in this laboratory.[7]

We determined the nitrogen content in the several ingredients of the food. Urinary *nitrogen*, in the leading forms, was determined as follows: ammonia, each day; total, urea, creatin and creatinin (as creatinin), every third day; purins, at the end of each period. The urine was preserved with thymol. Total nitrogen was determined by the Kjeldahl process; ammonia and creatinin by the Folin methods;[8] urea[9] by Benedict's method;[10] purin nitrogen by a combination of the Arnstein[11] and Salkowski[12] methods.

The authors used two specimens of the colorless "adrenalin chlorid" (1 : 1,000) of Parke, Davis and Co. They were purchased in the open market. Each was tested for its pressor action at the conclusion of the corresponding injection experiments and was then found to be practically as active as ever. Varying amounts and concentrations of "adrenalin chlorid" were injected into the peritoneal cavity; in the first experiment the concentration was 1 : 10,000—in the second, 1 : 1,000. In one injection period of

[6] Löwi: *Von Noorden's Metabolism and practical medicine*, 1907, iii, p. 1181.

[7] Mead and Gies: *Amer. Journ. Physiol.*, 1901, v, p. 106; also Gies and collaborators: *Biochemical Researches*, 1903, i, Reprint No. 21; Gies: *Amer. Journ. of Physiol.*, 1905, xiv, p. 403; Gies: *Amer. Journ. of Physiol.*, 1901, v, p. 235; also Gies and collaborators: *Biochemical Researches*, 1903, i, Reprint No. 1; Gies: Proc. Amer. Physiol. Soc., *Amer. Journ. of Physiol.*, 1904, x, p. 22; Hawk and Gies: *Amer. Journ. of Physiol.*, 1904, xi, p. 177.

[8] Folin: *Amer. Journ. of Physiol.*, 1905, xiii, p. 45.

[9] No glycosuria occurred. Examinations were made repeatedly.

[10] Benedict: *Journ. of Biol. Chem.*, 1910, viii, p. 405.

[11] Arnstein: *Zeit. f. physiol. Chem.*, 1897, xxiii, p. 417.

[12] Salkowski: *Salkowski's Manual of physiol. chem. and path.*, 1904; *Arch. d. ges. Physiol.*, 1897–98, lxix, p. 268.

eighteen days, a total of 62 c.c. of 1 : 10,000 solution was given intraperitoneally; in another injection period of six days, a total of 29 c.c. of 1 : 1000 solution was administered. The accompanying tables contain the metabolic data obtained in this study.

TABLE I. FIRST METABOLISM EXPERIMENT (JUNE 12–JULY 18, 1912)

A. Daily Records

I. Fore Period. Normal Condition

Number of the day	1	2	3	4	5	6	7	8	9	10
Body weight (kilos)	6.3	6.33	6.3	6.3	6.31	6.3	6.28	6.27	6.3	6.33
Urine, volume (c.c.)	270	210	270	190	192	212	275	240	188	170
Urine, sp. gr.	1,017	1,025	1,020	1,024	1,024	1,021	1,020	1,018	1,021	1,026

II. Dosage Period. Intraperitoneal Injections

Number of the day	1	2	3	4	5	6	7	8	9
Body weight (kilos)	6.34	6.27	6.28	6.26	6.35	6.25	6.33	6.26	6.31
Urine, volume (c.c.)	135	157	80	100	165	224	160	245	180
Urine, sp. gr.	1,027	1,031	1,042	1,040	1,030	1,020	1,030	1,020	1,025
Adrenalin solution (1 : 10,000) c.c.	5	5	5	5	8	–	5	–	5

Number of the day	10	11	12	13	14	15	16	17	18
Body weight (kilos)	6.26	6.33	6.41	6.40	6.42	6.37	6.35	6.34	6.43
Urine, volume (c.c.)	200	105	153	175	255	230	205	270	167
Urine, sp. gr.	1,023	1,037	1,025	1,023	1,019	1,017	1,020	1,020	1,023
Adrenalin solution (1 : 10,000) c.c.	2	3	3	–	4	–	4	4	4

III. After Period. Normal Conditions

Number of the day	1	2	3	4	5	6	7	8
Body weight (kilos)	6.34	6.40	6.44	6.40	6.44	6.41	6.40	6.46
Urine, volume (c.c.)	135	145	200	245	220	185	214	214
Urine, sp. gr.	1,027	1,020	1,022	1,020	1,018	1,022	1,019	1,020

B. Analytical Totals and Daily Averages of Urinary Data for Each Period

Period	Volume, total, c.c.	Volume, daily average, c.c.	Nitrogen		Urea N		Ammonia N		Creatin and creatinin N		Purin N	
			Total, grams	Daily average, grams	Total, grams	Daily average, grams	Total, grams	Daily average, grams	Total, grams	Daily average, gram	Total, gram	Daily average, gram
Fore (10 days)	2,217	221	41.594	4.159	36.85	3.685	1.511	0.1511	1.046	0.1046	0.067	0.0067
Dosage (18 days)	3,206	178	74.225	4.123	63.58	3.532	2.945	0.1636	1.714	0.0952	0.147	0.0081
After (18 days)	1,558	195	33.077	4.134	28.63	3.578	1.195	0.1493	1.101	0.1376	0.081	0.0101

TABLE 2. SECOND METABOLISM EXPERIMENT (JULY 21–AUGUST 9, 1912)

A. Daily Records

I. Fore Period. Normal Conditions

Number of the day	1	2	3	4	5
Body weight (kilos)	6.52	6.53	6.53	6.54	6.57
Urine, volume (c.c.)	190	190	230	200	193
Urine, sp. gr.	1,020	1,023	1,019	1,020	1,021

II. Dosage Period. Intraperitoneal Injections

Number of the day	1	2	3	4	5	6
Body weight (kilos)	6.54	6.69	6.68	6.63	6.70	6.71
Urine, volume (c.c.)	150	134	215	275	185	240
Urine, sp. gr.	1,028	1,028	1,019	1,018	1,020	1,016
Adrenalin solution (1 : 1,000) c.c.	4	5	5	5	5	5

III. After Period. Normal Conditions

Number of the day	1	2	3	4	5	6	7	8	9
Body weight (kilos)	6.68	6.72	6.72	6.76	6.78	6.77	6.78	6.83	6.80
Urine, volume (c.c.)	245	232	230	200	202	240	200	223	235
Urine, sp. gr.	1,019	1,018	1,018	1,019	1,017	1,018	1,019	1,017	1,019

B. Analytical Totals and Daily Averages of Urinary Data for Each Period

Period	Volume, total, c.c.	Volume, daily average, c.c.	Nitrogen		Urea N		Ammonia N		Creatin and creatinin N		Purin N	
			Total, grams	Daily average, grams	Total, grams	Daily average, grams	Total, grams	Daily average, gram	Total, grams	Daily average, gram	Total, gram	Daily average, gram
Fore (5 days)	1,003	201	20.997	4.199	18.22	3.643	0.767	0.1533	0.671	0.1342	0.039	0.0076
Dosage (6 days)	1,199	199	24.678	4.113	21.46	3.5766	0.967	0.1611	0.7314	0.1219	0.039	0.0065
After (9 days)	2,007	223	34.937	3.882	29.39	3.266	1.499	0.1664	1.266	0.1407	0.059	0.0066

TABLE 3. PARTITION OF THE URINARY NITROGEN

Period	Urea N, per cent.	Ammonia N, per cent.	Creatin and creatinin N, per cent.	Purin N, per cent.	Undetermined N, per cent.
I. Fore period	88.5	3.64	2.51	0.16	5.19
Injection period	85.7	3.75	2.50	0.20	7.86
Post injection period	86.5	3.61	3.33	0.24	6.32
II. Fore period	86.7	3.65	3.20	0.19	6.26
Injection period	87.0	3.92	3.73	0.16	5.19
Post injection period	84.2	4.29	3.62	0.17	7.72

III. CONCLUSIONS

The results of these experiments show conclusively that *intraperitoneal* injections of "adrenalin chlorid" solutions were without appreciable effect on the *proportions* of nitrogen (in the forms of urea, ammonia, creatin and creatinin, purins, and undetermined substances) in the urine of a healthy dog.

THE BIOCHEMICAL SOCIETY, ENGLAND

In a previous note, which appeared in the BIOCHEMICAL BULLETIN (1: 484), it was stated that the recently founded Biochemical Club would probably develop into a society with a journal of its own.

This is now an accomplished fact, and the Biochemical Society of England has been launched into being. It has been instituted for the purpose of facilitating intercourse between those biologists and chemists who are interested in problems common to both, such as the chemical questions connected with agriculture, brewing, animal and vegetable physiology and pathology, etc. Meetings are held at different centers throughout the country for the communication of papers and demonstrations.

The Honorary Secretary is Dr. R. H. A. Plimmer, University College, London, W. C., from whom further information can be obtained.

The *Bio-Chemical Journal,* which has hitherto been under the editorship of Professor Moore, F.R.S., of Liverpool, will in the future be conducted by the Biochemical Society, and will be issued by the Cambridge University Press, Fetter Lane, London, E. C.

The editors are Professor W. M. Bayliss, F.R.S., University College, London, W. C., and Professor A. Harden, F.R.S., Lister Institute, Chelsea Gardens, London, S. W. The first issue of the Journal under these editors is expected in January next. The price is £1.1.0 ($5) per volume.

One feels sure that our American confrères will heartily support the new enterprise.

W. D. HALLIBURTON

King's College, London.

MEETINGS OF THE SECTION (II) ON DIETETIC HYGIENE AND HYGIENIC PHYSIOLOGY OF THE FIFTEENTH INTERNATIONAL CONGRESS ON HYGIENE AND DEMOGRAPHY, WITH ABSTRACTS OF SOME OF THE PAPERS

PROCEEDINGS REPORTED BY THE SECRETARY,
LAFAYETTE B. MENDEL

The meeting of the Congress was noteworthy for the unusual opportunity which it afforded to American men of science to meet some of their foreign colleagues, particularly those from the Continent, in a personal way. It can scarcely be said that the proceedings of the Section on Dietetic Hygiene and Hygienic Physiology were unique in any way; nor could they be expected to attract the chief interest where so many important disciplines and conflicting or overlapping scientific fields were involved. The symposium on the specific dynamic action of foodstuffs deserves special comment, however, both on account of the new views which were forcefully presented there for the first time, and the preëminent part played by all of the referees in the development of this field of study.

I. OFFICIAL LIST OF PRESIDENTS AND VICE-PRESIDENTS OF THE SECTION

Honorary Presidents: DR. MAX RUBNER, Professor of Physiology and Director of the Physiological Institute, Berlin, Germany; DR. ARTUR SCHATTENFROH, Professor of Hygiene in the University of Vienna, Austria; DR. AXEL HOLST, Professor of Hygiene, University of Christiania, Norway; DR. A. B. MACALLUM, Professor of Biochemistry, University of Toronto, Canada.

President: DR. RUSSELL H. CHITTENDEN, Professor of Physiological Chemistry, Sheffield Scientific School of Yale University, New Haven, Conn.

Vice-presidents: Dr. Graham Lusk, Professor of Physiology, Cornell University Medical College, New York City; Dr. David L. Edsall, Professor of Clinical Medicine, Harvard Medical School, Boston, Mass.

II. OFFICIAL PROGRAM[1]

All the meetings of the section were held on Sept. 23 to Sept. 27, inclusive, in Washington, D. C., at the new National Museum, Room 376.

1. Monday afternoon, September 23. THE PHYSIOLOGICAL SIGNIFICANCE OF SOME SUBSTANCES USED IN THE PRESERVATION OF FOOD: *Dr. John H. Long,* professor of chemistry, Northwestern University Medical School, Chicago, Ill. (page 132); *Dr. Artur Schattenfroh,* professor of hygiene in the University of Vienna, Austria.

2. Tuesday morning, September 24. THE SPECIFIC DYNAMIC ACTION OF FOODSTUFFS: *Dr. Max Rubner,* professor of physiology and director of the Physiological Institute, Berlin, Germany. (*A*) The work of digestion and specific dynamic action: *Dr. N. Zuntz,* Direktor des tierphysiologischen Laboratoriums der landwirtschaftlichen Hochschule, Berlin, Germany (*presented by Prof. F. G. Benedict*).—(*B*) The influence of the ingestion of food upon metabolism: *Dr. Francis G. Benedict,* director of the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass. (page 134).—(*C*) The influence of foodstuffs and their cleavage products upon heat production: *Dr. Graham Lusk*, professor of physiology, Cornell University Medical College, New York City (page 135).

3. Tuesday afternoon, September 24. NUTRITION AND GROWTH. (*A*) An anatomical analysis of growth: *Dr. Henry H. Donaldson*, The Wistar Institute of Anatomy, Philadelphia, Pa.—(*B*) Nutrition of the embryo: *Dr. John R. Murlin,* assistant professor of physiology, Cornell University Medical College, New York City.—(*C*) The nutrition and growth of bone: *Dr. Francis H. McCrudden,* chemist at the Hospital of the Rockefeller Institute for Medical Research, New York City (page 137).—(*D*) The rôle of proteins in growth: *Dr. Lafayette B. Mendel,* professor of physiological chemistry, Sheffield Scientific School of Yale University, New Haven, Conn. (page 138).—(*E*) The influence of the quantity

[1] Abstracts of the papers appear on the pages indicated by the numerals in parenthesis.

and quality of food upon the growing organism: *Dr. Hans Aron,* director of the scientific laboratory of the University Children's Clinic, Breslau, Germany (*presented by Prof. Lafayette B. Mendel*).—(*F*) Direct calorimetry of infants, with a comparison of the results obtained by this and other methods: *Dr. John Howland,* professor of pediatrics, Johns Hopkins University, Baltimore, Md. (page 139).

4. **Wednesday morning, September 25.** THE RÔLE OF INORGANIC SUBSTANCES IN THE NUTRITION OF MAN. (*A*) The antagonistic action of salts: *Dr. Jacques Loeb,* head of the department of experimental biology, Rockefeller Institute for Medical Research, New York City.—(*B*) The distribution of soluble salts in living cells and the forces controlling it: *Dr. Archibald B. Macallum,* professor of biochemistry, University of Toronto, Canada (page 140). —(*C*) The rôle which common salt and water assume in the nutrition of man: *Dr. Hermann Strauss,* professor of clinical medicine, University of Berlin, Germany (page 141).

5. Thursday morning, September 26. PRACTICAL DIETETICS. (*A*) Cost and nutritive value of foods: *Dr. C. F. Langworthy,* expert in charge of nutrition investigations, U. S. Department of Agriculture, Washington, D. C.—(*B*) The influence of the preparation of food on its nutritive value: *Dr. Max Rubner,* professor of physiology and director of the Physiological Institute, Berlin, Germany.—(*C*) The choice of foods, with regard to disease: *Dr. Carl von Noorden,* professor of internal medicine and director of the First Medical Clinic, Vienna, Austria (page 143).—(*D*) Diet in relation to disease, chiefly in relation to some forms of partial underfeeding (beriberi and scurvy): *Dr. Axel Holst,* professor of hygiene, University of Christiania, Norway.—(*E*) Diet and metabolism in fever: *Dr. Warren Coleman,* Cornell University Medical College, New York City (page 145).

6. Thursday afternoon, September 26. VENTILATION IN ITS HYGIENIC ASPECTS. (*A*) Organic matter in the expired air: *Dr. Milton J. Rosenau,* professor of preventive medicine, Harvard Medical School, Boston, Mass.—(*B*) A consideration of the unknown factors in the ill-effects of bad ventilation: *Dr. Yandell Henderson,* professor of physiology, Yale Medical School, New Haven, Conn. (page 146).—(*C*) The hygienic physiology of work in compressed

air: *Dr. J. J. R. Macleod,* professor of physiology, Western Reserve Medical School, Cleveland, Ohio (page 147).

7. **Friday morning, September 27.** THE HYGIENIC PHYSIOLOGY OF EXERCISE. (*A*) The influence of exercise on the nervous system: *Dr. Leon Asher,* a. o. professor of physiology, Bern, Switzerland (*presented by Prof. L. B. Mendel*).—(*B*) The influence of exercise on the heart: *Dr. R. Tait McKenzie,* director of physical education, University of Pennsylvania, Philadelphia, Pa.—(*C*) Certain aspects of the influence of muscular exercise upon the respiratory system: *Dr. Theodore Hough,* professor of physiology, University of Virginia, Charlottesville, Va. (page 148).—(*D*) Physical training in the United States Naval Service: *Dr. J. A. Murphy,* surgeon, U. S. N., U. S. Naval Academy, Annapolis, Md.

ADDITIONAL PAPERS. The prevention of arteriosclerosis and heart disease in otherwise healthy individuals past middle life: *Dr. Louis F. Bishop,* New York City.—Tuberculosis and metabolism: *Dr. Diesing,* chief physician, Recreation and Convalescent Home, Gross-Hansdorf, Hamburg, Germany.—On the nature and importance of the diet as the most important factor of causal therapy in severe diseases of the stomach and intestines, in nervous and mental diseases, and in disorders of the circulation and of the metabolism: *Dr. W. Plönies,* Hanover, Germany.—Public baths: *Dr. Simon Baruch,* president, American Association for Promoting Hygiene and Public Baths, New York City.—The significance of hydrotherapy for hygiene, therapeutics and medical instruction: *Prof. Dr. L. Brieger,* Hydrotherapeutische Universitäts-Anstalt, Berlin, Germany.—The importance of the nutritive salts for healthy and sick people: *Dr. R. Peters,* Hanover, Germany.

III. ABSTRACTS OF SOME OF THE PAPERS[2]

The physiological significance of some substances used in the preservation of food

JOHN H. LONG

This paper dealt with the action on the human organism of a number of substances employed as food preservatives, or otherwise, in the preparation of food.

[2] Reprinted from the official pamphlet containing "abstracts of papers to be read at the . . . . . congress," Sept. 23–28, 1912 (pp. 11–28).

Something of the history of food preservatives was recited, and it was shown that a considerable number of substances are added to food largely because of their preservative properties, rather than because of flavors they may impart. Some of the so-called "natural" preservatives come under this head. Modern conditions of living and modern scientific advances have called for the introduction of more efficient substances, the so-called "chemical" or "artificial" preservatives. Many of these substances have been condemned, and perhaps properly, but frequently the condemnation is solely on the ground of their origin. This basis of condemnation has no justification in fact, as all preservatives are as truly chemical as are those of recent introduction made by industrial processes. The active principles in cloves, cinnamon, allspice, etc., are true chemical compounds, and in their action on the body and final disposition are much like benzoic acid, now made largely by laboratory processes.

A number of important investigations on the physiological action of sodium benzoate have been carried out in the last few years, and the results of these were discussed. The effects of large and small amounts of benzoic acid are known, and it has been clearly shown that the use of the small quantities employed in the ordinary protection of the condimental foods is quite unobjectionable. Such small amounts are normally disposed of in the human body without ill effects.

The use of copper salts in coloring vegetables was next discussed. There is an enormous literature on the subject, especially from France and Germany, where copper has long been used in the canning industries. Several commissions have pronounced in favor of permitting the use of copper salts, although others have opposed it. But all authorities have come to agree that the toxicity of these salts is much less than was at one time assumed. This toxicity depends somewhat on the combinations in which the salts are ingested. The effects of copper as used in young peas or string beans are far less marked than are those of its inorganic salts. It is, therefore, not quite justifiable to draw conclusions as to the behavior of copper from experiments with copper sulfate alone. If only very young and fresh vegetables, with plenty of chlorophyl, were treated

with copper, and if the amount were strictly limited, there might be but little fault found. But with older vegetables the combination is far less stable and the effects approach those of the inorganic salts. The amounts of copper taken up by the liver and other organs from inorganic salts may be considerable, and such absorption cannot be held free from danger. The use of these salts serves no real good purpose and should be condemned.

The paper touched also on the employment of sulphurous oxide and sulphites in certain food industries.

The influence of the ingestion of food upon metabolism

FRANCIS G. BENEDICT

Three interpretations of the increase in metabolism following the ingestion of food are current: first, the theory in which the mechanical work of the digestive processes plays the most prominent rôle; second, the less sharply defined theory in which the conception of the development of free heat unavailable to the cells is the dominant note, and, finally, the opinion expressed by Friedrich Müller, that there is absorbed out of the food certain substances which are carried by the blood to the cells and there stimulate the cells to a greater metabolic activity. The evidence used for the evaluation of these views in this paper is based almost exclusively upon experiments made upon men in our laboratory.

It was found that although the ingestion of sodium sulfate produced a powerful peristalsis, no measurable increase in the metabolism as measured by the oxygen consumption was noticed. Similarly, the ingestion of large amounts of agar-agar produced very voluminous, bulky stools, but did not increase metabolism measurably. As subsidiary evidence, in unpublished experiments on dogs with deficient pancreatic secretion, it was found that although the decreased assimilation of protein and fat resulted in large, bulky, fatty stools, there was not an increase in the carbon dioxide production. The evidence points strongly to the fact that the ingestion of meat by depancreatized dogs, accompanied as it is with large, voluminous, bulky stools, results in absolutely a smaller increase in metabolism than is experienced with the ingestion of meat by normal dogs.

Both of these pieces of evidence, therefore, can be taken as strongly contrary to the work of digestion as an explanation of any considerable proportion of the increased metabolism generally noted after the ingestion of food.

Experiments both on dogs and on men show that following the ingestion of food there is an increased muscle tonus as indicated by the pulse rate, and frequently by the respiration rate, showing that the animal is living on a higher metabolic plane than formerly. The increased heat is thus a product of cell action, and the question as to its economic value acquires a new significance. A man asleep, with lowest heat production, is of little value to the world; awake, with no external muscular activity, he has increased internal activity and is capable of intellectual life.

The ingestion of protein alone stimulates metabolism with the possibility of some differences in the kinds of protein. Carbohydrates show rapid effects, not so great as protein, and different carbohydrates give different results. The metabolism 12 hours after the last meal of a carbohydrate-free, fat-rich diet, with moderate amounts of protein, is much greater than the metabolism under similar time-conditions after a mixed diet with the same amount of protein. Diabetics with varying degrees of intensity of the disease show marked differences in the total metabolism 12 hours after the last meal. A high acidosis is coincident with a high metabolism.

A carbohydrate-free, fat-rich diet, eaten by a normal individual, is accompanied by the presence of an acidosis and an increased metabolism. The evidence suggests that coincidental with what is commonly termed a "state of acidosis" there is present in the blood a substance or substances, probably of an acid nature, that stimulate the cells to a greater metabolism.

The influence of foodstuffs and their cleavage products upon heat production

GRAHAM LUSK

If meat in large quantity be given to a dog, the heat production rises in the second hour almost to its maximum, reaches its maximum in the third hour and continues at this level through the tenth

hour when it begins to fall. In one instance the heat production during a morning hour was 22.3 calories, and after the ingestion of 1,200 grams of meat it had risen in the second hour to 36 calories, reaching 40 calories in the third hour at which level it remained through the tenth hour, after which it gradually fell to 25 calories in the twenty-first hour. During the second hour the nitrogen elimination was one third the maximal nitrogen output as evenly maintained between the third and tenth hours. The second hour also showed that the calculated non-protein respiratory quotient ranged between 90 and 99, which indicated that that part of the metabolism which was not due to protein, as calculated from urinary nitrogen, originated largely from carbohydrate. During the later hours, the increased heat production is proportional to the nitrogen in the urine. During a period of 15 hours, protein carbon was retained in the organism and when the oxygen absorption as computed on the basis of such retention in the form of dextrose is compared with the actual oxygen absorption, the two agree within 0.9 per cent., whereas computed on the basis of carbon retained as fat, there is a discrepancy of 10 per cent. between the calculated and actual value.

Administration of 50 grams of dextrose in 150 c.c. of water to a dog causes a rise of heat production from 16.2 to 20 calories, at which level it is maintained during the second, third and fourth hours, falling nearly to the basal level in the fifth hour. The skin temperature rises to a greater extent than the rectal temperature. The absorption from the intestine is completed in the fourth hour. The urine is scanty until the fourth hour when 100 c.c. are suddenly eliminated. The sugar content of the blood in per cent. rises in the first hour but becomes normal after that. After the first hour the percentage of hemoglobin in the blood falls but returns to normal subsequent to the fourth hour. Hence, after sugar ingestion, osmotic phenomena cause an increased volume of blood. When the absorption is complete, the glycogenic function removes the dextrose from the blood, and the blood returns to its normal composition through the elimination of water by the kidney. Water alone or a solution of salt or of urea have no effect on the metabolism, hence the increase in metabolism is probably due to the increased number of molecules of dextrose carried to the cells and not to

changes due to osmosis. Liebig's extract of beef is without influence on the metabolism. Fifty grams of olive oil cause a considerable increase in heat production. Glycocoll causes a very great increase in heat production, alanin also acts powerfully, leucin and tyrosin less so and glutamic acid not at all.

It is concluded that the heat production may be increased by increasing the quantity of sugar and fat reaching the cells, or it may be increased through the direct stimulation of the cells by amino-acids, notably glycocoll and alanin.

Nutrition and bone growth

FRANCIS H. MCCRUDDEN

The question of the nature of bone metabolism in health and disease is one that until recently can hardly be said to have been attacked experimentally. The pathologists, with one or two exceptions, have generally considered bone as a dead tissue not undergoing metabolism, once it is laid down. This opinion has, of course, colored their views regarding the nature of the process in various bone diseases. In osteomalacia, for example, a disease in which there is a decrease in the mineral content of the bone, it has been supposed that the process is due to the action of an acid which dissolves out the mineral constituents.

Numerous investigations, chemical, histological and clinical, during the last few years have shown that these views regarding the nature of the process in osteomalacia and the nature of normal bone metabolism cannot be correct. Bone, like the other tissues, undergoes metabolism throughout life. Old bone is continuously being resorbed and new bone laid down. If the new bone laid down is not qualitatively of the right composition, the result may be rickets, osteomalacia, osteoporosis, or osteitis deformans, depending on the age of the patient and other factors. The bones act as a store of lime salts to be called on in time of need just as the subcutaneous tissue acts as a store of fat and the liver as a store of glycogen; and a flux of calcium from the bones started by a growing fetus, a hardening callus, metastatic bone formation, etc., may, under certain circumstances, lead to decalcification enough to result in osteo-

malacia and similar conditions. An important factor is the degree to which overproduction takes place, a factor involved also in immunity and, in fact, in all tissue repair.

Other disturbances which may be said to involve quantitative disturbance in bone growth,—the rate of growth,—rather than disturbances in the qualitative character of the bone produced, are the various types of dwarfism. In some of these the failure to grow seems to depend on an absence of the "growing tendency" on the part of the bones; in others, some disturbance in the supply of lime salts available for bone growth seems to be at fault.

The rôle of proteins in growth

LAFAYETTE B. MENDEL

Some of the views held in the past regarding the interrelation of the food supply and growth are no longer tenable. Growth has often been associated in a causal way with the relative abundance of protein in diet. The parallelism between the protein content of the milk of various species and rate of growth may, in the familiar cases be an example of correlation rather than of causation. Recent investigations have shown that the assumed association of growth with high protein intake is not confirmed by the evidence at hand.

Growth is a function of the cells. This inherent capacity apparently cannot be exaggerated by feeding; but growth can be held in abeyance by various conditions. These include inadequacy of the food supply in respect to both quantity and quality of the nutrients. Attention must be directed to the chemical as well as the energetic aspects of the problems involved. In the past physiologists have largely disregarded the relative values of the individual members of different groups of food substances in nutrition, owing to an ignorance of the chemical characteristics of the individuals.

In considering the uses of protein in the organism, the distinction between the requirement for maintenance and that for growth must be clearly kept in mind. The development of a successful method of investigation by Osborne and Mendel has made it easy to approach some of the problems experimentally. The method was explained in detail. Normal rate of growth has been induced in

rats with dietaries containing various single purified proteins. But not all proteins suffice to promote growth under otherwise favorable conditions. Some suffice for maintenance without growth, whereby a prolonged period of stunting, or suppression of growth, can be induced; still other proteins are alone insufficient for the maintenance requirement.

The capacity to grow is not lost even after comparatively long periods of dwarfing and a subsequent normal unimpaired rate of growth may be attained with a suitable protein dietary. Aside from the apparent nutritive inequalities of the different proteins, other incidental findings, such as the synthetic features in growth, and diverse questions raised thereby, present a multitude of viewpoints which may serve to direct further research in this field.

Direct calorimetry of infants, with a comparison of the results obtained by this and other methods

JOHN HOWLAND

A discussion of the various methods for determining the exchange of energy in infants with their advantages and disadvantages and their limitations. The results of direct calorimetry obtained with four children by means of a modified Atwater-Rosa-Benedict calorimeter. The carbon dioxide excretion, oxygen consumption and heat production of two essentially normal children. The effect of the ingestion of food, and especially of an excess of protein. The effect of eighteen hours' fasting. The heat production and respiratory exchange of two extremely emaciated children.

Difficulty of comparing results with those obtained by other methods on account of the different conditions under which the experiments have been conducted, the different information that has been supplied and also on account of the unsatisfactory formulas for determining the surface areas of infants, which give errors of 20 per cent. and more. A new, more accurate and simple formula for determining this.

The distribution of soluble salts in living cells and the forces controlling it

ARCHIBALD B. MACALLUM

1. The distribution of salts in living matter is held to be due to the forces that make the distribution of salts uniform in an ordinary solution. These forces are the same as those which determine the distribution of the molecules of a gas in an enclosed space. Consequently, in a living cell the salts are supposed to be uniformly distributed throughout the fluid of the cytoplasm, that is, the osmotic force, or the pressure exercised by the molecules and ions throughout the fluid, is due to this uniform distribution of the solute throughout the system. The quantity of a soluble salt present in living matter is, therefore, a measure of the osmotic pressure therein and hence exchange between the salts within and those without would, in all cases, if the cell membrane were permeable, develop so as to adjust the pressure equally within and without the cells.

2. This conception leaves wholly out of account the action of surface tension. Every particle of the colloid of which living matter is composed presents to the fluid in which it is suspended an interface where the surface tension of the fluid is lower than such a fluid has at its free surface. In consequence the Gibbs-Thomson principle comes into operation and there results a condensation of the molecules and ions of the solutes on the interfaces of all particles. As the united interfacial surfaces must, in relation to the total volume of the solution or fluid, present a very great area, a very large proportion of each of the solutes must be so condensed, and the general concentration is, accordingly, greatly reduced or brought to the vanishing point. This would reduce the osmotic pressure due to such solutes to a very low value or even to nil.

3. The degree to which concentration on surfaces or on interfaces obtains depends on the degree of diminution of the tension of the fluid at an interface, but it also depends on the nature of the solute, for the concentration in case of certain salts greatly exceeds the value demanded by the Gibbs equation, while in other salts the ascertained value approximates the theoretical value.

4. Such surface condensation of the salts of living matter can,

in a number of cases, be demonstrated microchemically. In the case of potassium salts this is especially feasible. They are in this way found condensed in interfaces inside of living cells, and also on surfaces in tissues, *i. e.,* on the external surfaces of nerve cells, of renal, pancreatic and salivary tubules, and thereby relations are established which determine the processes of excretion and secretion of these salts. In such cases the potassium salts in the tissue fluids elsewhere than at the surfaces or interfaces are scarcely detectible microchemically.

5. Surface tension is, therefore, an all-important factor in determining the distribution of salts in living matter.

The rôle which common salt and water assume in the nutrition of man

HERMANN STRAUSS

Common salt plays an important part as a regulator of the osmotic processes in the human organism, whereby the latter with greatest tenacity holds fast the percentage concentration of its fluids. Man can get along on relatively small quantities (½ gm.) of "salt required by the tissues." But the majority of civilized men consume much greater quantities of common salt, and the principal quantity taken in food plays the part of a "seasoning salt." Therefore, the reduction, in the diet, of common salt has its limits, since disturbances may ensue from too great a reduction. Where the supply is too abundant, the excess is excreted. As a result of reduction of ingested common salt, a diminution in the secretion of gastric juice has been noted in dogs. In diseases of the stomach in men, it has been proposed, in the case of lack of hydrochloric acid in the gastric juice, to introduce copious quantities of common salt; in the case of increase in the secretion of gastric juice, to decrease the quantity of common salt in the food. But, in practice, with such a procedure, it has been possible to obtain only inconstant results.

I myself have pointed out, that an excretory insufficiency of the renal function may be traced to a retention of common salt. Through the retention of water, this condition favors the development of dropsy, since the principal amount of the retained salt finds

lodgement in the organism in the form of a "seroretention," while only a small part is deposited in the form of a "historetention." In consideration of these established opinions, for a decade, I have recommended a limitation of the supply of salt in the food, and a medicinal stimulation of salt-elimination, in the prevention and treatment of hydronephrosis.

The situation, with regard to uncomplicated diseases of the heart (as well as incipient compensation disturbances in subjects of heart disease) is different from that in cases of parenchymatous nephritis. Also, in inflammatory discharges, and in ascites resulting from cirrhosis of the liver, the circumstances are otherwise. In these conditions, the results of a deprivation of chlorin are very inconstant. For alimentation in diabetes insipidus, there have been established certain correct requirements similar to those laid down for parenchymatous nephritics with an inclination toward dropsy. The significance of a limitation of salt as a means of lessening the thirst, in all cases in which there is a question of a decrease of fluid in the aliment, is now more highly appreciated than formerly.

The question of dry retention of chlorin is now not wholly clear. At present, exact investigations as to the salt-content of the skin are lacking. Also, the relation of salt-retention to the development of uremia has not yet been fully explained. I should be inclined at this time to state only that, *cæteris paribus,* uremia occurs more readily in the nephritic organism which is poor in water, than in one where water is abundant, and I may also state that, in the vomited matter of uremics, an extraordinary quantity of common salt is found.

Through recent researches, a marked relationship between bromin and chlorin has also been brought to light. Bromid poisoning may be successfully treated by means of an abundant supply of common salt.

In complete deprivation of salt, and, likewise, in thorough limitation of fluids, an increase in the disintegration of protein may be noted. On the other hand, an increase in the combustion of fat cannot be shown. As a rule, salt-equilibrium is restored in 24–48 hours. On the contrary, following a previously sharp decrease in the supply of salt, it requires several days for the restoration of salt-

equilibrium; and, in extreme retention of salt, increased elimination may be checked for many days.

It cannot be denied that many healthy persons consume too great quantities of common salt. Moderate amounts are not injurious. A certain quantity of salt, as seasoning, is permissible for civilized individuals accustomed to substances which stimulate the sense of taste.

The choice of foods, with regard to disease

CARL VON NOORDEN

The discussion pertained to the lessons dietetic therapy holds for us in its connection with various diseases and disease groups, and to the foods that are serviceable or a hindrance to the attainment of the end desired. Only the major groups of food substances, such as proteins, fats, carbohydrates, spices and salts were considered.

1. Obesity. Principle: Decrease in the caloric value of the food. This is best attained through a decrease or total exclusion of the supply of fat. Carbohydrates, where relatively plentiful in the diet, should be barred out. In anti-fat treatments, the amount of contained protein should, where possible, amount to not less than 100 grams. The supply of water must be curtailed only if the obesity is accompanied by disturbances of the circulation.

2. Forced alimentation. Principle: Increase of the caloric supply over the diet for maintenance. Theoretically, it is all the same, whether the center of gravity rests upon a large supply of carbohydrates or fat. In reality, 250 gm. of carbohydrate is seldom exceeded, because most carbohydrate foods possess a very great volume. The supply of protein may not ordinarily be increased beyond 100–120 gm. By means of these, approximately 1,300 calories, no satisfactory alimentative results may be obtained. The practical results depend always upon the increase in the supply of fat. In most cases, the latter may be increased to 250 or 300 gm. daily, and then increases in weight of about 2 kilos per week may be gained.

3. Gout and uric acid diatheses. Principle: Decrease in animal foods; eventually total exclusion of the same. It is useful, in the

case of every gouty patient, to undertake a separate "test of toleration," and, from the result of this test, to establish the patient's diet.

4. Diabetes mellitus. Principle: Avoidance of such foods as incite the organ of sugar-production, the liver cells, to increased formation of sugar. Every undue stimulation of the sugar-forming organ has, as its result, not only an immediate lavish production of sugar, but also increases for the future its morbid excitability; while systematic care of the organ renders its recovery possible. Therefore, decrease, and, under some circumstances, total exclusion of the carbohydrates. Moreover, decrease of protein substances. It is essential, in every case of diabetes, to exactly determine under what dietetic régime and manner of living the least amount of superfluous sugar is formed. That order of diet is best under which the patient continues free from superfluous sugar.

5. Feverish diseases and morbus Basedowii. Principle: In both these diseased conditions there occurs an abnormal increase in caloric production. Simultaneously ensues a heightened sensibility in relation to the specific dynamic influence of proteins. In order to limit as far as possible the caloric production and the loss in weight, practical empiricism and theory likewise call for a scanty protein supply, while weight is gained by an ample provision of carbohydrates.

6. Diseases of the digestive organs. Principle: A food supply which is sufficiently nourishing, while imposing as little tax as possible upon the diseased organs. A discussion of the injurious effect of certain combinations of foods. Report upon enterotoxic neuritis. Attack by means of protracted pure milk diet.

7. Kidney diseases. Principle: As much rest as possible for the kidneys. The amount of the intake of those nutrient media whose products of metabolism leave the body through the kidneys, should be reduced. The proteins come first in this regard. But this limitation should not be carried too far, since patients with chronic kidney diseases become anemic and weak if strict curtailment of the proteins is too long continued. Many spices irritate the kidneys, and indulgence therein must be limited; the same is the case with regard to alcohol. Common salt and water severely tax the kidneys.

Final words: Warning against schematic employment of dietary precepts. An effort must be made, on the one hand, to hold fast to the basic rules of nutrition-therapy, but, on the other, to duly take into account the individuality of the patient.

Diet and metabolism in fever

WARREN COLEMAN

Empiricism has been a signal failure as a basis for the fever diet. Through studies of metabolism the solution of this problem appears to be at hand. With diets containing large amounts of carbohydrate it is possible to bring typhoid fever patients into nitrogen equilibrium, or nearly so. The apparently excessive quantities of food required for the purpose are almost completely absorbed.

The heat-production in typhoid fever, as determined by indirect calorimetry, averages about 35 calories per kilogram at absolute rest. Diets furnishing only sufficient energy to cover the heat-production do not protect the body against nitrogen or weight loss. The explanation of this discrepancy has not yet been found.

Respiratory quotients in typhoid fever below 0.65 to 0.70 appear to be due to errors of technique. The lowest quotient we obtained in the fasting state, during the febrile period, was 0.70. During the same period, patients on a full diet gave quotients varying from 0.75 to 0.95 at short intervals after food. The quotient rises during the later stages of the fever and reaches 1.0 to 1.15 early in convalescence. During a relapse, a quotient of 1.04 was obtained while the patient had a temperature of 102° F. (37.7° C.).

The oxygen consumption during the febrile stage varies between 4 and 6 c.c. per kilogram a minute. Compared with the amount used in the fasting stage, the oxygen consumption is not greatly increased by the quantity of food administered.

The body burns carbohydrate by preference during fever as long as it is available. As indicated by a falling quotient, from 100 to 120 grams of lactose is, for the most part, consumed, or deposited in the glycogen depôts, after 4 to 5 hours. The optimum amount of carbohydrate in the fever must be determined for each patient individually, but is always large. The optimum amounts of fat and protein have not yet been determined.

A consideration of the unknown factors in the ill-effects of bad ventilation

YANDELL HENDERSON

The facts regarding ventilation present an extraordinary contradiction. Fresh air, sunlight and dry cool climates exert a decidedly beneficial effect upon health. Ill-ventilated dwellings decrease vitality. In some persons under certain conditions even a few minutes in a crowded room may produce acute ill-effects. As to how these effects are produced physiology has up to the present time afforded no satisfactory explanation. The evidence is almost entirely negative. The ill-effects of bad ventilation can not be due to lack of oxygen. It is probable that they are not due in any considerable degree to excess of CO_2. The idea that they are due to some poisonous substance contained in the expired air has in recent years been regarded as untenable. Recently this conception has been revived in a novel form by the brilliant work of Rosenau. Even Rosenau's investigations do not appear, however, to afford the solution of this problem. The recent investigation of Hill in England and of Flügge and his pupils in Germany makes it highly probable that the effects of fresh or vitiated air are brought about not by a direct action upon the lungs but indirectly through the skin. It appears probable that the temperature and moisture of the air surrounding the body are the essential elements.

According to the explanation to be suggested in this paper the condition of the skin exerts a potent influence upon the lungs. This may be in part a vaso-motor reflex acting upon the pulmonary circulation. More probably it is a chemical or hormone influence upon certain pulmonary processes. The evidence accumulated during recent years indicates that the lungs are not mere passive organs through which gases diffuse as through non-living membranes. The investigations of Bohr, of Haldane and his co-workers and of the recent Pikes Peak expedition all tend to indicate that the lungs are the seat of vital activities of great importance to health. Thus under certain conditions the lungs secrete oxygen into the blood, and it appears that considerable oxidation may take place in the blood during its passage through the pulmonary vessels. The evidence

available, although still far from complete, suggests that these pulmonary activities are indirectly but powerfully influenced through conditions affecting the skin, and that it is in this manner that ventilation influences health.

The hygienic physiology of work in compressed air

J. J. R. MACLEOD

Although it is now a well-established fact that the symptoms of caisson disease and diver's palsy are due to the sudden liberation of bubbles of nitrogen in the blood and tissue fluids, on account of too sudden decompression, there are several peculiarities regarding the conditions which influence the safety of decompression about which there is still a certain degree of uncertainty. This is the case more particularly with regard to: (1) Whether the decompression should be uniform or in stages; (2) how long it should take in proportion to the time of the shift and the pressure employed; (3) the degree to which the breathing of oxygen increases the safety of decompression. Although, as insisted on by Haldane and others, it is no doubt the case that "the absolute air pressure can always be reduced to half the absolute pressure at which the tissues are saturated without risk" yet, in practice, it has not been found that the method is in any way superior to that of gradual decompression.

The time that should be taken in decompression depends on the length of the shift in the caisson, because the saturation of the remoter parts of the body with nitrogen continues for a long time after this has been attained in the blood and the more accessible tissues. Tables indicating what time should be allowed have been prepared by Haldane and by Japp.

The advantages of breathing oxygen are not only that it accelerates the diffusion of nitrogen out of the lungs and, therefore, out of the blood; but, if symptoms have already appeared, it supplies enough oxygen to keep life going when the circulation is dangerously obstructed by nitrogen bubbles. In using oxygen at higher pressures, its toxic action must however be kept in mind.

Recompression, either by placing the caisson worker in a pressure chamber or by having the diver descend again to a certain depth

whenever the first symptoms appear, is by far the most efficient treatment, as both experiment and the experience of engineers testify.

On account of the heat and the high relative humidity of compressed air, the worker in a caisson is under conditions which tend to lower his efficiency. Not only this, but his appetite is likely to suffer and his general condition after some time to deteriorate so that he becomes liable to infections if not to caisson disease itself. The caissons should therefore be well ventilated and the wet bulb thermometer kept as low as possible. Means for doing this were discussed. In the choice of men for caisson work attention should be paid to age, body weight and fatness and while engaged in the work the men should be kept in good training.

Certain aspects of the influence of muscular exercise upon the respiratory system

THEODORE HOUGH

Muscular activity increases the respiratory exchange from three- to tenfold, thus making demands on the system comparable only with those of the more severe forms of dyspnea. In meeting the respiratory needs of the tissues there are secondary effects of hygienic importance, such as the increased aspiration of the thorax upon the return of venous blood to the heart and also upon the flow of lymph in the larger lymphatics; this increased lymph flow is felt in the interstitial spaces of every organ in the body, thus favorably influencing the environment of every cell.

The introduction with more vigorous exercise of physiological strain makes it important to inquire into the exact condition of the organism revealed by the accompanying respiratory phenomena.

Of the conditions known to increase the work of the respiratory center Geppert and Zuntz have excluded, as exciting causes of the increased breathing movements of muscular activity, afferent impulses from the working muscles and deficiency of oxygen in the arterial blood; their work also shows a decrease of the total (*i. e.,* free and combined) carbon dioxid of the arterial blood; it does not establish a diminished tension of this gas in the respiratory center, and it is possible (Haldane) that there may be increased tension of this gas in

the center along with a fall of the total amount in the blood. No direct determinations of the condition of the blood in this respect have been made.

Determinations of the alveolar tensions of oxygen and carbon dioxid during and at varying periods after muscular activity show that with increasing intensity of work there is first a rise of CO_2 tension, then a fall to and below normal. In the latter case the CO_2 tension sinks still further after the cessation of the exercise and may remain subnormal for over half an hour or even an hour.

Review of the evidence as a whole leads to the conclusion that during more vigorous exercise the main cause of the increase of breathing movements is some catabolite (other than CO_2) of the working muscle. During moderate exercise the increase of CO_2 tension of the blood is probably an adequate explanation; the respiratory condition of the organism would thus differ not only in degree but also in kind with moderate and with more vigorous exercise.

Theory that the distress which is relieved by "second wind" is due to excessive CO_2 tension not well established by the evidence at hand, but worthy of further study. Bearing upon this question is the effect of previous inhalation of oxygen in lessening the distress of maximal effort.

Should not the measurement of respiratory power in physical examination be extended so as to include not only the anatomic features of chest expansion and vital capacity, but also the ability of the respiratory system to meet successfully the conditions of the more vigorous forms of muscular activity?

Yale University,
New Haven, Conn.

MEETINGS OF THE SECTION ON BIOCHEMISTRY, INCLUDING PHARMACOLOGY (VIII, D), OF THE EIGHTH INTERNATIONAL CONGRESS OF APPLIED CHEMISTRY

PROCEEDINGS REPORTED BY THE SECRETARY,
JOHN A. MANDEL

I. LIST OF OFFICERS OF THE BIOCHEMICAL SECTION

President, John J. Abel; *Vice President,* William J. Gies; *Secretary,* John A. Mandel; *Executive Committee:* Reid Hunt, Thomas B. Osborne and the officers.

II. SECTIONAL PROGRAM[1]

The meetings of the Section were held on September 6 to September 12, inclusive, at Columbia University, in Room 301 of Havemeyer Hall. The morning sessions were opened at 10 o'clock and the afternoon sessions at 1 o'clock.

Friday morning, September 6. IN THE CHAIR: THE VICE PRESIDENT. *Julius Stoklasa:* Ueber die photochemische Synthese der Kohlenhydrate unter Einwirkung der ultravioletten Strahlen.—*L. Marchlewski:* The present state of our knowledge of the relationship of the chemistry of the blood coloring matter and chlorophyll.—*L. Marchlewski* and *C. A. Jacobson:* On the quality of chlorophyll and the variable ratio of the two constituents, and on methods for determining this ratio.—**Guido M. Piccinini:* Il manganese del punto di vista delle funzioni enzimatiche.—**Jules Wolff:* Sur la résistance de la peroxydase à l'ammoniaque et sur son activation par contact avec l'alcali.—**Jules Wolff:* Sur une nouvelle fonction du catalyseur dit "peroxydase" et sur le transformation biochemique de l'orcine en orceine (page 53).—*Walter Jones:* Some new phases

[1] The asterisks indicate the papers which were actually presented. Some of the titles were received after the official program had been printed and are included here informally. Abstracts of most of the papers were published in Volume 19 of the preliminary report of the proceedings of the Congress.

of the nuclein fermentation.—*Carl Voegtlin:* Further studies in biologic oxidations.—**Walter R. Bloor:* Fatty acid esters of glucose.—*C. C. Guthrie:* A comparative study of the action of solutions on the preservation of the vitality of tissues.—**R. Delaunay* and *O. Bailly:* Les pepsines fluides etude du sediment qui se produit dans certaines d'entre elles.—*William J. Gies:* Modified collodion membranes, with demonstrations.

Saturday morning, September 7. In the chair: Prof. Mauthner, of Vienna. **Gabriel Bertrand* and *F. Medigreceanu:* Sur la présence normale du manganèse chez les animaux.—**M. Lindet:* Sur les elements mineraux de la caseine du lait.—**P. Malvezin:* La question de l'acide sulfureux dans les vins blancs.—**Z. Mimuroto:* Ueber das Vorkommen von Adenin und Asparaginsäure in Maulbeerblättern.—*U. Suzuki* and *S. Matsunaga:* Ueber das Vorkommen von Nikotinsäure (*m*-Pyridinkarbonsäure) in der Reiskleie.—*Zozo Sakaguchi:* Ueber den Fettgehalt des normalen und pathologischen Harns.—*W. N. Berg:* Effect of sodium chlorid and cold storage upon the activities of proteolytic enzymes.—**Thomas B. Aldrich:* The iodine content of the small, the medium and the large thyroid glands of beef, sheep and hogs.—**Lewis W. Fetzer:* The chemical changes taking place in milk under pathological conditions.—**Max Kahn:* A study of the chemistry of renal calculi.

Monday morning, September 9. In the chair: The President. **M. Nicloux:* Moyen de caractériser de petites quantités d'alcool methylique dans le sang et dans les tissus.—*Zennoshin Hatta:* Zur Kritik der Zuckerbestimmungsmethode nach Ivar Bang.—*Munemichi Tamura:* Zur Prüfung der Kumagawa-Sutoschen Fettbestimmungsmethode in Bezug auf die Oxydation der fettsäuren und unverseifbaren Substanzen im Verlaufe des Verfahrens.—*Yuji Suéyoski:* Eine neue approximative Eiweissbestimmungsmethode bei Albuminurie.—**Franz Herles:* Schnelles Verfahren zur Bestimmung der Harnsäure im Harn.—*W. Worth Hale* and *Atherton Seidell:* The comparative estimation of epinephrin in suprarenal glands and in its solutions, physiologically and by color tests.—*Lyman B. Stookey:* The Cammidge reaction.—**Herbert H. Bunzel:* Oxidase determinations.—*J. P. Atkinson:* On the separation of certain alkaloids from nerve tissue.—**W. H. Schultz* and *Atherton*

Seidell: The determination of thymol in dog feces.—**Shiro Tashiro:* A new apparatus for the detection and estimation of exceedingly minute quantities of carbon dioxide in biological materials.—**Shiro Tashiro:* Carbon dioxide production in the nerve fibre during an excitation. Its application for detection of life in protoplasm.—**F. Klein:* Die selenige Säure—ihr Verhalten gegen Eiweiss und tierische Haut.—**Gabriel Bertrand* and *H. Agulhon:* Sur la présence normale du bore chez les animaux.

Monday afternoon, September 9. In the chair: The President. **Felix Ehrlich:* Ueber einige chemische Reaktionen der Mikroorganismen und ihre Bedeutung für chemische und biologische Probleme.—**Gilbert T. Morgan* and *E. Ashley Cooper:* The influence of the chemical constitution of certain organic hydroxyl and aminic derivatives on their germicidal power.—*Takaoki Sasaki:* Ueber den Abbau einiger Polypeptide durch Bakterien. II. Untersuchungen mit nicht verflussigenden Bakterien.—*Nagamichi Shibata:* Zur Frage der Fettzersetzung durch einige Saprophyten.—**A. Trillat:* Influence des impuretés gazeuses de l'air sur la vitalité des microbes.—**M. Javillier:* Influence exercée par le zinc sur l'*aspergillus niger* au point de vue de l'utilisative par la plante.—**Carl L. Alsberg* and *O. F. Black:* Biochemical and toxicological studies upon *Penicillium stoloniferum.*

Tuesday morning, September 10. In the chair: The President. **M. Maze:* Relations de la plante avec les élements fertilisants; loi du minimum et loi des rapports physiologiques.—**M. Gerber:* Etude comparée des pressures des l'*amanite phalloide* et de l'*amadouvier.*—**R. Dubois:* Sur l'atmolyse et sur l'atmolyseur.—**R. Dubois:* Recherches sur les vacuolides de la purpurase.—**R. Dubois:* La biophotogenese réduite a une action zymasique.—*Oswald Schreiner:* The physiological rôle of organic constituents in plant metabolism.—**C. F. Langworthy:* The study of problems of vegetable physiology by means of the respiration calorimeter; a progress report.—**Howard S. Reed:* The enzyme activities involved in certain plant diseases.—**Ernest D. Clark:* Origin and significance of starch.—*William J. Gies:* Studies of diffusion, with demonstrations.

Tuesday afternoon, September 10. In the chair: The

PRESIDENT. **P. Carles:* Les phosphates et le son de froment dans l'alimentation animale.—**P. Carles:* Entretien du tissu dentaire par une alimentation appropriée.—*Minoru Maéda:* Versuche über die Ausnutzung von "Konnyak" (einer japanischen Speise).—**Paul E. Howe* and *Philip B. Hawk:* The utilization of various protein foods by man after repeated fasting.—**L. F. Foster* and *Philip B. Hawk:* A study of the utilization of ingested food when undermasticated ("bolted") and overmasticated ("fletcherized").—*S. P. Beebe:* The influence of the thyroid on the excretion of ammonia.—**Andrew Hunter* and *Maurice H. Givens:* Purin metabolism in the monkey.—*William Salant* and *J. B. Rieger:* The influence of alcohol on protein metabolism.—*Jacob Rosenbloom:* Chemical and pharmacological studies of human duodenal contents.

Wednesday morning, September 11. IN THE CHAIR: THE PRESIDENT. **M. Sauton:* Nutrition minérale du bacille tuberculeux.—**Walter J. Dilling:* Charts of spectra representing visible and invisible bands of various hemoglobin derivatives, with explanatory booklet.—**G. O. Higley:* Some notes on the form of the curve of carbon dioxide excretion resulting from muscular work following forced breathing.—**G. O. Higley:* The influence of barometric pressure on the carbon dioxide excretion in man.—**Joseph L. Miller* and *Dean D. Lewis:* Physiological action of the various anatomical components of the hypophysis.—*Isaac Levin:* Immunity and specific therapy in experimental cancer.—*Lafayette B. Mendel:* The physiological behavior of lipoid-soluble dyes.—**B. B. Crohn:* Experiences with duodenal and stool ferments in health and disease.—*Herman M. Adler:* Experimental production of lesions resembling pellagra.—*William J. Gies:* Studies of edema, with demonstrations.

Wednesday afternoon, September 11. IN THE CHAIR: THE PRESIDENT. *George W. Crile:* Neuro-cytological changes resulting from the administration of certain drugs.—**G. A. Menge:* Some new compounds of the cholin type.—*Reid Hunt:* Physiological action of some new compounds of the cholin type.—*Arthur S. Loevenhart:* Further observations on the action of oxidizing substances.—*W. H. Schultz:* Pharmacological action of proteins and some of their derivatives.—**W. H. Schultz* and *Atherton Seidell:* Subcutaneous absorption of thymol from oils.

Thursday morning, September 12. IN THE CHAIR: THE PRESIDENT. **J. M. Fortescue-Brickdale:* The arylarsonates: their pharmacology considered from the experimental and practical standpoints.—**H. A. D. Jowett, V. F. L. Pyman* and *V. F. G. P. Remfry:* The relation between chemical constitution and physiological action, as exemplified by the glyoxalines, isoquinolines and acid amides.—*Walther Straub:* Pharmakologische Bedeutung der Zellmembranen.—*Charles Baskerville:* Inhalation anesthetics.—**Thomas B. Aldrich:* On feeding young white rats the anterior and posterior parts of the pituitary gland.—*J. A. E. Eyster:* The relation of calcium to the inhibitory mechanism of the heart.—*Clyde Brooks:* On the action of alcohol on the circulation.

Thursday afternoon, September 12. IN THE CHAIR: THE PRESIDENT. *Giovanni Bufalini:* Reazioni caratteristiche del veleno del rospo (*Bufo vulgaris.*)—*Giovanni Bufalini:* Meccanismo dell' azione narcotici del chloridrine.—**E. Fourneau* and *V. K. Ochslin:* Chlorure de l'acide dichloroarsinobenzoique; éthers des acides benzarsineux et benzarsinique.—**C. R. Marshall:* The pharmacological action of brom-strychnins.—**C. R. Marshall:* The influence of hydroxyl and carboxyl groups on the pharmacological action of nitric esters.—*Isaac Adler:* Studies on chronic adrenalin, lead and nicotin intoxications.—**Ivo Novi:* Il calcio e il magnesio del cervello in varie condizioni fiziologiche e farmacologiche.—**L. Launoy:* Action de quelques amines, en particulier du chlorure et de l'hydrate de tetramethylammonium sur la secretion pancreatique.—**R. Delaunay* and *O. Bailly:* Examen critique des conditions d'essai des pancreatines medicinales.

III. ATTENDANCE

Among the many in attendance at one or more sectional meetings, the Secretary noted the presence of the colleagues named below: John J. Abel, T. B. Aldrich, C. L. Alsberg, J. P. Atkinson, W. N. Berg, G. Bertrand (Paris), Samuel Bookman, Harold C. Bradley, H. H. Bunzel, Ernest D. Clark, F. C. Cook, F. Ehrlich (Breslau), Frank R. Elder, B. G. Feinberg, Lewis W. Fetzer, M. S. Fine, Harry L. Fisher, A. O. Gettler, Wm. J. Gies, A. J. Goldfarb, R. A. Gortner, Isidor Greenwald, M. L. Hamlin, G. A. Hanford,

Robert A. Hatcher, Philip B. Hawk, G. O. Higley, B. Horowitz, E. M. Houghton, Paul E. Howe, Reid Hunt, Max Kahn, F. Klein, P. A. Kober, W. M. Kraus, P. A. Levene, Isaac Levin, Alfred P. Lothrop, Wm. G. Lyle, John A. Mandel, Samuel Matthews, T. Mauthner (Vienna), F. Medigreceanu, G. M. Meyer, Jos. L. Miller, G. T. Morgan (Dublin), Max Morse, Victor C. Myers, W. A. Pearson, F. B. Power (London), Howard S. Reed, A. I. Ringer, C. J. Robinson, Anton R. Rose, Jacob Rosenbloom, William Salant, Emily C. Seaman, Atherton Seidell, B. Setlik (Prague), H. C. Sherman, Torald Sollman, Matthew Steel, M. X. Sullivan, Shiro Tashiro, Rodney H. True, H. Vieth (Ludwigshavn), Charles Weisman, Louis E. Wise.

University and Bellevue Hospital Medical College,
New York City.

SIXTH SCIENTIFIC MEETING OF THE COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION, AT THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK, JUNE 3, 1912

PROCEEDINGS REPORTED BY THE SECRETARY,
ALFRED P. LOTHROP

The *sixth scientific session* (*third "annual" meeting*) of the Columbia University Biochemical Association was held at the Columbia Medical School on the evening of June 3, 1912. The executive proceedings of this session were published on pages 570–573 of Volume I of the BIOCHEMICAL BULLETIN (June number).

The scientific proceedings consisted of research communications by members of the Association. Abstracts of the papers are presented here (pages 158–187) in two groups: (I) *Abstracts of papers on research by non-resident members*[1] and (II) *abstracts of papers from the Columbia Biochemical Department and affiliated laboratories.* The appended summary will faciliate reference to the abstracts (1–44).

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS

I

WILLIAM N. BERG. The physico-chemical basis of striated-muscle contraction. (1)

WILLIAM N. BERG, WITH L. A. ROGERS, C. R. POTTEIGER AND B. J. DAVIS. Factors influencing the flavors of storage butter. (2)

ISABEL BEVIER, FOR ANNA W. WILLIAMS. A study of ropy bread. (3)

ALLAN C. EUSTIS. On the toxicity of guinea pig urine and its relation to anaphylaxis. (4)

ALLAN C. EUSTIS. On the physiological action of some of the amins produced by intestinal putrefaction. (5)

ALLAN C. EUSTIS. Solubilities and action of β-imidazolylethylamin and the relation to asthma and anaphylaxis. (6)

A. J. GOLDFARB. On the production of grafted multiple embryos. (7)

MAX MORSE. Non-toxicity of inorganic colloid solutions upon protozoa. (8)

[1] Members of the Association who were not *officially* connected with the Columbia biochemical department when the research was conducted.

MAX MORSE, FOR L. B. RIPLEY. Larvae of Lepidoptera obtained with sulfuric acid. (9)

ANTON RICHARD ROSE. A study of the metabolism and physiological effects of certain phosphorus compounds in milk cows. (10)

II

DAVID ALPERIN. Contribution to the knowledge of nucleoprotein metabolism, with special reference to uricolysis and to the properties of uricase. (11)

GEORGE D. BEAL AND GEORGE A. GEIGER. The comparative diffusibility of various pigments in different solvents. (12)

STANLEY R. BENEDICT. The occurrence and estimation of creatinin in urine. (13)

LOUIS E. BISCH. An endeavor to prepare phrenosin from protagon. (14)

LOUIS E. BISCH. Mucoid-silver products. (15)

SIDNEY BORN. Protein-copper products. (16)

J. J. BRONFENBRENNER AND HIDEYO NOGUCHI. A biochemical study of the phenomena known as complement splitting. (17)

ERNEST D. CLARK. Notes on the chemical nature of Lloyd's "tannin mass." (18)

WALTER H. EDDY. A study of some protein compounds. (19)

WALTER H. EDDY. The preparation of thymus histon. (20)

FRANK R. ELDER AND WILLIAM J. GIES. The influence of proteases on the swelling of collagen and fibrin particles in alkalin and acid media containing a biological electrolyte. (21)

WILLIAM J. GIES. A convenient form of apparatus for demonstrations of osmotic pressure exerted by lipins. (22)

WILLIAM J. GIES. Some interesting properties of thymol. (23)

WILLIAM J. GIES. A convenient method of preparing starch that swells rapidly in water. (24)

R. F. HARE. A study of the carbohydrates of the prickly pear and its fruits. (25)

HENRY H. JANEWAY AND WILLIAM H. WELKER. The relation of acapnia to shock. (26)

MAX KAHN. Biochemical studies of sulfocyanate. (27)

MAX KAHN. The chemical constitution of renal calculi. (28)

MAX KAHN AND JACOB ROSENBLOOM. The colloidal nitrogen in urine from a dog with a tumor of the breast. (29)

MAX KAHN AND FREDERIC G. GOODRIDGE. A non-protein, colloidal, nitrogenous substance in milk. (30)

JOHN L. KANTOR. A biochemical test for free acid, with a review of the methods for estimating the various factors in gastric acidity. (31)

MARGUERITE T. LEE. A study of modifications of the biuret reagent. (32)

ALFRED P. LOTHROP. A chemical study of salivary mucin. (33)

C. A. MATHEWSON. A study of some of the more important biochemical tests. (34)

JACOB ROSENBLOOM. A quantitative study of the lipins of bile obtained from a patient with a biliary fistula. (35)

JACOB ROSENBLOOM AND WILLIAM WEINBERGER. Effects of intraperitoneal injections of epinephrin on the partition of nitrogen in urine from a dog. (36)

OSCAR M. SCHLOSS. A case of allergy to common foods. (37)

CARL A. SCHWARZE. The comparative enzyme content of green and variegated leaves of *Tradescantia*. (38)

EMILY C. SEAMAN. Biochemical studies of beryllium sulfate. (39)

CLAYTON S. SMITH. Chemical changes in fish during long periods of cold storage (40)

WILLIAM WEINBERGER. An attempt to sharpen the end point in Benedict's method for the quantitative determination of sugar in urine. (41)

WILLIAM H. WELKER. Diffusibility of protein through rubber membranes, with a note on the disintegration of collodion membranes by common ethyl ether and other solvents. (42)

CHARLES WEISMAN. A further study of the Bardach test for protein. (43)

HAROLD E. WOODWARD. A study of the surface tension of dog blood-serum by the drop-weight method. (44)

I. ABSTRACTS OF PAPERS ON RESEARCH BY NON-RESIDENT MEMBERS[2]

1. The physico-chemical basis of striated-muscle contraction. WILLIAM N. BERG. (*Washington, D. C.*). *Part I was published in the June issue of the* BIOCHEMICAL BULLETIN; *part II is presented in this issue.*[3]

2. Factors influencing the flavors of storage butter. WILLIAM N. BERG, with L. A. ROGERS, C. R. POTTEIGER, and B. J. DAVIS. (*Dairy Division Research Laboratories, Bureau of Animal Industry, Washington, D. C.*) The official government bulletin on this subject is in press.

3. A study of ropy bread. ISABEL BEVIER, for ANNA W. WILLIAMS. (*Research Laboratory, Department of Household Science, University of Illinois, Urbana, Ill.*) *Published in full in the June issue*[4] *of the* BIOCHEMICAL BULLETIN.

4. On the toxicity of guinea pig urine and its relation to anaphylaxis. ALLAN C. EUSTIS. (*Laboratory of Clinical Medicine, Department of Nutrition, Tulane University, New Orleans, La.*) The urine of guinea pigs, fed on *Kohlrabi* or cabbage, contains a great excess of indican, which readily oxidizes to indigo. Such urine also contains excess of putrefactive amins. Tests for β-imidazolylethylamin, as well as efforts to isolate it, have been negative. Experiments on fifteen guinea pigs weighing 300 grams each, with different specimens of guinea pig *urine,* indicate that 1.5 c.c. constitutes a lethal dose when injected intravenously. In these

[2] Members of the Association who were not *officially* connected with the Columbia biochemical department when the research was conducted.

[3] Berg: BIOCHEMICAL BULLETIN, 1912, i, pp. 535–7; ii, pp. 101–10.

[4] Williams: BIOCHEMICAL BULLETIN, 1912, i, pp. 529–534.

animals, the symptoms were identical with those observed after injections of β-imidazolylethylamin. There was no delay in coagulation of the blood, but there was marked lowering of blood pressure and lowering of body temperature.

After intravenous injections of filtered guinea pig *urine* into three dogs, symptoms resembling those of anaphylactic shock were exhibited, but the fall in blood pressure was not constant as it is after anaphylactic shock, and there was no delay in the coagulation of the blood. There is evidently some relation between the occurrence of putrefactive amins and anaphylactic shock, but the writer's results do not bear out Pfeiffer's opinion regarding that relation.

5. On the physiological action of some of the amins produced by intestinal putrefaction. ALLAN C. EUSTIS. (*Laboratory of Clinical Medicine, Department of Nutrition, Tulane University, New Orleans, La.*) *Putrescin* (*tetramethylendiamin*) and *cadaverin* (*pentamethylendiamin*), in doses as small as 0.1 mg., are instantly fatal when injected intravenously into guinea pigs. Non-fatal doses produce marked lowering of blood pressure, dyspnea from edema of the lungs, salivation and prostration. The pulse is quickened.

Phenylethylamin is immediately fatal to a guinea pig weighing 300 gm. when 0.05 gram is injected intravenously; 0.03 gram was fatal in two minutes when injected intravenously into a 300 gram guinea pig, with immediate prostration and paralysis of the respiratory center; 0.02 gm. produced a distinct chill in a 300 gram guinea pig, followed by prostration but with ultimate recovery.

β-imidazolylethylamin, in doses of 0.01 gram intravenously, caused death in three minutes with typical anaphylactic symptoms, the animals dying in attacks of forcible inspiratory effort, the heart continuing to beat after the respiration had ceased.

Parahydroxyethylamin, as well as *isoamylamin,* produced marked rise in blood pressure.

6. Solubilities and action of β-imidazolylethylamin and the relation to asthma and anaphylaxis. ALLAN C. EUSTIS. (*Laboratory of Clinical Medicine, Department of Nutrition, Tulane University, New Orleans, La.*) I. A specimen of chemically pure β-imidazolylethylamin, obtained through the courtesy of Dr. Dale

of the research laboratory of Burroughs, Welcome & Co., was insoluble in cold chloroform, benzene, toluene, amyl alcohol, but slightly soluble in xylol, easily soluble in methyl alcohol, and soluble in cold carbon disulfide and hot amyl alcohol.

Aqueous solutions were tested with several reagents, to discover if possible some means of detecting the presence of β-imidazolylethylamin in the tissues or blood, as follows: *Bromine water,* no precipitate, no coloration; *copper sulfate,* negative; *potassium ferrocyanid,* negative; *Pauly's reagent,* cherry red coloration; *picric acid,* yellow precipitate insoluble in water, alcohol, ether, xylol and toluene, but which gave the positive Pauly reaction; *phosphotungstic acid,* gray-blue precipitate, soluble in barium chloride solution, and in barium hydroxid solution, which gave a positive Pauly reaction; *sodium nitrite,* negative; *magnesium sulfate,* negative; *mercuric chlorid,* negative; *gold chlorid,* negative.

Efforts to detect, by microchemical means, the presence of β-imidazolylethylamin in the bronchioles of guinea pigs dying from anaphylactic shock, were without results.

II. Tests of the *physiological action* of β-imidazolylethylamin were conducted upon rabbits, guinea pigs and dogs by intravenous, subcutaneous and intraperitoneal injections. *Intravenous injections* of 0.5 mg. in guinea pigs caused immediate respiratory embarrassment, lowered blood pressure and diminished body heat, the animal dying in six minutes from suffocation due to complete occlusion of the bronchioles; it being impossible to either force air into the lungs or to withdraw air, after the contraction had become complete. The symptoms were typical of anaphylactic shock, and the post-mortem examination revealed the presence of enormous emphysema, the heart continuing to beat long after respiration had ceased. In dogs and rabbits there was also a lowering of the blood pressure and some respiratory embarrassment, but the occlusion of the bronchioles was not as complete as in guinea pigs.

Subcutaneous and intraperitoneal injections were much less toxic and, in some instances, were entirely negative, suggesting that the tissues are able to utilize β-imidazolylethylamin.

The writer has seen many cases of asthma relieved entirely along dietetic lines by a "low protein" diet, and empirically has

found that red meats predispose to asthmatic attacks. β-imidazolylethylamin is produced in the putrefaction of histidin, and hemoglobin yields a large percentage of histidin on decomposition. It is possible, therefore, that β-imidazolylethylamin causes asthma. Unlike clinical asthma, however, experimental asthma produced by β-imidazolylethylamin is not relieved by injections of epinephrin ("adrenalin chlorid").

7. On the production of grafted multiple embryos. A. J. GOLDFARB. (*Marine Biological Laboratory, Woods Hole, Mass., and the Department of Natural History, College of the City of New York.*) Grafted multiple embryos were first successfully produced in considerable numbers by Driesch, with the eggs of either of two genera of echinoderms, namely, *Echinus* and *Sphaerechinus*. Though several investigators have endeavored to repeat these experiments with American echinoderms they have failed completely. By slightly modifying the Herbst-Driesch method as described below, an unusually large number of grafted multiple embryos and larvae were produced from the eggs of *Arbacia punctulata*.

After removing the fertilization membranes, the eggs were placed either directly into a sodium hydroxid solution, or first placed in calcium-free sea water, then in an alkaline liquid of the following composition: 4 to 20 drops of 0.5 per cent. sodium hydroxid solution in 200 c.c. of sea water. This treatment sufficed in Driesch's experiments with *Echinus* and *Sphaerechinus*, giving rise to about 4 per cent. of agglutinated and fused embryos. For *Arbacia* eggs it was necessary to supplement this treatment by centrifuging the eggs in tubes with very narrow bores, so that the eggs whose outer surfaces had previously been gelatinized were compressed against one another. These eggs gave rise to about 40 per cent. of agglutinated and fused embryos and larvæ.

The multiple embryos of *Arbacia*, so produced, were of the same general character as those described by Driesch, such as true twins, incomplete fusions, and complete fusions of the respective embryos.

8. Non-toxicity of inorganic colloid solutions upon protozoa. MAX MORSE. (*Boardman Laboratories, Trinity College, Hartford, Conn.*) Colloidal platinum prepared by the Bredig

method, in which the house current of 110 volts was reduced to 70 volts by lamps in parallel and passed through glass-distilled water by means of platinum electrodes, was used as a medium in which cultures of *Paramecium* and other protozoa were permitted to rest. Drop-culture slides were also made of these cultures in hanging drops of the platinum black. In all cases there was no augmentation of division-frequence or size of the organism, nor any evidence of toxicity. Attempts with a solution of mastic in ether and alcohol, which gave beautiful pictures under the *Dunkelfeldtbeleuchtung* of Zeiss, were not clear in their results. The colloidal solution was dialyzed for seven days in a fish-bladder, which freed it from the ether and alcohol, leaving a colloidal mass with excellent brownian movement. However, there is good reason to believe that this is not toxic in any way on protozoa. No attempt was made to "ultra-filter" the colloidal solutions, in order to study the effects of small and larger colloidal particles upon protozoa, because of the apparent indifference of the organisms to the mixed solution.

9. Larvae of Lepidoptera obtained with sulfuric acid. MAX MORSE, FOR L. B. RIPLEY. (*Boardman Laboratories, Trinity College, Hartford, Conn.*) Larvae were obtained from unfertilized eggs of the moth, *Cecropia,* by painting them with Baker's conc. sulfuric acid (sp. g. 1.84) for from 3 to 6 seconds and immediately washing in pure water until entirely free from the acid. They were then left to dry and to develop. Checks were made by treating one-half of the batch from a given female with the acid and leaving the other half untouched. The females had been raised and isolated, from cocoons. The typical blueing of the developing eggs could be observed in the early stages of the eggs treated with acid while the control eggs remained white. The larvae emerged several days later in the case of the artificially fertilized eggs than in those normally fertilized. The percentage of errors was low.

The larvae after emerging from the eggs were fed upon wild cherry, but thus far they have not been carried to the adult stage. This is now being tried. Petrunkevitch, Tichomorow and others have succeeded in obtaining larvae from silk-worm eggs by artificial means, but *Cecropia* has thus far failed to yield larvae under artificial conditions. Short exposure and thorough washing may be the key to the success obtained in the present case.

10. **A study of the metabolism and physiological effects of certain phosphorus compounds in milk cows.** ANTON RICHARD ROSE. (*New York Agricultural Experiment Station, Geneva, N. Y.*) The phosphorus requirement of a cow, aside from the milk phosphorus, would seem from the results of this experiment to be about 26 mg. per kilo of body weight. When the phosphorus supply is less than this amount, the physiological functions are continued at the expense of the phosphorus previously stored in the tissues. Storage takes place when a greater amount than that indicated above is ingested. When the ingested insoluble phosphorus did not exceed 14 grams per day, there was approximate regularity in the phosphorus elimination in the feces independent of the ingestion, suggesting that all the forms of phosphorus were digested, with liberation of phosphates; also that the fixed phosphorus of the feces was entirely due to the cellular matter from the mucosa and the intestinal flora. The soluble organic phosphorus in the feces was relatively slight in quantity, even in the periods when "phytin" was fed in liberal amounts. The calcium phytate added to the washed-bran ration was not utilized as economically as the "phytin" of the whole bran, and the "phytin" of the partially washed bran also gave a lower digestion coefficient.

The addition of "phytin" to the "low phosphorus" ration increased the potassium output in both feces and urine. The fecal potassium dropped in quantity when the "phytin" was withheld, but the urinary potassium did not. The amount of fecal magnesium was constant through the several periods except in the fourth, when it seems to have been influenced by the increased intake of calcium phytate. At the beginning of the experiment the magnesium in the urine was equal to half that in the feces, but continually decreased until the mobile magnesium of the body had been largely eliminated. The calcium in the urine increased remarkably when the phosphorus intake decreased. In the calcium phytate period, the calcium increase in the feces was approximately equivalent to the calcium increase in the rations.

In all cases the addition of organic phosphorus to the "low phosphorus" ration was followed by a decrease in the milk flow, and the withdrawal of this phosphorus from the ration was followed by a

larger yield of milk. The percentage of fat in the milk fluctuated regularly with the changing amount of phosphorus ingested. The response was immediate, but the quantities of milk-fat bear no constant ratio to the amount of phosphorus in the rations. Aside from those pertaining to the fat, there were practically no changes in the composition of the milk, not even in the percentage of phosphorus in the fat-free solid matter.

The moisture relations in the problem seem significant, though the intake and outgo of water could not be accurately measured in this experiment. The margin, after allowing for the influence of temperature, leads one to suspect a large retention of water in the last two periods.

Up to the sixtieth day there was no outward sign of any physiological disturbance, but about that time the appetite began to wane. On the seventy-seventh day the milk-flow declined rapidly and serious trouble developed. A few days later the cow was placed in a box-stall and fed alfalfa, silage and wheat bran, which caused all signs of malnutrition to disappear in the course of a week and also increased the milk-flow.

II. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEMICAL DEPARTMENT AND AFFILIATED LABORATORIES

11. Contribution to the knowledge of nucleoprotein metabolism, with special reference to uricolysis and to the properties of uricase.[5] DAVID ALPERIN. The author studied the relative efficiency of the Wiener, Rosell, Croftan, Wiener and Wiechowski, and Galeotti methods for the preparation of uricase, and indicated the properties of the products. Wiener and Wiechowski have suggested that the subcutaneous or intravenous administration of uricase preparations is an effective procedure for the cure of gout and allied diseases. The author concludes that "practical demonstration of the efficiency of this method of treatment has not been made."

12. The comparative diffusibility of various pigments in different solvents. GEORGE D. BEAL and GEORGE A. GEIGER. (*Published in full in this issue of the* BIOCHEMICAL BULLETIN.)[6]

[5] Alperin: *Dissertation,* Columbia University, 1912.

[6] Beal and Geiger: BIOCHEMICAL BULLETIN, 1912, ii, pp. 78–86.

13. **The occurrence and estimation of creatinin in urine.**[7] STANLEY R. BENEDICT. The work contemplates a thorough investigation of the question as to whether the Jaffé reaction in urine is due entirely, as is usually assumed, to the form of creatinin which is ordinarily isolated from urine, or whether other substances may not be partially responsible for the reaction. The results indicate that there are two (or more) forms of creatinin in urine, both of which yield the Jaffé reaction and also a zinc chlorid compound, but which differ from each other in certain specific properties. A change in the ratio between these two forms of creatinin in the urine has been observed in certain abnormal conditions. The most marked change was noted in inanition. There is probably a third substance contributing to the creatinin reaction of urine which is in no wise related to creatinin, but appears to be a weak acid. The study is in progress.

14. **An endeavor to prepare phrenosin from protagon.**[8] LOUIS E. BISCH. Thudichum's method[9] of isolating phrenosin has apparently never been reviewed. It was assumed that this method could be applied with success directly to protagon. The author was unable to do so, however. Repetitions of each of the numerous steps in the process, with as much as 1450 grams of protagon at a time (in faithful accord with Thudichum's description), failed to yield sufficient material with which to complete the directions. It is possible that losses, which seem to have occurred at all stages of the process, totally consumed any phrenosin that existed in the original protagon. It is Dr. Gies' intention to study this possibility further.

15. **Mucoid-silver products.**[10] LOUIS E. BISCH. Moist, *acid-free* tendomucoid, triturated with a moderate amount of moist, *alkali-free* silver oxid, yields a brown to black mixture which becomes very viscid when a small volume of ammonium hydroxid solution is stirred into it. A mechanical excess of 10 per cent. ammonium hydroxid solution converts the viscid mass into a brown

[7] Under the auspices of the George Crocker Special Research Fund.

[8] Bisch: *Dissertation* (Part I), Columbia University, 1912.

[9] Thudichum: *A treatise on the chemical constitution of the brain,* 1884, pp. 136–8.

[10] Bisch: *Dissertation* (Part II), Columbia University, 1912.

to black solution, from which free alkali and free silver can be removed by dialysis. The neutral solution thus prepared appears to contain argent-ammonium-mucoid, which may be obtained by precipitation with alcohol or by direct desiccation. The aqueous solutions of these products are similar to those of argyrol in many respects yet appear to keep indefinitely. The purified material is antiseptic, and retards the growth of plants, but is seemingly non-irritant to the cornea or other animal tissues. Fairly large quantities fail to induce toxic effects when injected subcutaneously or intravenously into dogs. The product in aqueous solution is decomposed by acidification. The purified material yields about 16 per cent. of ash. The silver content will be given special attention in the near future.

16. Protein-copper products. SIDNEY BORN. Concentrated aqueous solutions of various *indiffusible* proteins, when rendered slightly alkalin with sodium hydroxid solution and treated with a moderate quantity of copper sulfate solution, exhibit the typical biuret reaction in marked degree, but the excesses of alkali and copper may be removed by dialysis and, as the process continues (although no color may appear in the diffusate), the deep "biuret color" slowly changes until finally a blue or green persists. The resultant protein-copper product was isolated by precipitation of such a solution with alcohol or by its direct desiccation. The proportion of copper in six products made from edestin, gelatin, and serum protein ranged from 4.2 to 6.3 per cent. Injected subcutaneously into frogs, the edestin and gelatin products (1.3 c.c. of concentrated aqueous solution in each case) caused death in three hours. The properties of the dialyzed solutions and the products therefrom will be described in some detail later.

17. A biochemical study of the phenomena known as complement splitting. J. J. BRONFENBRENNER AND HIDEYO NOGUCHI.[11] **A.** It is generally accepted that complement may be split into a mid-piece and an end-piece. The mid-piece is thought to be in the globulin fraction, and the end-piece in the albumin fraction.

[11] Bronfenbrenner: *Dissertation,* Columbia, 1912; Bronfenbrenner and Noguchi: *Journal of Experimental Medicine,* 1912, xv, 598–643. Most of the work was conducted at the Rockefeller Institute for Medical Research.

The restoration of complement activity by putting together the albumin and globulin fractions does not prove, however, that each fraction contained a part of the complement, for the albumin fraction can be reactivated in the absence of the globulin fraction.

Complement-splitting as brought about by hydrochloric acid, carbon dioxid, and dialysis, is really an inactivation of the whole complement by certain acids or alkalis, either added in the free state to the serum, or liberated as a result of the dissociation of certain electrolytes.

That the whole complement, and not a part only, is present in the albumin fraction of the serum can be demonstrated by the removal of the inhibitory action of the acid or alkali. This can be effected by the addition, not only of alkali or acid, but also of any amphoteric substance. When hydrochloric acid, carbon dioxid, or dialysis are employed to produce the phenomenon known as complement splitting, the complement is merely inactivated, not split.

B. Thus far, most investigators have made but little distinction between the splitting phenomenon obtained by chemical interference and that which takes place in the biological phenomenon known as complement fixation. In this study we have shown that these two sets of phenomena exhibit certain fundamental differences and that the so-called complement splitting by physical conditions leading to chemical interaction, or directly by chemical means, is not a real splitting of the complement, but an inactivation of the active principle of complement through an alteration in the reaction of the medium caused by an excess of either anions or cations. The modification of the reaction of the medium may cause a more or less definite combination of the complement with the free ions, but the latter can readily be removed by an appropriate number of opposite ions, and render the complement active once more. The fluids that have hitherto been regarded as containing the end-piece of complement, contain, as a matter of fact, the whole complement temporarily deprived of its activity by certain ions derived either from the salt constituents of the serum itself under a modified physical condition (dialysis against water or dilution with water) or introduced in the form of dissociable electrolytes.

On the other hand, the splitting of complement in the fixation

reaction seems far more complicated than that caused by the physical or chemical procedures. The supernatant fluid from the fixation test differs from all the other end-pieces prepared by chemical methods in being active upon persensitized sheep corpuscles only (not upon human corpuscles). The addition of various mid-pieces, obtained by different methods, to sensitized sheep corpuscles does not render the Wassermann supernatant fluid active. It is quite remarkable that the persensitized sheep corpuscles are, on the other hand, easily attacked, not only by the supernatant fluids of fixation tests, but also equally well by the other end-pieces. It is not at all improbable that in the fixation reaction, where so many factors come into play, there is a most complicated physical as well as chemical interaction leading to such an entangled mixture of factors that a substance carrying one set of ions alone cannot reverse the activity of complement, and hence the reversion takes place only when certain electrolytes with both ions are employed. At all events there seems to be no doubt that the inactivation of complement is far more complicated in the Wassermann reaction or the Bordet-Gengou phenomenon than in the inactivation by physical or chemical means. Nevertheless, no one has as yet proved conclusively that the supernatant fluid of a fixation test necessarily contains the end-piece of complement.

18. Notes on the chemical nature of Lloyd's "tannin mass." ERNEST D. CLARK. Chemical studies were made upon "tannin masses" prepared by Lloyd from the fruit of the persimmon. The original material dissolved in alkalies to form a purple jelly-like solution. In dilute mineral acid solutions the "masses" turned bright red in color and no swelling was observed. Upon hydrolysis with 0.2 per cent. and 2.0 per cent. hydrochloric acid solutions, cherry-red colorations were obtained. Such solutions contained both tannin and phloroglucin in considerable proportions. The presence of phenolic substances like vanillin was also indicated. An insoluble gelatinous substance was removed, by filtration, from the hydrolyzed acid mixture and seemed to be cellulose or a related material. Hydrolysis with 0.5 per cent. and 5.0 per cent. sodium hydroxid solutions gave thick, dark-colored liquids and large amounts of insoluble gelatinous residue. Alkaline hydrolysis pro-

duced the same kinds of materials as those that resulted from acid hydrolysis. The "tannin masses" seem to be combinations of tannin and phloroglucin associated with cellulose-like substances. With ferric chlorid, phloroglucin gives a dark blue product but not the blackish precipitate characteristic of the tannin-ferric chlorid reaction. Therefore, "iron reagents" do not detect tannin in the presence of phloroglucin.

19. **A study of some protein compounds.** WALTER H. EDDY. (*Published in full in this issue of the* BIOCHEMICAL BULLETIN.)[12]

20. **The preparation of thymus histon.** WALTER H. EDDY. As outlined by Bang, the properties of histon may be summarized as follows: Water-soluble, non-coagulable by heat, precipitated by ammonia in the presence of salts, precipitated from *neutral* solution by "alkaloidal reagents," produces precipitates of several soluble proteins from their aqueous solutions.

The current method of preparing thymus histon, as recommended in standard handbooks such as Abderhalden's and Oppenheimer's, may be summarized as follows: Extraction of the minced glands with water. Precipitation of the water extract by acid or calcium chlorid, and extraction of this precipitate with 0.8 per cent. hydrochloric acid solution. Precipitation of the hydrochloric acid extract with ammonium hydroxid solution, either before or after removing free hydrochloric acid by dialysis. Washing the "ammonia precipitate" free from ammonia with alcohol and ether.

Kossel, who discovered histon in goose blood, obtained it by saturating the hydrochloric acid extract with sodium chlorid. He alone calls attention to the anomaly noted in our experiments, viz., that treatment with ammonium hydroxid solution results invariably in the precipitation of a substance that is practically insoluble in water. In a series of many preparations, extending in time over a period of two years and involving materials obtained from many calves, we have come to the conclusion that the "ammonia-precipitation" of a hydrochloric acid solution of thymus histon results invariably in a water-insoluble product. Furthermore, our experiments show that of two fractions of the same hydrochloric acid solution, the fraction saturated with sodium chlorid invariably

[12] Eddy: BIOCHEMICAL BULLETIN, 1912, ii, p. 111–22.

yields a product which (when free from sodium chlorid) is water-soluble, gives all the qualitative histon tests, and contains less nitrogen than the "ammonia precipitate" from the other fraction; the "ammonia precipitate" being *water-insoluble* and apparently a very different substance. Finally, when the "sodium chlorid precipitate" of histon is dissolved in water, and the aqueous solution is treated with a few drops of ammonium hydroxid solution, a precipitate is produced which is insoluble in water. Quantitative studies now under way show marked differences in the nitrogen content of the two products.

The results suggest that "histon" as commonly prepared is an adsorption product or a salt, rather than a simple protein.

The following method is suggested as a means of obtaining water-soluble histon from thymus: Mince fresh thymus glands and extract the hash with distilled water for 24 hours (best in the cold). Precipitate the aqueous extract with acetic acid solution and extract the precipitate with 0.8 per cent. hydrochloric acid solution (after *Lilienfeld*); or add sufficient calcium chlorid to the aqueous extract to make its content of that substance 0.2 per cent. and extract the precipitate with 0.8 per cent. hydrochloric acid solution (after *Huiskamp*); or add sufficient hydrochloric acid to the aqueous extract to make its content of the acid 0.8 per cent. and let stand 24 hours (after *Kossel and Kutscher*). Filter off the hydrochloric acid extract, and either remove the free acid or precipitate the histon directly by saturation with sodium chlorid. Remove admixed sodium chlorid by dialysis. Filter the resultant salt-free solution and evaporate it to dryness at 45° C. This material, ground to a powder, may be heated to 105° C. without loss of water-solubility.

21. The influence of proteases on the swelling of collagen and fibrin particles in alkalin and acid media containing a biological electrolyte. FRANK R. ELDER AND WILLIAM J. GIES. (*Published in full in the June issue of the* BIOCHEMICAL BULLETIN.)[13]

22. A convenient form of apparatus for demonstrations of osmotic pressure exerted by lipins. WILLIAM J. GIES. The

[13] Elder and Gies: BIOCHEMICAL BULLETIN, 1912, i, pp. 540–545.

writer repeated the demonstration described on page 59.[14] Instead, however, of using a thin rubber bag in a muslin sheath, he employed a 12-inch section of ordinary bunsen-burner tubing. The rubber tube had been swollen to its maximum extension by immersion in ether for about an hour previous to its use. It was then closed at one end by the insertion of a short, tightly fitting, section of a thick glass rod, which was fastened by a ligature. After the swollen tube had been filled with olive oil and a narrow glass tube about 10 feet in length (in two sections) had been tied into the open end and held upright, the rubber-oil portion of the vertical tubular apparatus was completely immersed in ether in a tall, narrow cylinder. The oil began to rise in the tube almost immediately, and rapidly proceeded upward until the liquid emerged from the open top.

23. Some interesting properties of thymol. WILLIAM J. GIES. During the course of recent experiments on enzymes as possible factors in the development of edema,[15] we had occasion to study the effect of trypsin on elastin in ammonium hydroxid solutions containing a biological electrolyte (NaCl). To our surprise we not only failed to obtain the swelling results which we had previously observed under similar conditions,[16] but the elastin particles in use gradually became green, ultimately blue. With repeated shaking, the elastin particles were more deeply colored, and the supernatant liquid slowly became green; finally, bluish green. The color of the particles slowly diminished in intensity as the pigment accumulated in the liquid. Unlike the elastin used in the previous experiments, this product had been prepared about 10 years before. The fresh-ligament hash had been put in water and preserved there with considerable alcoholic thymol solution; later, had been put in alcohol; ultimately, had been dried and bottled. The main supply of the dry elastin *smelled strongly of thymol.*

Some of the above-mentioned green and blue ammoniacal liquids, when shaken with ether or toluene, were quickly transformed into purplish, then reddish mixtures. *The ether layer on the quiescent liquid was bright red—all green and blue had disappeared from the*

[14] Gies: BIOCHEMICAL BULLETIN, 1912, ii, p. 55.
[15] Elder and Gies: *Ibid.*, 1912, i, p. 540.
[16] Tracy and Gies: *Ibid.*, 1912, i, p. 472.

alkalin liquid underneath, which was colorless. By spontaneous evaporation, the ether extract yielded a purplish-red oily product, with a pronounced thymol odor.

When a small quantity of thymol (Kahlbaum) was mixed with 10 per cent. ammonium hydroxid solution, the liquid became greenish in about 2 hours; then gradually turned blue. Alcohol appeared to accelerate the transformation. Shaken with ether, the blue was wholly removed and a beautiful, red, ether-layer obtained. Such ether extracts yielded, by spontaneous evaporation, a purplish-red oily product, which dissolved readily in ether, toluene and alcohol, the solutions being bright red. In some cases the oily product became crystalline, due apparently to the presence of unchanged thymol (?). The red alcoholic solution was turned deeply bluish by a drop of $n/10$ sodium hydroxid solution; the red was restored by a drop of $n/10$ hydrochloric acid solution. These transformations could be elicited repeatedly in the same solution. The changes were so sharp that the material may prove to be a valuable indicator for use in the titration of alcoholic liquids. Concentrated alcoholic solutions yielded reddish white precipitates when they were diluted with water—a ready means of isolating the substance. The reddish white precipitate dissolved promptly in alcohol, ether and toluene, and formed a red solution in each case.

An *excess* of thymol, added to a green or blue ammoniacal solution in its original condition, completely changed the green or blue to red, and wholly dissolved the red material, behaving, in this respect, like toluene and ether.

These phenomena did not appear to be due to impurities in the thymol. A general survey of thymol literature has not revealed the explanation of these results, although certain inferences are suggested by several color reactions of thymol.

The chemical nature of the colored substances derived from thymol in these preliminary experiments, the possible utility of the products—their probable antiseptic, pharmacologic and other relationships, suggest numerous interesting biochemical inquiries which will be undertaken in the near future.

24. A convenient method of preparing starch that swells rapidly in water. WILLIAM J. GIES. For the purpose of study-

ing the effects of amylases on the power of starch to imbibe water (prior to hydrolytic cleavage), the writer prepared markedly hydrophylic starch in the following way: A *very thick* starch paste was speedily prepared by *rapidly* pouring a thick potato-starch suspension through muslin into *boiling* water while the latter was being vigorously stirred. The vessel containing the paste was immersed in ice water *immediately* after the last portion of starch suspension had been added. By constant stirring of both liquids, and by the maintenance of a low external temperature, the paste was speedily cooled,[17] when it was poured into, and thoroughly stirred in, a large excess of 95 per cent. alcohol. After the sedimentation of the product, and the decantation of the alcoholic liquid, the snow white material was treated with fresh portions of alcohol until its viscidity disappeared and it became firmly granular. After several washings with ether, to remove alcohol, the product was rapidly freed from ether in a current of air from an electric fan. Although somewhat hygroscopic, the material formed hard, snow-white masses which could be granulated easily in an ordinary pulverizer.

Placed in water, the particles swell very rapidly into bloated glassy forms. "Starch paste" may be made almost instantly from the product. The powder can easily be freed from its soluble carbohydrate impurities by dialysis. The material promises to be of special service in many connections. Mr. Nathan Rosenthal has undertaken a study of the effects of amylases on the swelling of material of this kind in various anti-hydrophylic media, such as dilute alcohol.

25. A study of the carbohydrates of the prickly pear and its fruits. R. F. HARE.[18] The difficulties encountered in the practical laboratory separation of the sugars from the mineral matter, mucilages, gums and dextrinoid substances have been numerous, and the operations time-consuming. Many attempts to obtain the sugars free and in crystalline form have usually resulted unsuccessfully; so that it became necessary to make the individual tests not on the sugar crystals, but on the syrups previously purified as much as possible by different methods.

[17] The operations were conducted rapidly in order to prevent undue hydrolysis. It is probable that satisfactory results can be obtained by pouring the hot paste directly into alcohol.

[18] Hare: *Dissertation,* Columbia University, 1911.

The juice of the ripe fruit contains 1.57 per cent. of *pentosans* and only traces of *galactan.* After precipitation with lead acetate, the juice gave the aniline acetate reaction for *pentose,* but none for *galactose.* The presence of *fructose* and *glucose* in considerable amounts was quite definitely established by several reactions characteristic of these sugars.

The dried *mucilage* of the prickly pear, when separated by precipitation with alcohol from a two per cent. solution, contained 15 per cent. of galactan, 31 per cent. of pentosan and 12 per cent. of ash. The mucilage in the aqueous extracts could not be separated completely from cell fragments, starch, crystals of calcium oxalate and other solid particles that caused opalescence and turbidity. A dilute solution containing 1.5 per cent. of solid matter, rendered fairly clear by repeated filtration through silk, had no effect on polarized light. This was true of all the solutions of mucilage obtained in this work, both before and after subjecting them to acid hydrolysis. Harley[19] reports having found a specific rotation of $+38°$ for *Opuntia* mucilage, but places little confidence in his own results, since the reading was made on a very dilute opalescent solution and calculated from an observed rotation of $+6$ minutes. Hydrolysis of the mucilage by digestion for several hours with 1.25 per cent. sulfuric acid solution produced a sugar that had properties similar to *arabinose.* When its osazone was formed, oily globules rose to the surface. The precipitate was darker than glucosazone, readily soluble in hot water and melted at about 160° C.

A 95 per cent. alcoholic extract of the dried stems, previously treated with ether, contained a *sugar* with specific rotations made on three separate solutions of $-6.6°$, $-8.25°$, and $-7.1°$. The osazone produced from this sugar had properties similar to those of glucosazone. These results indicate the presence of glucose and fructose in this extract.

A 60 per cent. alcoholic extract of the dried stems contained *a substance apparently intermediate in character between mucilage and sugars.* It did not reduce Fehling solution before hydrolysis, but was very readily hydrolyzed by dilute acid solutions. Alcohol stronger than 60 per cent. reprecipitated this material as a flocculent

[19] Harley: *Journal de Pharmacie,* iii, pp. 6–193.

mass, quite different in appearance and properties from the precipitate of the mucilage obtained with alcohol. The precipitate was readily soluble in water, but its solution was not mucilaginous. When hydrolyzed, it gave a plus rotation to polarized light.

The *coloring matter* can be concentrated and made into a marketable product, of value for coloring certain foods, by first removing mucilages and gums with alcohol, and precipitating the pigment from the filtrate with acetone. The pigment is evidently a *glucoside*. When separated from the juice with alcohol and acetone, and then precipitated with lead acetate, the coloring matter liberated by sulfuric acid gave a glucose-like sugar on hydrolysis. The lead salt produced by precipitating the purified pigment with lead acetate contains 61.42 per cent. of lead.

26. The relation of acapnia to shock.[20] HENRY H. JANEWAY AND WILLIAM H. WELKER. Henderson has published a number of papers on the relation of acapnia to shock. He maintains that a diminution of the normal amount of carbon dioxide in the blood to a sufficient degree, and maintained for a sufficient length of time, produces an irreparable disturbance of the normal balance of osmotic forces between the blood and the cytoplasm of the body cells, and that this disturbance leads to tissue asphyxia, acidosis, and fatal oligemia, accompanied by symptoms indistinguishable from shock. He believes that the essential cause of shock is acapnia. He supports this theory, not only by very thorough work on the relation of acapnia to shock from several different standpoints, but also by furnishing control experiments, as it were, in which shock is prevented by conservation of the animal's store of carbon dioxide and also by successful treatment of animals, already in a condition of shock, with injections of Ringer solution containing carbon dioxide. Whether this theory fails to stand in whole or in part, its originator deserves the greatest credit for calling attention to the possibility that harm may arise from neglect to conserve the body's store of carbon dioxide, the important functions of which, in the body, have long oxide, the important functions of which, in the organism, have long been appreciated by physiologists. This theory has been of the greatest interest to one of us because of the relation of acapnia to

[20] Some of the work was done in the Surgical Research Laboratory of the College of Physicians and Surgeons.

artificial respiration, and to the production of shock in connection with intrathoracic surgery. It has prompted us to investigate the degree of acapnia and the associated shock produced by excessive artificial respiration.

We soon found that the diminution of carbon dioxide in the blood in ordinary intrathoracic insufflation was negligible. On the other hand, it has been quite an easy matter for us to reduce the amount of carbon dioxide in the blood to from one-third to one-half the normal amount by forced rapid inflation and deflation of the lungs. The artificial respiration was performed 45 to 90 times a minute and was continued for periods varying from 30 minutes to 3 hours. These experiments have differed from those of Henderson in that the animals were allowed to recover. The trachea was not divided but respiration was performed by inserting a large, rather tightly fitting, tube through the larynx into the uninjured respiratory tract. The blood pressures in our experiments were not accurately measured, the animals being left as nearly normal as possible after the operations. The degree of shock was estimated entirely from the condition of the animals after the operation and the manner in which they recovered from it. Judged in this manner there was nothing about these animals to indicate a serious degree of shock or any greater disturbance than could be accounted for by *three other factors* to which we desire to call attention in connection with these experiments and which, unless guarded against, can alone cause considerable depression and even death.

(1) In all experiments in which excessive artificial respiration is employed there is a great reduction in the animal's body heat. The temperature can easily fall to 85° F. (2) There is a very evident possibility (which we believe to be a fact) that the rapid and complete filling of the lungs exercises a definite interference with the return of the blood to the heart. The fall of the blood pressure, as estimated with the finger, and the rapidity of the heart's action coincide closely with the pressures used to inflate the lungs; indeed, a scarcely perceptible pulse may be immediately improved by slightly lowering the latter pressures. (3) The duration of the apnea following these experiments depends as much upon the amount of morphin and ether administered as upon any other factor. We do not

believe that it is possible to produce death by apnea, caused in turn by acapnia, without the assistance of the toxic effects of morphin and ether. The toxic effects of these drugs must be included as factors contributing to the shock.

This report deals with only one of the phases of the relation of acapnia to shock, namely the relation of acapnia, produced by excessive artificial respiration, to shock; and as it is only a *preliminary report,* it is not intended as an answer to Henderson's contention. Its purpose is mainly to record two general facts: (A) That we have reduced the amount of carbon dioxide in the blood to nearly 40 per cent. of the normal amount, and have maintained this reduction for a period of 3 hours, without producing symptoms of shock; and (B) that there are other factors than depletion of the store of carbon dioxid, which, unless properly guarded against, can in themselves cause the death of the animal under experimentation.

27. Biochemical studies of sulfocyanate.[21] MAX KAHN. **A.** The ferric chlorid colorimetric test for sulfocyanate in saliva is inexact and unreliable. A negative result by the Bunting suction method is no evidence of the absence of sulfocyanate but a positive result is suggestive of the presence of a comparatively large amount. The pink color spontaneously disappears from the ethereal layer in positive tests by the Bunting suction method. Various medicinal substances, and also certain compounds that result from biological transformations of proteins and carbohydrates, if excreted in the saliva, give a very marked red coloration in the ferric chlorid test, similar to that produced by sulfocyanate.

B. Sulfocyanate occurs in the saliva and salivary glands of man, in the salivary glands of oxen, but apparently not in the salivary glands of dogs. It occurs in the blood, but the spleen, the pancreas, the thymus, the thyroid and the testicles of dogs do not contain it. The liver seems to be the gland in the body that contains most sulfocyanate, which is also present in bile and in the small intestines. The stomach contents of dogs on an ordinary diet were free from sulfocyanate. When, however, sodium sulfid was given, the gastric mixture contained sulfocyanate.

[21] Kahn: *Dissertation,* Columbia University, 1912. Conducted under the auspices of the Dental Society of the State of New York.

C. Sulfocyanate is excreted in the urine and feces. Its elimination in the urine is not dependent upon the amount in the saliva. Although dog saliva is apparently always free from sulfocyanate, dog urine invariably contains it. The ingestion of amino acids (alanin) and of nitriles (acetonitrile) increases the amount of sulfocyanate in the body, as well as in the excreta. Sulfocyanate seems to be produced in the body from protein. Results with a fasting dog harmonize with this conclusion. The ingestion of sulfur, sodium sulfid, thioacetic acid, thiourea and taurin did not increase the output of sulfocyanate.

D. Potassium sulfocyanate is toxic to both plants and animals. Its toxicity is so marked that indiscriminate dispensation of the substance to people is dangerous. The growth of molds is enhanced by potassium sulfocyanate. Yeast fermentation is not affected or is stimulated by moderate proportions of potassium sulfocyanate. Biological proportions of potassium sulfocyanate have no inhibiting influence on the growth of bacteria. The souring of milk is inhibited by large proportions of sulfocyanate.

28. The chemical constitution of renal calculi. MAX KAHN. Sixteen stones of nephric origin were analysed according to the method of Mackarell, Moore and Thomas.[22] Most of the stones were composed mainly of salts of calcium. All of the stones contained uric acid or urates in varying amounts, but no stone was wholly composed of urates. The shape, color and consistency of a stone are not criteria of its chemical composition. Three gouty tophi were examined by the murexid test for urates. A negative response was obtained in each case, showing that not all gouty deposits are composed of uric acid salts.

29. The colloidal nitrogen in urine from a dog with a tumor of the breast. MAX KAHN AND JACOB ROSENBLOOM. (*Published in full in this issue of the* BIOCHEMICAL BULLETIN).[23]

30. A non-protein, colloidal, nitrogenous substance in milk. MAX KAHN AND FREDERIC G. GOODRIDGE. Since the figure obtained for "total" nitrogen in milk exceeds the sum of the values for the known nitrogenous constituents, unknown nitrogenous sub-

[22] Mackarell, Moore and Thomas: *Bio-Chemical Journal,* 1910, iv, p. 179.
[23] Kahn and Rosenbloom: BIOCHEMICAL BULLETIN, 1912, ii, p. 87.

stance must be present. The urines of man and dog contain colloidal nitrogenous material.[24] It was thought probable that such material is present in all the secretions.

After a careful process, including the removal of protein without hydrolysis, substance was obtained from milk which is white, amorphous, odorless and tasteless; insoluble in the lipin solvents, but forms in water an opalescent solution which fails to flocculate on boiling. This material does not respond to any of the protein "color tests." It contained about 5.3 per cent. nitrogen; also carbon, hydrogen, oxygen, and sulfur, but no loosely combined ammonia radicals.

31. A biochemical test for free acid, with a review of the methods for estimating the various factors in gastric acidity.[25] JOHN L. KANTOR. The author presented details along the lines of our original publication on this subject.[26] The test is a microscopic one and depends upon the immediate expansion of moist collagen fibrils when they are immersed in aqueous solutions containing free organic or mineral acids of the kinds that ordinarily appear in gastric contents. "Combined" acid[27] and acid salts fail to induce such effects. The test may be satisfactorily conducted with a drop of liquid and a single collagen fibril.

Comparative observations indicate that for free *mineral* acid (HCl) the collagen-fibril test is equal in delicacy to the Töpfer and Günzberg tests, but that for free *organic* acid (lactic), or for mixtures of free mineral and organic acids, it is more delicate than the latter tests. Comparative studies of common factors of interference with the several tests indicate that the collagen-fibril test exhibits the greater delicacy. The *color* of the solution under examination had no effect on the test. Further details from the clinical standpoint, and an abstract of the historical discussion, will be published at an early date.

32. A study of modifications of the biuret reagent. MARGUERITE T. LEE. This investigation was made in the endeavor to

[24] Kahn and Rosenbloom: BIOCHEMICAL BULLETIN, 1912, ii, p. 87.

[25] Kantor: *Dissertation,* Columbia University, 1912.

[26] Kantor and Gies: *Proceedings of the American Society of Biological Chemists,* 1911, ii, p. 20; *Journal of Biological Chemistry,* 1911, ix, p. xxvi.

[27] Goodridge and Gies: *Proceedings of the Society for Experimental Biology and Medicine,* 1911, viii, p. 107.

discover, if possible, a more effective alkali for the biuret reagent than the standard sodium hydroxid—or a *combination* of alkalies that might be better.

Fairly strong solutions of the following alkalies, when substituted for sodium hydroxid in the biuret reagent,[28] yield solutions that give the biuret test when they are added to dilute solutions of Witte peptone: potassium hydroxid, ammonium hydroxid, calcium hydroxid, sodium carbonate, conin, piperidin, ethylene di-amin, trimethyl amin, piperazin, and tetra-ethyl ammonium hydroxid.

Sodium hydroxid, potassium hydroxid, ammonium hydroxid, trimethyl amin, and tetra-ethyl ammonium hydroxid are excellent as alkalies in the biuret reagent. Tri-methyl amin appears to be more effective than sodium hydroxid. Tetra-ethyl ammonium hydroxid is seemingly as effective as sodium hydroxid when the reagent is fresh, but the efficiency of the solution decreases on standing. Piperazin and tetra-ethyl ammonium hydroxid give most satisfactory tests when an excess of copper is present. There is apparently an optimum amount of copper (sulfate) for each alkali. The study is in progress.

33. **A chemical study of salivary mucin.** Alfred P. Lothrop. Salivary mucin from the submaxillary glands of oxen was prepared by the Hammarsten-Levene method. It is a white powder, insoluble in water, acid in reaction and readily soluble in dilute alkalin solutions.

The sodium salt can be prepared by dissolving mucin in nine parts of 0.5 per cent. sodium bicarbonate solution plus one part of 0.5 per cent. sodium carbonate solution. The thick solution is then dialysed until it no longer reacts alkalin to phenolthalein but is still alkalin to litmus. (Prolonged dialysis completely hydrolyses the salt and precipitates the mucin.) The dialysed solution may be precipitated by the addition of about six volumes of alcohol, although electrolyte (NaCl) must be present for complete flocculation. The product, washed with alcohol and ether, dries to a fine powder.

[28] Gies: *Proceedings of the American Society of Biological Chemists,* 1910, i, p. 273; *Journal of Biological Chemistry,* 1910, vii, p. lx. Also, Kantor and Gies: Biochemical Bulletin, 1912, i, p. 264.

The salt, having an ash content of 2.7–3.3 per cent., is completely soluble in water. A 0.2 per cent. solution is very much like a relatively thick natural saliva. The solution is faintly alkalin to litmus, gives all the usual protein tests, including the Molisch test for the carbohydrate group, and is precipitated in stringy masses by acetic acid.

Quantitative determinations of nitrogen and ash in mucin preparation III and its sodium salt gave the following typical results:

	Ash	*Nitrogen*	
	Per Cent.	Found Per Cent.	Calculated (Ash Free) Per Cent.
Preparation III	0.28	12.49	12.53
Sodium salt III	3.27	12.20	12.61

The potassium salt was prepared in the same manner. Frequent reprecipitations by alcohol render the salts decreasingly soluble in water.

These products have been made preparatory to experiments on the possible relation of salivary mucin to dental caries, in continuance of our studies under the auspices of the Section on Stomatology and Research, of the First District Dental Society, State of New York.

34. A study of some of the more important biochemical tests.[29] C. A. MATHEWSON. Representative substances from the following groups were studied in their influence on the tests named below: neutral inorganic salts, neutral organic compounds, acids, acid salts, bases, basic salts, biological mixtures and miscellaneous materials. Over seventy-five substances or products were used in each case. It was found that the ten tests under examination could be arranged in the following sequence according to the *percentage* of factors causing interference with them: Sudan III, 0; xanthoproteic, 4; Hopkins-Cole, 4; Seliwanoff, 5; Molisch, 6.5; iodine, (for starch) 6.5; Fehling-Benedict, 10; biuret, 13; Millon, 22; Barfoed, 60.

The acid salts were the most potent interfering substances, the neutral organic compounds the least potent. Of the salts, ferric chlorid was the most active agent of interference. An extension of the study is in progress.

[29] Mathewson: *Dissertation,* Columbia University, 1912.

35. **A quantitative study of the lipins of bile obtained from a patient with a biliary fistula.** JACOB ROSENBLOOM. Through the kindness of Dr. William Weinberger, of the Lebanon Hospital, there was placed at my disposal 3180 c.c. of human bile obtained from a patient with a biliary fistula. The fluid had the appearance of typical human bile. Its specific gravity was 1.020. The following data were obtained in a quantitative lipin analysis, the results being expressed in parts *per thousand:* Water, 970.2; total solids, 29.8; cholesterol, 2.61; lecithans, 6.42; fat, 6.85; fatty acids, 1.2; soaps, 2.6. Total lipins, 19.68 (1.97 per cent.).

36. **Effects of intraperitoneal injections of epinephrin on the partition of nitrogen in urine from a dog.** JACOB ROSENBLOOM AND WILLIAM WEINBERGER. (*Published in full in this issue of the* BIOCHEMICAL BULLETIN.)[30]

37. **A case of allergy to common foods.**[31] OSCAR M. SCHLOSS. In a boy now 8 years old marked urticarial lesions were caused by the ingestion of eggs, almonds and oatmeal. The idiosyncrasy to egg was not congenital but was acquired at some time between the ages of 10 days and 14 months. Symptoms due to the ingestion of oats appeared some time after the child had first eaten oatmeal when he was 22 months old. As far as can be ascertained, the idiosyncrasy to almonds was manifested the first time this food was eaten.

It was found that cutaneous inoculation of these and certain related food substances produced an urticarial wheal at the site of inoculation. The cutaneous reaction was produced only by the protein constituents of eggs, almonds and oats. Different proteins from the same source varied in activity, some being incapable of causing a reaction. Some of the active proteins caused urticaria by mere contact with the unbroken skin. It was possible passively to sensitize guinea-pigs to ovo-mucoid (one of the active proteins from eggs) by intraperitoneal injections of the patient's blood-serum. By feeding ovo-mucoid, in gradually increasing doses, the patient became immune to egg. At the same time immunity to oatmeal and an apparently decreased susceptibility to almonds occurred.

[30] Rosenbloom and Weinberger: BIOCHEMICAL BULLETIN, 1912, ii, p. 123.
[31] Schloss: *American Journal of Diseases of Children,* 1912, iii, p. 341.

38. The comparative enzyme content of green and variegated leaves of Tradescantia.[32] CARL A. SCHWARZE. The results of the experiments made to determine the relative enzyme content of green and variegated leaves of *Tradescantia* show that there is a marked difference between juices expressed from them. Etiolated leaves are yellow in their rudimentary stage; that is, an entirely yellow leaf presents this condition when first formed. The etiolated leaves are free from chloroplasts and therefore possess no starch. The juice extracted from yellow leaves gives a negative Fehling test; that from green portions, a positive test. When yellow leaves are ground in a mortar, and the juice is expressed through cheese cloth, a dark brown liquid results. Green leaves similarly treated yield a dark green liquid. Alcoholic extracts of crushed green and yellow leaves, when filtered, assume a brown color. The filtrate from yellow leaves is at first pink but the liquid gradually assumes a brown color. The filtrate from the green leaves comes through brown immediately. The juice of yellow and green leaves, when filtered, gives in both cases a brown filtrate, that from the yellow leaves being a reddish brown. When unfiltered green juice desiccates, a glossy dark green residue is deposited, at the periphery of which a few needle-shaped crystals are seen. The juice from the yellow leaves, upon desiccation, deposits a brown crystallin mass, the long crystals of which make a figure which resembles a polyaster seen in plant cells. Extracts in alcohol (80 per cent.) deposit the greatest amount of crystals. The crystals from yellow leaves are darker than those from green leaves.

Such reagents as guaiac and trikresol show the presence of oxidase and peroxidase in yellow and green juices. The yellow juice seems to be richer in oxidase and peroxidase. When green juice was heated to 72° C., and tested the following day, oxidase proved to be present, that temperature having failed to destroy it. (Subjecting green juice to high temperatures results in the production of a flocculent precipitate, which sediments promptly under a clear supernatant liquid.)

Juice from yellow leaves was injected into the nodes and inter-

[32] Conducted in the Botanical Laboratory under Dr. Gies' guidance.

nodes of healthy green *Tradescantia* stems. No discoloration or yellowing of the injected stem could be detected.

39. Biochemical studies of beryllium sulfate.[33] EMILY C. SEAMAN. The experiments with beryllium sulfate have shown very conclusively that the substance has a marked effect on biochemical processes. When administered with the food it produced in dogs decided nutritive disturbances, which manifested themselves in *loss* of body weight, total inorganic matter, nitrogen, sulfur and phosphorus. When large doses were administered *per os* the substance caused vomiting before a sufficient amount was absorbed to produce any other obvious toxic symptoms.

When the calculated lethal dose was administered by a single *subcutaneous injection,* the substance produced edema and necrosis of the tissue extending over a large area. No other decided symptoms were produced by this method.

Very gradual *intravenous injections* of the salt produced decided toxic effect. The action of the heart became irregular—unusually rapid and very weak: the respiration also became irregular and shallow. During the course of the injection, there was decided tremor but this disappeared soon after the operation. As a direct effect of the injection the temperature increased, sometimes to 105° F., but about 24 hours before the death of the animal the temperature began to decrease and steadily fell. After intravenous injections there was increased elimination of urine followed by retention. The feces became diarrheal and bloody. Vomiting began about the time the dog refused food or water.

Beryllium sulfate had a decided inhibitory effect on the action of ptyalin, pepsin, and trypsin. It also retarded the action of sucrase but not to so great an extent. Solutions of the salt (1 per cent. or less) did not precipitate proteins from neutral or acid solutions. Below the concentration of M/512 solution, beryllium sulfate did not inhibit the growth of lupin or timothy seedlings, but more concentrated solutions prevented growth. When present in proportions less than 0.5 per cent., beryllium sulfate had very little, if any, bactericidal action.

40. Chemical changes in fish during long periods of cold storage. CLAYTON S. SMITH. Fresh fish were delivered directly

[33] Seaman: *Dissertation,* Columbia University, 1912.

from the boat. Specimens of the same catch were immediately placed in storage and delivered to us, at intervals, in a frozen state, when they were thawed under uniform conditions and promptly subjected to analysis.

Comparative data were obtained regarding moisture, organic matter, inorganic matter, and total solids; ammonia nitrogen, soluble nitrogen, insoluble nitrogen, coagulable nitrogen, non-coagulable nitrogen and total nitrogen; "proteose" nitrogen, both before and after autolysis; fat content and fatty-acid number; and the reducing power of the aqueous protein-free extract, as determined by the Benedict method.

The flesh of fish which had been refrigerated not less than four months and not more than six months was unaltered in composition. After a period of nine months in cold storage there was a slight, *almost imperceptible,* increase in the content of ammonia nitrogen, but no other change was noted. The work is in progress.

41. An attempt to sharpen the end point in Benedict's method for the quantitative determination of sugar in urine. WILLIAM WEINBERGER. In Benedict's modification of Fehling's sugar titration method "instead of the reduced copper being precipitated as the red sub-oxid, which of its own color obscures the end point of the reaction, the copper is precipitated as cuprous sulfocyanate, a snow white compound, which is rather an aid than a hindrance to accurate observation of the disappearance of the last trace of blue color." However, in applying Benedict's method to urine of low sugar content (below 0.5 per cent., as it frequently occurs in cases of glycosuria), one is struck by the fact that the blue color of the mixture does not persist until the reaction is ended, for the contents of the porcelain dish assume a dirty brownish-green hue that gradually merges into brown. This renders the correct estimation of the end point very difficult if not impossible.

Clarifying the urine by the addition of lead acetate previous to the titration might overcome the difficulty, but this procedure would require additional manipulations and calculations; and there is also the danger of a chemical change in the copper solution. None of these objections apply to the simple method proposed by the author. It consists in the addition, just before heating, of approximately 10

grams (two heaping teaspoonsful) of powdered calcium carbonate to the contents of the porcelain dish (25 c.c. of Benedict's solution, 5–10 grams of anhydrous sodium carbonate, and a small amount of powdered pumice). The titration is then made in the usual manner.

The snow white calcium carbonate, insoluble and suspended in the alkalin solution, appears to act like the copper sulfocyanate in that it effectively obliterates all colors except the blue color of the copper solution. The end point obtained is sharp, the blue color being visible up to the addition of the last two drops of urine that are necessary for complete reduction. A sufficient amount of calcium carbonate (10 grams) must be added, otherwise the precipitate will be gray and the end point less distinct. In order to prevent sudden ebullition of the concentrated solution, it is advisable to dilute the latter with a little distilled water. Experiments have shown that the addition of the calcium carbonate does not introduce any noticeable error.

The author demonstrated these facts.

42. Diffusibility of protein through rubber membranes, with a note on the disintegration of collodion membranes by common ethyl ether and other solvents. WILLIAM H. WELKER. (*Published in full in this issue of the* BIOCHEMICAL BULLETIN.)[34]

43. A further study of the Bardach test for protein. CHARLES WEISMAN. (*Published in full in the June issue of the* BIOCHEMICAL BULLETIN).[35]

44. A study of the surface tension of dog blood-serum by the drop-weight method.[36] HAROLD E. WOODWARD. These experiments, about twenty in number, were planned to answer the question whether ordinary variations in the blood supply and nutritive condition of an individual affect the surface tension of the blood.[37] Serum could be handled better than blood and serum from

[34] Welker: BIOCHEMICAL BULLETIN, 1912, ii, p. 70.

[35] Weisman: *Ibid.*, 1912, i, p. 538.

[36] The animals were fed and controlled, and the blood was withdrawn and the serum collected, by Dr. Gies and Mr. Chris Seifert. The drop-weights were made by the author in the laboratory of physical chemistry under the direction of Prof. J. L. R. Morgan.

[37] These experiments were a logical preliminary to the work described elsewhere: Woodward, *Dissertation*, Columbia University, 1912.

clotted blood was more satisfactory than serum obtained by centrifuging defibrinated blood.

The normal surface tension of dog serum (five dogs), from the blood of animals on the usual diet in metabolism experiments in this laboratory, is about 45.5 dynes per centimeter. A daily hemorrhage of 3 per cent. or more of the body weight, on two successive days, was without material effect on the surface tension.[38] Small additions of salt to the food raised, whereas additions of sugar lowered, somewhat the surface tension. The ingestion of extra quantities of meat, several hours before blood was withdrawn, caused a decrease of about 1.5 per cent. in the surface tension. Fasting (1–2 days) raised the surface tension about 1 per cent. Copious water drinking (2 hours before withdrawal of blood) and the administration of magnesium sulfate, with resultant marked diarrhea (a short time prior to removal of blood from another dog), were without appreciable effect on the surface tension of the serum. These results suggest that the nutritive state of a given individual must be definitely established before accurate conclusions can be drawn regarding the significance of data for surface tension of the subject's blood (or serum).

[The December issue of the BIOCHEMICAL BULLETIN will present abstracts of the scientific communications at the meeting of the Biochemical Association to be held on December 6, at the Columbia Medical School.]

Biochemical Laboratory of Columbia University,
College of Physicians and Surgeons,
New York.

[38] When the second bleeding occurred in much less than 24 hours after the first, the surface tension was above normal.

BIOCHEMICAL NEWS, NOTES AND COMMENT

I. General

Necrology. Dr. W. W. Daniels, emeritus professor of chemistry at the University of Wisconsin.—Thomas Doliber, president of Mellin's Food Co., and one of the best known manufacturing druggists in America.—Dr. Morris Loeb, professor of chemistry at New York University and president of the Chemists' Club.—Dr. Hermann Munk, formerly professor of physiology at the veterinary college in Berlin.—Dr. E. A. Holmström, Sweden's foremost pharmacist.—Dr. Edmund von Neusser, professor of internal medicine at Vienna.—Prof. Melville Amasa Scovell, director of the Kentucky Agricultural Experiment Station and dean of the College of Agriculture of the Kentucky State University.—Dr. Henry Adam Weber, professor of agricultural chemistry, Ohio State University.—Dr. Thomas Winter, professor of agriculture in University College of North Wales, Bangor.

In memoriam. *Lord Lister.* A memorial to Lord Lister will be established at University College Hospital. It was in 1843 that Joseph Lister entered the college as an arts student and graduated bachelor of arts in 1847. He then became a student of medicine and entered the hospital to complete his studies. A special committee has been formed under the presidency of the Duke of Bedford, president of the hospital. The exact nature of the tribute will be largely decided by the amount of the subscriptions received, but it has been suggested that either a bust or a tablet should be placed in both the hospital and the college. It is understood that the memorial will be entirely local in character, and only those who have been

in some way connected with University College or the hospital are being asked to subscribe.

The presidents of the Royal Society and the Royal College of Surgeons some weeks ago took the necessary steps for the formation of a large and representative committee for the purpose of establishing a memorial to the late Lord Lister. A meeting of the committee, which was largely attended, was held on July 22 at the rooms of the Royal Society, under the chairmanship of Sir Archibald Geikie. The following were appointed an executive committee to recommend to a future meeting of the general committee a scheme for the memorial to Lord Lister and to organize an appeal for subscriptions: The Archbishop of Canterbury, the Lord Chancellor, Lords Iveagh, Rayleigh, Rothschild and Alverstone, the dean of Westminster, the Lord Mayor, the Lord Provosts of Edinburgh and Glasgow, the Master of the Rolls, Mr. Lewis Harcourt, M.P., Sir T. Barlow, Sir W. W. Cheyne, Sir R. J. Godlee, Sir H. Morris, Sir A. Geikie, Sir D. MacAlister, the Hon. Sir C. Parsons, Sir W. Turner, Sir J. Wolfe-Barry, Sir J. R. Bradford, Sir A. P. Gould, Sir A. Kempe, the Hon. W. F. D. Smith, Mr. F. M. Fry and Mr. Edmund Owen. Lord Rothschild and Sir W. W. Cheyne were appointed treasurers and Sir J. R. Bradford was appointed secretary of the Lister Memorial Committee.

Dr. Paul C. Freer. The Bureau of Science of the Philippine Government has adopted resolutions in memory of Dr. Paul C. Freer, director of scientific work in the bureau, who died last April. The resolutions express the sense of his associates that "the Bureau of Science has suffered a very great loss and that the cause of science in the Philippine islands has been deprived of one of its most zealous and conscientious advocates."

Anniversary celebrations. *June 30:* Professor Gad, formerly director of the Physiological Institute at Graz, a pupil of Du Bois-Reymond, celebrated his seventieth birthday.—*July 1:* Prof. Carl Binz, formerly director of the Pharmacological Institute at Bonn, celebrated his eightieth birthday.—*August 3:* Professor Bernstein, formerly director of the Institute of Physiology at Halle, celebrated the fiftieth anniversary of his doctorate.—*September 14:* Prof. W.

O. von Leube, the distinguished clinician, celebrated his seventieth birthday. Professor Leube has been living at Stuttgart since last year, when he resigned his directorship of the Würzburg medical clinic.

Honors. *Awards of prizes.* Dr. Alexis Carrel has been awarded the *Nobel prize* in medicine, in recognition of his achievements in the suture of blood-vessels and the transplantation of organs.—The Vienna Academy of Sciences has conferred its *Lieben prize* for 1912 on Dr. Oswald Richter for his work on the food of algæ.

Honorary degree. The University of St. Andrews, Dundee, Scotland, has conferred the degree of LL.D. on Dr. S. J. Meltzer.

Foreign associates. Sir William Ramsay and J. Reverdin have recently been elected foreign associates of the Paris Académie de Medécine.

Retirements, resignations and appointments. *Retirements.* Col. *Martin V. Calvin,* for the past six years director of the Georgia Agricultural Experiment Station.—Prof. *H. J. Wheeler,* former acting-president of the Rhode Island State College, at Kingston, R. I., and, during the past eleven years, director of the government agricultural experiment station at that institution.

Leave of absence. Dr. *W. P. Bradley,* professor of chemistry at Wesleyan University, has been granted leave of absence for the year 1912–13, to organize a department of research for the United States Rubber Goods Company.—Dr. *A. F. Blakeslee* has a year's leave of absence from the Connecticut Agricultural College. He has a temporary appointment on the staff of the Carnegie Station for Experimental Evolution at Cold Spring Harbor, L. I., where he will study lower fungi.

Appointments have lately been announced, as follows:[1]

Bryn Mawr College: Dr. *Don R. Joseph* (associate in physiology and pharmacology at the Rockefeller Institute), associate professor of physiology.

Carnegie Institution, Boston Nutrition Laboratory: Mr. *Joseph C. Bock* (instructor in chemistry at Michigan Agricultural College), chemist.

[1] In the appended summary, institutions from which *resignations* occurred are named in parenthesis.

College of Agriculture and Mechanic Arts (Mayaguez, P. R.): Dr. *B. E. Ray* (N. C. Experiment Station and College of Agriculture), professor of chemistry.

Columbia University: Mr. *Ernest L. Scott* (University of Kansas), instructor in physiology; Dr. *Otto von Huffman* (Cincinnati Hospital and Ohio Miami Medical College), instructor in clinical pathology.

Commission for the study and prevention of malaria in the South: Dr. *William S. Thayer,* member.

English Government Laboratory, London: Mr. *E. Grant Hooper,* deputy-government chemist (promoted), vice Mr. H. W. Davis, retired.

Hamburg Botanical Institute: Dr. *Hans Winkler* (associate professor of botany at Tübingen), director.

Harvard University: Dr. *Geo. R. Lyman* (assistant professor of botany in Dartmouth College) will take the work of Professor Roland Thaxter during a sabbatical leave of absence.

Institute for experimental research on cancer, established by the Kaiser Wilhelm Society for the promotion of science: Prof. *A. von Wassermann,* director.

Margaret Morrison School for Women of the Carnegie Institute (Pittsburgh): Miss *Mary D. MacKenzie* (professor of biology at Western College, Oxford, Ohio), head of the department of biology.

McGill University (Montreal): Prof. *Francis E. Lloyd* (professor of botany in the Alabama Polytechnic Institute and plant physiologist to the Alabama Experiment Station), MacDonald professor of botany; Dr. *F. R. Miller,* lecturer in physiology.

Medico-Chirurgical College (Philadelphia): Dr. *H. Lowenberg,* assistant professor of infantile dietetics and also pediatrist to Mount Sinai Hospital, succeeding the late Dr. Edwin Rosenthal.

Munich medical clinics: Prof. *Friedrich Müller,* instead of retaining the second clinic, has taken the first, left vacant by the death of Professor Bauer; Prof. *E. v. Romberg* (Tübingen) succeeds Professor Müller.

N. Y. State Food Laboratory (Ithaca): Mr. *J. T. Cusick* (assistant in nutrition investigations, N. Y. Agricultural Experiment Station), analyst.

N. Y. State School of Agriculture (Alfred University at Alfred): Prof. *W. J. Wright* (Pennsylvania State College), director.

N. C. Agricultural Experiment Station (West Raleigh): Dr. *Joseph F. Brewster,* chemist.

Ohio State University: Dr. *W. G. Stover* (Oklahoma Agricultural Experiment Station), assistant professor of botany.

Ontario Agricultural College: Mr. *R. E. Stone,* lecturer in the botanical department.

Pennsylvania Chestnut-Tree Blight Commission: Dr. *F. D. Heald* (professor of botany in the University of Texas), pathologist; Miss *Caroline Rumbold* (Missouri Botanical Garden), physiologist in charge of tree medication; Mr. *Joseph Shrawder,* chemist.

Reed College (Portland, Oregon): Dr. *Harry Beal Torrey* (associate professor of zoology in the University of California), professor of biology.

Skin and Cancer Hospital of Maryland: Mr. *J. M. Codd,* chemist.

State University of Oregon Medical College (Portland): *John M. Connolly,* Ph.D., M.D. (Harvard Medical School), professor of physiological chemistry.

U. S. Bureau of Animal Industry: Dr. *Frederick J. Birchard* (assistant in chemistry at the Rockefeller Institute), research chemist in the Dairy Division.

U. S. Bureau of Mines (Pittsburgh): Dr. *I. K. Phelps* (U. S. Bureau of Chemistry, Washington, D. C.), chemist.

U. S. Bureau of Plant Industry: Dr. *R. Kent Beattie* (professor of botany in the State College of Washington), expert in the office of forest pathology; Dr. *Neil E. Stevens* (assistant pathologist in Kansas Experiment Station), forest pathologist.

University College, Reading: Dr. *S. M. T. Auld* (lecturer in the chemical department of the Southeastern Agricultural College at Wye), professor of agricultural chemistry; Mr. *John Goding* (Midland Agricultural College), research chemist in dairying.

University of Bonn: Professor *Johannes Fitting* (director of the State Botanical Institute at Hamburg), successor of Professor Strasburger.

University of Illinois: Dr. *J. Howard Beard,* instructor in physiology (promotion).

University of Maryland: Dr. *Isaac M. Macks,* pathologist.

University of Minnesota: Dr. *Robert B. Gibson,* assistant professor of physiological chemistry (promotion); Dr. *Rodney M. West,* assistant professor of agricultural chemistry (promotion).

University of South Dakota: Mr. *Herbert Otto Lussky* (assistant in physiology at the University of Chicago), director of the department of physiology in the college of arts and sciences and the college of medicine.

University of Vienna: Prof. *Wilhelm Türk,* temporary successor to Prof. E. von Neusser in the medical school (page 188).

Washburn College: Dr. *Edith M. Twiss,* head of the department of botany; Mr. *James P. Poole,* instructor in botany.

Washington State College (Pullman): Dr. *Ira D. Cardiff* (professor of botany in Washburn College), professor of plant physiology.

Prizes, grants, endowments and funds. *Prizes.* The College of Physicians, Philadelphia, announces that the next award of the *Alvarenga prize,* amounting to about $180, will be made July 14, 1913. Essays may be devoted to any subject in medicine but must not have been published, and should be received by May 1, 1913, by the secretary of the college, Dr. Thomas R. Neilson, 1937 Chestnut Street, who will furnish particulars, on request.—Madame Dieulafoy, widow of the late clinician, has given to the Academy of Medicine, of Paris, in memory of her husband, the sum necessary to found the *Dieulafoy prize* of $400, which will be awarded every two years to the author of the best work on the subject of internal pathology.—The *Riberi prize,* amounting to $4,000, will be awarded by the University of Turin, after the close of the year 1916, for the work which is adjudged to have most advanced the science of medicine.

Grants. Grants for research, at the recent meeting of the British Association: Mr. A. D. Hall, plant enzymes, £30; Prof. E. A. Schäfer, the ductless glands, £40; Prof. E. H. Starling, oxy-hemoglobin, £15; Prof. F. Gotch, mammalian heart, £20; Sir W. Ramsay, for the International Commission on Physical and Chemical Constants, £40.

Endowments and funds. The London School of Tropical Medicine is making an appeal for $500,000 to provide for the equipment and more efficient conduct of its work.—The late Dr. J. E. Robinson, first governor of Kansas, bequeathed $100,000 to the *University of Kansas.* The gift will be used for the medical school.—Mr. James B. Brady, of New York, has given the sum of $220,000 to the *Johns Hopkins Hospital,* for the establishment of a ward for the treatment of diseases of the kidney.—The late Mr. Allan Octavian Hume, well known as an ornithologist and botanist, lately bequeathed about £14,000 to the *South London Botanical Institute,* to which in 1907 he gave £10,000.—Under the will of the late Augustus W. Openhym, *Columbia University* will receive a third of a trust fund of

$275,000 for the endowment of research into the cause, prevention and cure of cancer. Mr. Openhym's will stipulates that if at any time further investigation of cancer is not required, the income of the fund may be used for research in any branch of medicine or surgery. The endowment under Mr. Openhym's will is to be known as the Openhym Research Fund, and the terms of the gift are substantially the same as those of the Crocker Research Fund, which amounts to $1,440,777.13.

Meetings of congresses and societies. *The Fifteenth International Congress on Hygiene and Demography* was officially opened in the Continental Memorial Hall on September 23 and continued until September 27. President Taft delivered an address at the opening exercises. The delegates numbered about 3,000, representing 33 foreign governments, every American state and territory, over 300 American cities, and leading colleges and universities and many scientific, medical and social institutions throughout the world. The congress was divided into eleven sections and four general sessions were held. President Taft was honorary president, Dr. Henry P. Walcott, of Massachusetts, was president, and Dr. John S. Fulton, of Maryland, was secretary-general, of the congress. A full account of the proceedings is given in the *Journal of the American Medical Association,* beginning at page 1207 (September 28). The proceedings of the biochemical section—"dietetic hygiene; hygienic physiology"—are reported at page 129 of this issue of the BIOCHEMICAL BULLETIN.

The Eighth International Congress of Applied Chemistry was officially opened at Continental Memorial Hall, in Washington, on September 4, and continued in New York from September 6–13, inclusive, where the work was centralized at Columbia University and the College of the City of New York. About 2,500 members were in attendance. Dr. Edward W. Morely was honorary president, Prof. William H. Nichols was president, and Dr. Bernhard G. Hesse was secretary, of the congress. The scientific work of the congress was organized in twenty-four sections. Among the general addresses was one by Prof. Gabriel Bertrand on "The part played by infinitely small quantities of chemicals in biological chemistry."

Professor W. H. Perkin delivered a lecture on "The polymerization of butadiene and isoprene," before the Sections on Organic Chemistry and India Rubber. Prof. Perkin outlined his original method of making *synthetic rubber*,[1] and then described the following new method: Take ethyl alcohol, which may be easily oxidized to acetaldehyde. This is condensed by means of potassium carbonate to aldol and the aldol can be quantitatively converted into butylidine gycol. All the yields of these reactions are practically quantitative. The butylidine glycol is then converted into a chlorid and passed over soda-lime, when practically the same product is produced as the isoprene from isoamyl chlorid and, when treated with sodium, gives even better rubber than isoprene. Professor Perkin exhibited samples of what he called the first synthetic rubber ever made (the product of Tilden).

A general review of the proceedings of the Congress will appear in the October issue of the *Journal of Industrial and Engineering Chemistry* (pages 706–719). The proceedings of the biochemical section are reported at page 150 of this issue of the BIOCHEMICAL BULLETIN.

The eighty-second annual meeting of the *British Association for the Advancement of Science,* which opened at Dundee on September 4, had a registration of 2,504 members, which is considerably larger than the average. At the opening session the President, Prof. E. A. Schäfer, delivered a notable address on the "Nature, origin and maintenance of life," which has been published in *Nature* (90: 7–19) and *Science* (36: 289–312). It was announced that Dr. J. K. Caird, of Dundee, had given £10,000 to the funds of the association. A general account of each sectional meeting will appear in *Science* (36: 446–452).

The *Royal Society* recently celebrated its 250th anniversary.

The 14th meeting of the *Australasian Association for the Advancement of Science* will be held in Melbourne in January, 1913.

Buildings and general equipment. The work of the *Herriman Dispensary* of the Brooklyn Hospital was inaugurated on July 17. The dispensary will be open daily. It is a two and one-half story

[1] BIOCHEMICAL BULLETIN, 1912, i, p. 566.

brick and marble structure and was given by Mr. William H. Herriman in memory of his wife. Mr. Herriman donated $100,000 for this purpose, $25,000 of which will be used as an endowment fund. —Messrs. Jacob H. Schiff, Sol. R. Guggenheim, Ferdinand Sulzberger and Samuel Sach have each given $50,000 to a fund for the construction of a private hospital for persons suffering from chronic diseases, to be built by the *Montefiore Home,* in the Bronx, New York City.—The Medical Faculty of the *University of Utah* is requesting the Regents of the University to ask the Legislature for a special appropriation of $25,000 for the medical school. It is not generally known that the State of Utah is doing better by its University, proportionately, than any other state, in that this institution receives 28 per cent. of the state's income in taxes. The State of Utah contains about 400,000 inhabitants.

Acts of Congress. *Public Health Service.* The following is the text of the *act of congress* concerning the Public Health Service: *Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled.* That the Public Health and Marine-Hospital Service of the United States shall hereafter be known and designated as the Public Health Service, and all laws pertaining to the Public Health and Marine-Hospital Service of the United States shall hereafter apply to the Public Health Service, and all regulations now in force, made in accordance with law for the Public Health and Marine-Hospital Service of the United States, shall apply to and remain in force as regulations of and for the Public Health Service until changed or rescinded. The Public Health Service may study and investigate the diseases of men and conditions influencing the propagation and spread thereof, including sanitation and sewage and the pollution either directly or indirectly of the navigable streams and lakes of the United States, and it may from time to time issue information in the form of publications for the use of the public.

Amendment to the food and drug act. Congress, before adjournment, passed an amendment to the food and drug act which the President has signed, making it illegal "if its package or label shall bear or contain any statement, design, or device regarding the curative or therapeutic effects of such article, or any of the ingredi-

ents or substances contained therein, which is false and fraudulent." It will be remembered that the act of 1906 declared that a drug is misbranded "the package or label of which shall bear any statement . . . which shall be false or misleading in any particular . . ."; but the supreme court, by a majority of five to three, decided that this did not refer to false statements regarding the curative effect of a drug.

Miscellaneous items. *Proposed state medical service in England.* During the recent meeting of the British Medical Association at Liverpool, a State Medical Service Association was formed under the inspiration of Dr. B. Moore, professor of biochemistry at the University of Liverpool. Prof. Moore lately produced a book entitled "The dawn of the health age," in order to demonstrate the necessity for entirely remodeling the present system of medical practice in the interests of the whole community. The object of the new association is to advocate a state medical service on the following basis: (1) the whole profession to be organized on the lines of the other state services now in existence; (2) entry to the profession to be by one state examination; (3) each member of the service to be paid an adequate salary, increasing gradually according to the length of service and position in the service, and to be entitled to a pension after a specified number of years or in case of permanent disablement; (4) members of the public to have, as far as possible, free choice of physicians, but no physician to be called on to have charge of more than a specified number of patients; (5) one of the primary objects of the state service to be to unite preventive and curative medicine; all hospitals to be nationalized and used for the purpose of consultative, operative and therapeutic work at the request of and in conjunction with the patient's own physician; (6) the services of the state physicians to be open to every one, rich or poor; (7) the state medical service to be administered by a board of health under a minister of public health with cabinet rank, assisted by expert medical advisers. This movement was started before the insurance act was passed and is quite independent of the present *impasse.* It is intended that the work of the association shall form a branch of sociologic science, and membership will be open to all prominent sociologists, whether lay or medical.

(London correspondent, *Journal of the American Medical Association,* 1912, lix, p. 663: August 10).

Detection of formaldehyde in foods. In view of the introduction of a mixture of nitrite and formaldehyde with the object of masking the reactions of the latter when used as a food preservative, the following experiments may be of interest. A sample of fresh mixture was divided into four portions and treated as follows: (1) A small amount of commercial formaldehyde solution was added; (2) small amounts of formaldehyde and sodium nitrite were added; (3) a small amount of sodium nitrite was added; (4) no addition was made. Portions of each of these were tested with Rimini's test (phenylhydrazin hydrochlorid, sodium nitroprussid and sodium hydroxid). Prompt reactions for formaldehyde were obtained in 1 and 2; negative results in 3 and 4. Other portions of the samples were tested with the well-known test for nitrite (sulfanilic acid and alphanaphthylamin). The responses of 2 and 3 were prompt and distinct. No color was produced in 1 and 4. The original mixtures were allowed to stand 24 hours at room temperature and the tests repeated with the same results as obtained at first. It seems easy, therefore, to unmask nitrite and formaldehyde in the presence of each other. Henry Leffmann. (*Journal of Industrial and Engineering Chemistry, 1912, iv, p. 626: August.*)

Journalistic. With the September number Prof. A. R. Cushny, of University College, London, becomes joint editor with Prof. John J. Abel, of Johns Hopkins University, Baltimore, of the *Journal of Pharmacology and Experimental Therapeutics.* At the same time, Sir T. Lauder Brunton, of London, Professors J. T. Cash, of Aberdeen, W. E. Dixon, of Cambridge, J. A. Gunn, of Oxford, Sir Thomas R. Fraser, of Edinburgh, J. N. Langley, of Cambridge, C. R. Marshall, of the University of St. Andrews, R. Stockman, of Glasgow, F. Ransom, of London and Dr. H. H. Dale, of London, join the board of associate editors. By this arrangement the ablest representatives of pharmacology in Great Britain unite with the American and Canadian colleagues in the conduct of the *Journal* and the publishers feel confident that it will henceforth serve as the medium of publication for the best pharmacological researches of the

english-speaking countries. (Publisher's announcement, September number, vol. iv, no. 1.)

Visiting agriculturalists. Mr. Paul Korchoof, agricultural expert, department of the Russian ministry of agriculture, and Mr. Vaseelie Yurieff, assistant director, Kharkow Central Agricultural Experiment Station, have been visiting the agricultural colleges and stations in this country.—Dr. E. B. Copeland, dean of the College of Agriculture, Los Baños, P. I., who has been visiting the United States, recently returned to the Philippines.

Parsons in Washington. Dr. Charles L. Parsons, secretary of the American Chemical Society, moved from Durham, N. H., to Washington on September 1. The headquarters of the American Chemical Society may now be addressed, Box 505, Washington, D. C.

Remsen to remain at Hopkins. Owing to the difficulty of finding a suitable occupant for the post, Dr. Ira Remsen will remain at the head of Johns Hopkins University for the ensuing session, or part of it at least.

Petroleum production in the United States, in 1911, surpassed its own record (made in 1910) by an increase of nearly 11,000,000 barrels. In 1910 the output was 209,557,248 barrels. The total production of the world also surpassed all previous records, amounting to over 345,000,000 barrels.

Johns Hopkins limits enrolment. The dean of Johns Hopkins Medical School announces that it has become necessary to limit the number of students owing to the restricted space and facilities in the various laboratories. The present enrolment is 355, the largest in the history of the school, and fifty other students were refused admission prior to the beginning of the session.

Standard rations for nutrition experiments. A conference was held at the Graduate School of Agriculture, Lansing, Mich., on July 24, to discuss the formulation of standard rations for experimental work in determining the comparative value of feed stuffs. Mr. B. H. Rawl, chief of the dairy division, U. S. Department of Agriculture, President H. J. Waters, of Kansas Agricultural College, Prof. C. H. Eckles, of Missouri Experiment Station, and other leading workers in this field were present and led the discussion.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

1. General notes

Miscellaneous items. Dr. *Carl L. Alsberg* was one of the distinguished non-resident scientists to participate, by invitation, in a series of lectures, during the late summer at Fordham University Medical College, in New York, on nervous and mental diseases.—The following members of the Association conducted investigations at Woods Hole, Mass., during the summer: Cora J. Beckwith, H. B. Goodrich, Louise H. Gregory, Charles Packard, Alwin W. Pappenheimer, Henry J. Spencer, Charles R. Stockard, Isabel Wheeler, and L. L. Woodruff.—Dr. *A. Richard Bliss* is editor-in-chief of *The Mask,* the official national organ of the Kappa Psi Fraternity.—Prof. *R. Burton-Opitz* is now in Europe, where he is spending a half-year leave of absence.

Officers of societies. Section (V) on Control of Infectious Diseases of the 15th International Congress on Hygiene and Demography (page 194): Dr. *Charles F. Bolduan,* secretary.—New York Post-Graduate Medical School and Hospital: Dr. *Arthur F. Chace,* secretary (reelected).—Section (IV) on Organic Chemistry of the 8th International Congress of Applied Chemistry (page 194) Dr. *Harry L. Fisher,* secretary.—N. Y. Entomological Society: Prof. *Raymond C. Osburn,* president.—American Association for the Study and Prevention of Infant Mortality: Dr. *Philip Van Ingen,* secretary.

Appointments. Jefferson Medical College (Philadelphia): Dr. *Philip B. Hawk* (professor of physiological chemistry, University of Illinois), professor of physiological chemistry and toxicology.—Rockefeller Institute for Medical Research: Dr. *Michael Heidelberger* (recently returned from Zürich), fellow in chemistry.—Johns Hopkins University: Dr. *John Howland* (professor of pediatrics in Washington University, St. Louis), director of the Harriet Lane Home for Invalid Children, professor of pediatrics, and physician in charge of the pediatric department of Johns Hopkins Hospital.—Cornell University Medical College, Loomis Laboratory: Miss *Jessie A. Moore* (assistant at the Rockefeller Institute for Medical Research), chemical assistant.—N. J. Agricultural Experiment Station: Mr. *Carl A. Schwarze,* assistant plant pathol-

Paul E. Howe

ogist.—Long Island Medical College: Dr. *Matthew Steel* (assistant professor of physiological chemistry, University of Missouri), assistant professor of physiological chemistry and pharmacology.—At a recent annual meeting of the Imperial Cancer Research Fund, in London, Dr. *William H. Woglom* was appointed first assistant in New York, a position maintained under the auspices of the Crocker Fund for the investigation of cancer. Dr. Woglom has returned from London, where he had been pursuing a course of study under Dr. Bashford, director of the Imperial Cancer Research Fund.

2. Proceedings of the Association.

Abstracts of the scientific proceedings of the third annual meeting (June) are published on pages 156–187 of this issue.

3. Columbia Biochemical Department.

The new Assistant Professor, Dr. Paul E. Howe, B.S., A.M., PhD.[1] MEMORANDUM WHICH WAS PRESENTED TO THE FACULTY OF MEDICINE WITH DR. HOWE'S NOMINATION TO THE ASSISTANT PROFESSORSHIP IN BIOLOGICAL CHEMISTRY.

Paul Edward Howe was born in Chicago, Illinois, on July 29, 1885. His early education was received in the public schools of Chicago, Champaign and Urbana, Illinois (1890–1901). He attended the Urbana High School (1899–1901) and spent a year (1901–'02) in the Preparatory School of the University of Illinois. At the end of a four-year course at the University of Illinois he received the degree of B.S. in Chemistry in 1906.

Since 1906 he has been a graduate student and officer at the University of Illinois, passing by promotion through the grades of scholar in chemistry in the graduate school (1906–'07), assistant chemist in the laboratory of physiological chemistry (1907–'08), assistant in physiological chemistry (1908–'10), and instructor in physiological chemistry (1910–'12).

In 1907 he received the degree of M.A.; in 1910, the degree of Ph.D. His major subject for the Ph.D. degree was physiological chemistry, with Professor P. B. Hawk; his minor subjects were physical chemistry, physiology and histology.

[1] BIOCHEMICAL BULLETIN: 1911–12, i, pp. 136, 570, 573 and 574.

Dr. Howe is a member of the American Society of Biological Chemists, American Chemical Society, American Society of Animal Nutrition, American Association for the Advancement of Science, Illinois Academy of Science, Sigma Xi, Phi Lambda Upsilon, and the Gamma Alpha Graduate Scientific Fraternity.

Dr. Howe's publications. **1907.** The electrolytic corrosion of brasses (with A. T. Lincoln and David Klein); *Journal of Physical Chemistry,* **11**, 501.

1908. Comparative tests of Spiro's and Folin's methods for the determination of ammonia and urea (with P. B. Hawk); *Proceedings of the American Society of Biological Chemists,* **1**, 104; *Journal of Biological Chemistry,* **4**, p. x.

1909. Comparative tests of Spiro's and Folin's methods for the determination of ammonia and urea (with P. B. Hawk); *Journal of Biological Chemistry,* **5**, 477.—On the preservation of feces (with T. A. Rutherford and P. B. Hawk); *Proceedings of the American Society of Biological Chemists,* **1**, 196; *Journal of Biological Chemistry,* **6**, p. xlix.

1910. On the preservation of feces (with T. A. Rutherford and P. B. Hawk); *Journal of the American Chemical Society,* **32**, 1683.—A study in repeated fasting (with P. B. Hawk); *Proceedings of the American Society of Biological Chemists,* **1**, 259; *Journal of Biological Chemistry,* **7**, p. xlvi.—Fasting studies on men and dogs (with H. A. Mattill and P. B. Hawk); *Proceedings of the American Society of Biological Chemists,* **1**, 260; *Journal of Biological Chemistry,* **7**, p. xlvii. —Nitrogen partition in repeated fasting; *Dissertation* (pp. 42), presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy (University of Illinois).

1911. On the differential leucocyte count during prolonged fasting (with P. B. Hawk); *Proceedings of the American Society of Biological Chemists,* **2**, 15; *Journal of Biological Chemistry,* **9**, p. xxi.—Fasting studies: I. Nitrogen partition and physiological resistance as influenced by repeated fasting (with P. B. Hawk); *Journal of the American Chemical Society,* **33**, 215.—Fasting studies: III. Nitrogen partition of two men through two seven-day fasts following the prolonged ingestion of a low-protein diet: Supplemented by comparative data from the subsequent feeding period (with H. A. Mattill and P. B. Hawk); *Journal of the American Chemical Society,* **33**, 568.—Fasting studies: V (Studies on water drinking: XI). Influence of an excessive water ingestion of a dog after a prolonged fast (with H. A. Mattill and P. B. Hawk); *Journal of Biological Chemistry,* **10**, 417.

1912. A metabolism study on a fasting man (with P. B. Hawk); *Proceedings of the American Society of Biological Chemists,* 2, 65; *Journal of Biological Chemistry,* 11, p. xxxi.—Hydrogen-ion concentration of fecal extracts (with P. B. Hawk); *Proceedings of the American Society of Biological Chemists,* 2, 66; *Journal of Biological Chemistry,* 11, p. xxxii.—Studies on water drinking: XIII (Fasting studies: VIII). Hydrogen-ion concentration in feces; *Journal of Biological Chemistry,* 11, 129.—A comparison of the data from two fasts each exceeding one hundred days in length and upon the same subject (with P. B. Hawk); *Proceedings of the American Physiological Society, American Journal of Physiology,* 29, p. xiv.—On the differential leucocyte count during prolonged fasting (with P. B. Hawk); *American Journal of Physiology,* 30, 174.—Fasting studies: VI. Distribution of nitrogen during a fast of 117 days (with H. A. Mattill and P. B. Hawk); *Journal of Biological Chemistry,* 11, 103.—The general aspects of fasting: Address before the Columbia University Biochemical Association, May 1, 1912; BIOCHEMICAL BULLETIN, 2, 90.—The distribution of urinary nitrogen as influenced by the ingestion of moderate and copious quantities of distilled water at meal time (with D. W. Wilson and P. B. Hawk); (in press) *Journal of the American Chemical Society,* 34, Proceedings, p. 33.—*Addendum.* The utilization of individual proteins by man as influenced by repeated fasting (with P. B. Hawk); *Proceedings of the Eighth International Congress of Applied Chemistry* (preliminary edition), 19, 145. (William J. Gies, *Secretary of the Faculty of Medicine.*)

Resignations and appointments. The following changes in the staff for the year 1912–13 were officially authorized *prior to October 1, 1912:* Dr. Paul E. Howe, assistant professor, vice Prof. Wm. H. Welker, resigned; Dr. Clayton S. Smith, instructor (promoted), vice Dr. Ernest D. Clark, appointed instructor in chemistry at the Cornell University Medical College; Dr. Frederic G. Goodridge, assistant, vice Reuben Ottenberg, resigned; Messrs. E. G. Miller, Jr., and Arthur Knudson, assistants, vice Dr. C. S. Smith (promoted) and Mr. A. R. Rose, resigned; and Misses Ethel Wickwire and Tula L. Harkey, assistants (at Teachers College), vice Mr. E. G. Miller, Jr. (promoted) and Miss Blanche Harris, resigned.

Summer session. *Courses.* Professor Gies kept the laboratory open daily throughout the summer and conducted courses (July 5–August 15) in nutrition at the College of Physicians and

Surgeons with Dr. C. S. Smith's assistance, and at Teachers' College with the aid of Dr. Emily C. Seaman and Miss B. E. Shaffer.

Investigators. The workers named below conducted research, in the biochemical laboratory at the College of Physicians and Surgeons, during all or part of the summer vacation: Louis Berman, R. J. Cook, Edward Cussler, F. R. Elder, N. B. Foster, Wm. J. Gies, Samuel Gitlow, Isidor Greenwald, W. M. Kraus, Alfred P. Lothrop, H. A. Mattill, H. O. Mosenthal, Jacob Rosenbloom, Emily C. Seaman, C. S. Smith, William Weinberger, Charles Weisman, Wm. H. Welker, Harry Wessler.

Miscellaneous notes. Professor *Gies* was vice president of the Section on Biochemistry including Pharmacology of the 8th International Congress of Applied Chemistry (page 150).—Dr. *H. O. Mosenthal* recently returned from Tübingen, where he had been working in the medical clinic under the direction of Prof. E. von Romberg.—Dr. *Jacob Rosenbloom* has resigned the affiliated position of assistant pathologist at the German Hospital.—Mr. *A. R. Rose* has lately completed the requirements for the Ph.D. degree and will be publicly examined in October. He has begun a special study of amylase with Prof. H. C. Sherman.—Mr. *Joseph Hepburn* has begun work as "university fellow" in biological chemistry.

EDITORIALS

Ernst Schulze

Ernst Schulze was one of the great pioneers in biological chemistry. He worked in an inspired way along the zone between the old zoöchemistry and the ancient phytochemistry, and achieved the distinction of removing the barriers between these two fields and uniting them in one great open biochemical territory. He brought light and understanding into large domains where darkness and doubt prevailed. His example in industry, patience, perseverance, devotion, enthusiasm, ability and productiveness has been an inspiration to biochemical workers the world over. Schulze's classical achievements and service will be forever linked with the history of fundamental developments in a great formative scientific era. His name and service will be justly remembered, as his memory will be venerated, for very many generations.

Important though unknown factors in nutrition

As the methods of chemical analysis become more delicate and refined there appears ever increasing evidence that the maintenance of health and nutrition depends not alone on the caloric values of food-stuffs and the relative proportions of nitrogen and carbon in the diet, but quite as much on other factors which we are beginning fully to appreciate. Scurvy has long been one of the indications that there are certain unappreciated factors in a normal diet, and the antiscorbutic action of vegetables and vegetable juices is strong emphasis on this point. The researches of Hart, McCollum, Steenbock and Humphrey,[1] on cattle, and of Osborne and Mendel,[2] on rats, are among the many recent studies that reveal the importance of such unknown though influential factors in their broad bearing.

The disease beriberi is a concrete example of the disturbance of such subtle influences. For a considerable time physicians in the

[1] Hart, McCollum, Steenbock and Humphrey: *University of Wisconsin Agricultural Experiment Station Research Bulletin*, No. 17 (June, 1911).

[2] Osborne and Mendel: *Carnegie Institution, Publication 156.*

Orient have believed that certain foods were responsible for this form of polyneuritis. Miura believed the noxious agent to be contained in a certain fish, which is much eaten raw; but more recently the blame has fallen on rice. It has been asserted that in the prisons of Java, beriberi occurs in one out of every forty prisoners when shelled rice is eaten; in one out of ten thousand, if the unshelled grain is used. The classical studies of Schaumann were suggested by observations of this kind. Schaumann believed that since polished rice is poor in phosphorus, beriberi is due to a deficiency of certain organic phosphorus compounds. This hypothesis had some support in the fact that materials which relieve the pain of neuritis, such as bran, are rich in phosphorus, but the later investigations of Wieland[3] cast doubt on the accuracy of these deductions, since it could not be shown that the total body-phosphorus was much influenced by feeding mice on polished rice. In this connection the researches of Fingerling,[4] and of McCollum and Halpin,[5] are suggestive, for they have shown a synthesis of organic phosphorus compounds from inorganic phosphates.

The latest contributions to the study of beriberi were made by Chamberlain and Vedder,[6] by whom it has been shown that extracts of rice-bran are effective as therapeutic agents and that these extracts contain mere traces of phosphorus. The active substance in the bran has not yet been identified, but the interesting feature disclosed by the present evidence as to the etiology of kakki is that a food stuff may contain an ingredient which is essential in order to prevent injury to the tissues by other components of such food material. Rice grain is harmless when eaten with the pericarp but, if the latter is removed by "polishing," a malady ensues which may be cured by extracts made from the pericarp. These facts present a new face to the idea of "balanced rations" and also remind us of the broad biological significance of Loeb's "balanced solutions."

N. B. F.

[3] Wieland: *Archiv für experimentelle Pathologie und Pharmakologie,* 1912, lxix, p. 293.

[4] Fingerling: *Biochemische Zeitschrift,* 1912, xxxviii, p. 448; xxxix, p. 239.

[5] McCollum and Halpin: *Journal of Biological Chemistry,* 1912, xi (Proceedings of the American Society of Biological Chemists, p. xiii).

[6] Chamberlain and Vedder: *Philippine Journal of Science,* 1912, vi, p. 251.

The coming of age of the Babcock test

In a circular with this title, Director Russell of the Wisconsin Agricultural Experiment Station[7] has recently given an interesting summary of the perfection of the Babcock quantitative test for milk-fat and the influence which it has exerted on dairy science and practice throughout the world. Milk and its numerous products play so important a rôle in the economy of the home and in the dietary of the sick that the significance of Professor Babcock's contribution cannot remain unnoticed in the annals of the medical world. The simple, yet highly accurate Babcock method of estimating the fat content of milk and cream finds daily application not only in dozens of analytic laboratories, but likewise in hundreds of creameries, in milk establishments, and even in the office of the busy practitioner of medicine, where a few inexpensive devices enable him to gauge the richness of a breast-milk or a modified milk mixture with facility.

Every pediatrist appreciates what the Babcock test means for the exigencies of practice and successful feeding. Today, twenty-two years after the introduction of this procedure which, as Ex-Governor Hoard remarked, has made dairymen more honest than the Bible because it has removed all opportunity for them to profit by any deceit, it is interesting to note that no change has been made in the essential features of the test during all this period. The technic of the operation remains the same as when the details were published by Dr. Babcock in 1890. The stimulus which it has given to scientific dairying, to the standardization and improvement of our milk-supplies, to the possibilities of rational infant-feeding, and to what these in turn involve in the direction of the public health, is scarcely appreciated by the medical profession. Director Russell has written that the Babcock test frees the dairy farmer from the fetters of past traditions, and removes him from the category of "mossbacks." The influences here referred to have in fact been even more far-reaching.

An additional feature deserves mention: No patent was taken out on either the method or the apparatus required to carry out the Babcock test. *There was no copyrighting of a name—no commer-*

[7] *University of Wisconsin Agricultural Experiment Station, Circular of Information,* No. 32, 1912.

cialism. In accord with a code of ethics now more generally recognized than at any time, the discoverer, *because of his connection with the state experiment station, gave his invention freely to the world.* We may gladly join in acknowledging our obligation to the man whom the grateful state of Wisconsin has presented a medal in recognition of "*his unselfish dedication of these inventions to the public service.*" (Editorial: *Journal of the American Medical Association,* 1912, lix, p. 544.)

Organo-therapy

The discovery and investigation of the specific secretions of the so-called ductless glands and of other organs make one of the most interesting chapters in physiology. Much has been learned concerning these secretions and their rôle in the body. These extracts, theoretically, should be of great value in the treatment of diseases in which a certain gland or glands are deficient or entirely lacking in function. But actual experience has been disappointing as a rule, for two reasons: (1) The diagnosis of insufficiency of secretion on the part of a certain gland or organ is usually most difficult; (2) and even when a correct diagnosis is made, it is rarely possible to administer the gland substances in such a way as to develop their specific activity.

A notable exception to this experience is the successful use of thyroid extract in thyroid insufficiency or myxedema. Suprarenal substance has also proved highly useful as a circulatory stimulant and hemostatic, but not for the treatment of Addison's disease. It can safely be said that the administration of gland substance from the thymus, hypophysis, ovaries, pancreas, testicles, etc., for diseases of these organs, has hitherto met with failure. Only harm can come from their promiscuous use before careful experimentation fully determines their value.

A wholesome skepticism concerning the efficiency of preparations of the *digestive enzymes* is likewise commended. After years of usage many of our best clinical observers believe that pepsin, "pancreatin" and the amylases are of little or no value. The use of secretin more recently has been similarly disappointing. The continued routine use of these preparations is due chiefly to the claims of manufacturers.

We congratulate our English confréres on the successful consummation of their plans for the formation of a biochemical society and the publication of a biochemical journal[8] under their associated control. In this country we have long derived great benefit from the meetings and activity of the American Society of Biological Chemists and are confident our English colleagues will have a similar experience. We felicitate the biochemical profession at large on this further evidence of the rapid growth in usefulness, and the prominent place of service, of biochemical art and science. The *Bio-Chemical Journal* has been highly esteemed in America, and we wish it long life and distinguished service under its new management. "Science is essentially mutualistic and *the success of one organization is the gratification of all*—the triumphs and discoveries of one are shared with the many, *and the feeling of pride in the progress of the one may be shared without loss by sister organizations.* As the discovery made in one branch of science may be the necessary foundation for the solution of some problem in another, so the contribution from one society may be the stepping stone to advancement in another. It is all hail then, greetings and felicitation—and Godspeed in the accomplishments of your future destiny."

Biochemical Society, England

The name of the writer of this note might suggest a strong partiality on his part for the incorporation into biochemical discussions, in English, of such words as "Baustein." He believes, nevertheless, that English phrases of equal force though of more abstract significance, such as "construction unit" for "Baustein," are more acceptable, especially to students receiving their introduction, in English, to the subject of protein synthesis and similar processes.

"Baustein" or "construction unit"

The foregoing remarks recall the common use, in English, of "splitting product" or "split product" as equivalents for "Spaltungsprodukt," when the substance referred to is neither "splitting nor "split," but *has resulted* from cleavage. Why not term such substances "cleavage products" in conformity with good usage in analogous relationships?

Splitting products or cleavage products

[8] Halliburton: Biochemical Bulletin, 1912, ii, p. 128.

A rare compliment

We received recently, with very great pleasure, a foreign money order for *twelve dollars* instead of *twelve shillings* in payment of Volume I of the BIOCHEMICAL BULLETIN. In view of the fact that this overpayment did not excite a desire to discontinue the subscription, we have proceeded with more enthusiasm than ever with our editorial work, in the hope that future volumes of the BULLETIN may be much more deserving of such a compliment.

X-Rays

The *doing* that makes commerce is born of the *thinking* that makes scholars.—*Ruskin.*

Perhaps the most valuable result of all education is the ability to make yourself do the thing you have to do, when it ought to be done, whether you like it or not.—*Huxley.*

The fabric of medical progress—indeed, of all progress—is woven from legitimate dreams to a greater extent than the "practical" man is wont to realize or willing to admit. *Editorial: Journal of the American Medical Association,* 1912, lix, p. 1195.

Who is it that, when years are gone by, we remember with the purest gratitude and pleasure? Not the learned or clever. But those who have had the force of character to prefer the future to the present, the good of others to their own pleasure.—*Stanley.*

A fig for yesterday's convictions! They were the cocksure beliefs of children lost in the dark. This is another day, and we've grown overnight. Do you plead the dignity of fixed opinion? It is enough for us to say: "We believed it when we affirmed it; we have learned and changed our minds."—*Ana Phylactic.*

The successful man, whether in business, in the professions and trades, or in politics, enjoys the game for its own sake. He is not a conscript in life's battles, but a volunteer. The way interests him as much as the goal. Not only the result, but the exercise of powers necessary to achieve it, gives him satisfaction.—*Al I. Phatic.*

Speaking mentalwise, overfed conceit equals the blind staggers. The easiest kind of intoxication is that which feeds upon the poisons distilled by a self-caressing imagination. Open the floodgates of self-approval and soon you won't know whether you are making good or not, for you won't be able to present an intelligent comparison of your own achievements with those of others.—*Jaun Dice.*

BOOKS RECEIVED

The BIOCHEMICAL BULLETIN will promptly acknowledge, under this heading, the receipt of all publications that may be presented to it. From time to time, selections will be made for review on pages of the volume to be appropriately indicated here. Reviews will be matter-of-fact statements of the nature and contents of the publications under consideration, and will be intended *solely to guide possible purchasers.* The wishes or expectations of publishers or donors of volumes will be disregarded, when they are incompatible with our convictions regarding the interests of our colleagues. *The size of the printed pages, in inches, is indicated in the appended notices.*

Practical physiological chemistry. *A book designed for use in courses in practical physiological chemistry in schools of medicine and of science.* By Philip B. Hawk, professor of physiological chemistry and toxicology in the Jefferson Medical College of Philadelphia. Fourth edition, revised and enlarged. Pp. 475—4½ × 8; $2.50 net. P. Blakiston's Sons & Co., Philadelphia, 1912.

The protein element in nutrition. (One of the *International Medical Monographs.*) By Major D. McCay, professor of physiology, Medical College, Calcutta. Pp. 216—4 × 7, with 8 full page portraits of human subjects; $2.00 net. Longmans, Green and Co., New York; Edward Arnold, London, 1912.

Oxidations and reductions in the animal body. (One of the *Monographs on Biochemistry.*) By H. D. Dakin, The Herter Laboratory, New York. Pp. 135—4½ × 8; $1.40 net. Longmans, Green and Co., 1912.

Researches on cellulose. III (1905–1910). By C. F. Cross and E. J. Bevan. Pp. 173—3½ × 6; $2.50 net. Longmans, Green and Co., 1912.

An introduction to the study of the protozoa, with special reference to the parasitic forms. By E. A. Minchin, professor of protozoology in the University of London. Pp. 517—4 × 7½; $6.00 net. Longmans, Green and Co., New York; Edward Arnold, London, 1912.

Studies from the Rockefeller Institute for Medical Research. Reprints: Volume xv; 1912. (48 reprints).

Collected reprints of papers. By Graham Lusk. (Researches, III; 1907–'11—11 reprints).

Studies from the Department of Physiology, Cornell University Medical College, 1911–1912. (11 reprints).

Studies from the Departments of Pathology, Bacteriology, Experimental Pathology and Experimental Therapeutics, Cornell University Medical College, 1911. (12 reprints).

Les produits biologiques médicinaux. By P. Byla and R. Delaunay. Pp. 466—3¾×6¼. Société d'éditions scientifiques et médicales, F. Gittler, Directeur, Paris, 1912.

E. Merck's Jahresbericht über Neuerungen auf den Gebieten der Pharmakotherapie und Pharmazie: 25 Jahrgang (1911). E. Merck, Chemische Fabrik, Darmstadt, 1912. Pp. 531—4 × 7, with a general index of volumes 1–25.

Optical instruments: Adam Hilger, Ltd. 75 a, Camden Road, London, 1912. (Catalogue).

OFFICERS OF THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY, 1912–1913*

OFFICIAL REGISTER, SEPT. 30, 1912

WILLIAM J. GIES: *Professor and Chairman of the Staff;* Consulting chemist, New York Botanical Garden; Pathological chemist, Bellevue Hospital; Member of the Faculties of N. Y. Teachers College and N. Y. College of Pharmacy. [B.S., Gettysburg College, 1893 and M.S., 1896; Ph.B., Yale University, 1894; Ph.D., 1897. Instructor, 1898–'02; adjunct professor, 1902–'05; professor, 1905–.]

PAUL E. HOWE: *Assistant Professor,* 1912–. [B.S., University of Illinois, 1906, A.M., 1907 and Ph.D., 1910.]

NELLIS B. FOSTER: *Associate;* Associate Physician, New York Hospital; Chemist, St. Luke's Hospital. [B.S., Amherst College, 1898; M.D., Johns Hopkins University, 1902. Instructor, 1906–'08; associate, 1908–.]

WALTER H. EDDY: *Associate and Secretary of the Staff.* [B.S., Amherst College, 1898; A.M., Columbia, 1908 and Ph.D., 1909. Assistant, 1908–'10; associate, 1910–.]

JACOB ROSENBLOOM: *Associate;* Pathological chemist, German Hospital. [B.S., University of Pittsburg, 1905; M.D. and Ph.D., Columbia, 1909. Assistant, 1909–'10; associate, 1910–.]

ALFRED P. LOTHROP: *Associate and Departmental Registrar.* [A.B., Oberlin, 1906 and A.M., 1907; Ph.D., Columbia, 1909. Assistant, 1908–'09; instructor, 1909–'12; associate, 1912–.]

HERMAN O. MOSENTHAL: *Instructor;* Assistant Attending Physician, Presbyterian Hospital; Assistant Physician, Vanderbilt Clinic; Instructor in medicine. [A.B., Columbia, 1899 and M.D., 1903. Assistant, 1908–'09; instructor, 1909–.]

EMILY C. SEAMAN: *Instructor.* [B.S., Adelphi College, 1899; A.M., Columbia, 1905 and Ph.D., 1912. Tutor, 1909–'10; instructor, 1910–.]

CLAYTON S. SMITH: *Instructor.* [B.S., Rutgers College, 1910 and M.S., 1912. Assistant, 1910–'12; instructor, 1912–.]

EDGAR G. MILLER, Jr.: *Assistant,* 1911–. [B.S., Gettysburg College, 1911.]

FREDERIC G. GOODRIDGE: *Assistant,* 1912–. [A.B., Harvard University, 1897; M.D., Columbia, 1901.]

ARTHUR KNUDSON: *Assistant,* 1912–. [A.B., University of Missouri, 1912.]

ETHEL WICKWIRE: *Assistant,* 1912–. [A.B., Tri-State College, 1909.]

TULA L. HARKEY: *Assistant,* 1912–. [A.B., Colorado College, 1909.]

CHRISTIAN SEIFERT: *Laboratory assistant,* 1898–.

STELLA WALDECK: *Recorder,* 1908–.

BLANCHE E. SHAFFER: *Laboratory assistant,* summer session, 1912.

JOSEPH S. HEPBURN: *University fellow,* 1912–'13. [A.B., Central High School, Philadelphia, 1903 and A.M., 1908; B. S., University of Pennsylvania, 1907 and M.S., 1907.]

* The work of the department was inaugurated in October, 1898, by Prof. R. H. Chittenden (lecturer and director), Dr. William J. Gies (instructor), Messrs. Alfred N. Richards and Allan C. Eustis (assistants), and Christian Seifert (laboratory assistant).

COURSES OFFERED BY THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY. 1911–1912

Courses 51 (109), 105 and 215 are given during the first half-year only. Course 101 is given during the first half-year and is repeated (102) during the second half-year. Courses 104 and 110 (52) are given only during the second half year. All other courses are conducted throughout the entire academic year. All courses not otherwise specified are given at the College of Physicians and Surgeons.

(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51 (109) ELEMENTARY ORGANIC CHEMISTRY. Introductory to courses 101, 102 and 110 (52). (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr. each section (2). Profs. Gies and Howe, Drs. Smith and Goodridge, and Messrs. Miller and Knudson.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101–102. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (Teachers College, School of Practical Arts.) L, 2 hr. R. 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Dr. Seaman and Misses Wickwire and Harkey. (This course is designated "Chemistry 51" and "Household Arts Education 125" in the Teachers College Announcement.)

This course is designated "*S*—H. A. 25" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies, Dr. Seaman and Miss Shaffer.

104. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (Teachers College, School of Practical Arts.) L, 1 hr. Prof. Gies. (This course is designated "Chemistry 52" in the Teachers College Announcement.)

110 (52). GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R. 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Dr. Smith, and Messrs. Miller and Knudson.

This course is designated "*S*—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Dr. Smith.

209–210. CHEMISTRY OF NUTRITION. (School of Pharmacy. *Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

211–212. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* L, 1 hr. Lw, 4 hr. Dr. Eddy.

213–214. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (Teachers College, School of Practical Arts.) L, 1 hr. Lw, 5 hr. Prof. Gies and Dr. Seaman. (This course is designated "Household Arts Education 127" in the Teachers College Announcement.)

215. GENERAL BIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* L, 1 hr. Lw, 7 hr. Prof. Gies, Dr. Lothrop and Messrs. Miller and Knudson.

217–218. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS AND URINARY ANALYSIS IN GENERAL. L, 1 hr. Lw, 7 hr. Profs. Gies and Howe, Dr. Lothrop, and Messrs. Miller and Hepburn.

219–220. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* L, 2 hr. Lw, 14 hr. Profs. Gies and Howe, Dr. Lothrop and Mr. Miller.

Courses in Nutrition (continued)

221–222. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies.

223–224. NUTRITION IN DISEASE. L, 1 hr. Profs. Gies and Howe, and Drs. Foster, Mosenthal and Goodridge.

225–226. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Howe, and Dr. Lothrop.

TOXICOLOGY

231–232. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. Lw, 6 hr. Prof. Gies and Mr. Miller.

BOTANY

235–236. CHEMICAL PHYSIOLOGY OF PLANTS. (New York Botanical Garden.) L, 1 hr. Lw, 5 hr. Prof. Gies.

BACTERIOLOGY

241–242. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* L, 1 hr. Lw, 7 hr. Prof. Gies.

SANITATION

105. SANITARY CHEMISTRY. (Teachers College, School of Practical Arts). L, 1 hr. Lw, 3 hr. Dr. Seaman and Miss Harkey. (This course is designated "Chemistry 57" and "Household Arts Education 129" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301–302. BIOCHEMICAL SEMINAR. 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance, in any of the departmental laboratories.

LABORATORIES FOR ADVANCED WORK IN BIOCHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College, New York Botanical Garden and Bellevue Hospital. Each laboratory is well equipped for research in nutrition and all other phases of biological chemistry.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all past and present workers in the Department.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds scientific meetings regularly on the first Fridays in December, February and April, and on the first Monday in June. These meetings are open to all students in the University.

SUMMER SCHOOL COURSES

Summer session courses are mentioned in the foregoing references to Courses 101–102 and 110 (52). Prof. Gies will have charge of both courses next summer. He will also conduct a special lecture course in nutrition. The laboratories will be open for research throughout the summer.

ANNOUNCEMENTS

Professional Assistance Offered to Biological Chemists

The Columbia University Biochemical Association will be glad to cooperate confidentially with all who desire the services of biological chemists and with all who seek positions in biological chemistry.

Address inquiries to William J. Gies, 437 West 59th St., New York.

Seventh Annual Meeting of the American Biochemical Society.

The seventh annual meeting of the American Society of Biological Chemists will be held in the buildings of the Medical Department of the Western Reserve University, Cleveland, Ohio, on Monday, Tuesday and Wednesday, December 30, 31, 1912, and January 1, 1913. The American Physiological Society and the American Society for Pharmacology and Experimental Therapeutics meet in Cleveland at the same time, and joint sessions will be held. The headquarters of the three societies will be at the Hotel Colonial. For particulars, address the Secretary, Prof. Alfred N. Richards, Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia.

THE BIOCHEMICAL BULLETIN

The BIOCHEMICAL BULLETIN is a quarterly biochemical review. It publishes results of original investigations in biological chemistry and presents miscellaneous items of personal and professional interest to chemical biologists.

Biological chemists everywhere are cordially invited to forward contributions of any character whatever that will increase the value and add to the interest of the BULLETIN. Original contributions to research, preliminary reports of investigations, abstracts of papers, addresses, reviews, descriptions of new methods and apparatus, practical suggestions to teachers, biographical notes, historical summaries, bibliographies, quotations, news items, personalia, views on current events in chemical biology, etc., are solicited.

The BULLETIN will present as much biochemical substance of as great variety and value, in as little space and for as little money, as possible. Contributors are accordingly requested to keep their papers within the bounds of 15 printed pages and, if possible, to *restrict them to 10 pages or less.* Recrystallize all the products, reject the "mother liquors" and send the BIOCHEMICAL BULLETIN "preparations of *tested purity*"!

Each volume of the BIOCHEMICAL BULLETIN will contain about 600 pages. *The price of Volume I is $6.00.* Single numbers may be purchased from a diminishing reserve supply at the following prices: No. 1, $1.50; No. 2, $2.50; No. 3, $2.00; No. 4, $1.50.

The reserve supply of copies of each number of Volume II of the BIOCHEMICAL BULLETIN will be closely restricted to the *indicated* desires of subscribers and the estimated needs of future members of the Association. Subscriptions for Volume II are accordingly solicited at the following rates, *payable in advance:*

Before October 1, 1912: domestic, $2.75; foreign, $3.00.
Between October 1 and December 1: $3.00; foreign, $3.25.
Between Dec. 1, 1912, and March 1, 1913: $3.50; foreign, $3.75.
Between March 1 and June 1, 1913: $4.25; foreign, $4.50.
After June 1, 1913: $6.00—to be sold only to subscribers.
Subscriptions may be renewed at any time on original terms.

Remittances, manuscripts and correspondence should be addressed to the **Biochemical Bulletin**, 437 West 59th St., New York City.

Vol. II January, 1913 No. 6

Biochemical Bulletin

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NEW YORK

Columbia University Biochemical Association.

Entered as second-class matter in the Post Office at Lancaster, Pa.

Honorary Members of the Columbia Biochemical Association

PROF. R. H. CHITTENDEN, *First Director of the Columbia University Department of Biological (Physiological) Chemistry; Director of the Sheffield Scientific School of Yale University*

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DR. JACQUES LOEB, *Member of the Rockefeller Institute for Medical Research; Head of the Department of Experimental Biology*

PROF. ALEXANDER SMITH, *Head of the Department of Chemistry, Columbia University*

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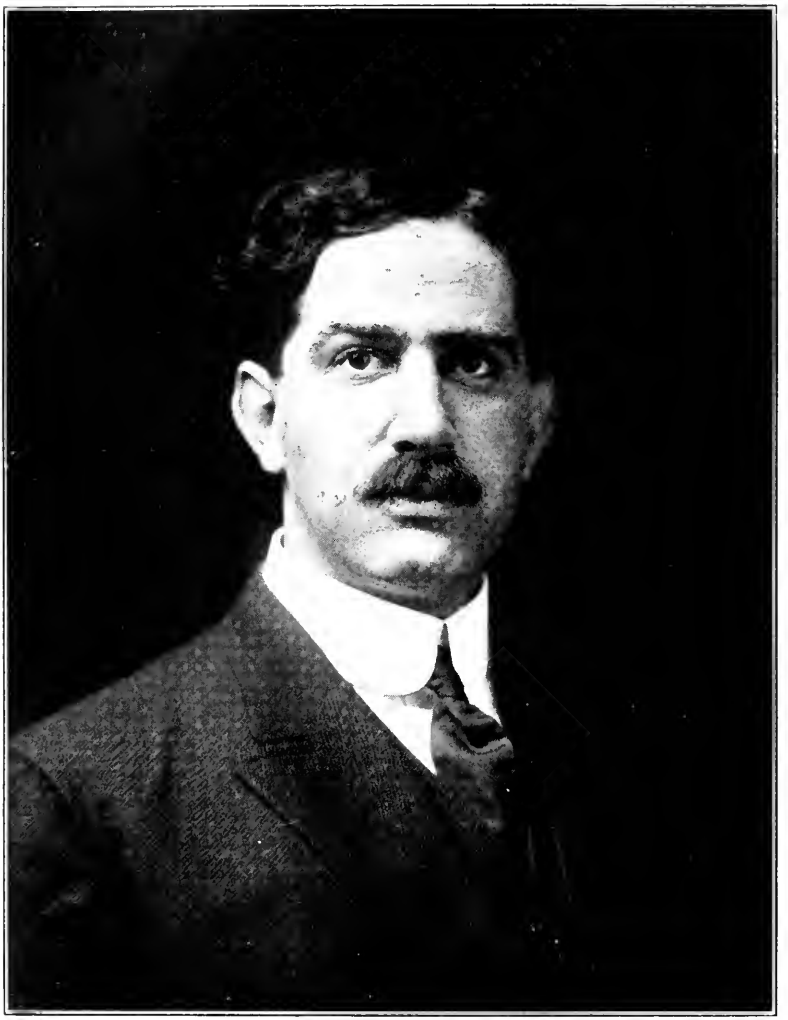

Carl L. Alsberg

BIOCHEMICAL BULLETIN

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CARL L. ALSBERG

Chief of the Bureau of Chemistry of the U. S. Department of Agriculture

Carl Lucas Alsberg was born April 2, 1877, in New York City, son of Bertha (Baruch) and Meinhard Alsberg. From early childhood Alsberg evinced an interest in natural science, especially biology. This was partly due to training and environment, and partly to a natural inclination.

His father, a chemist of distinction and one of the first to manufacture organic dye stuffs in this country, was graduated (Ph.D.) from the University of Jena, and trained under Wöhler (University of Göttingen), Bunsen (University of Marburg), and Geuther (University of Jena). He was assistant to Professor Geuther at Jena and Privatdozent in chemistry at that University. About 1865 he became assistant to Prof. Chandler in the School of Mines at Columbia College. Later he was chemist to the New York City Board of Health under Prof. Chandler. Subsequently he was occupied in chemical manufacture and chemical engineering. He died in 1897. He was one of the founders and first Secretary of the New York Chemical Society, from which the American Chemical Society developed. He had given up the academic career, in which he had unusual prospects, in order to support his wife and parents. But the spirit of research continued to influence the father, and he never ceased to have an active interest in the purely scientific side of chemistry This point of view was maintained in spite of a very active and rapidly growing business, and at a time when research in theoretical science did not receive the recognition in this country that later was conceded to it. It had an effect in

molding the growing mind of his son, which never could have been obtained otherwise.

With a true appreciation, however, of the values of a liberal education, this scientific interest was not allowed to exert a narrowing effect.[1] It was not until Alsberg entered the College of Physicians and Surgeons of Columbia University, however, that he allowed himself to devote his entire time to scientific work He graduated with the degree of M.D. in 1900, and received at the same time the degree of A.M. for special research in physiology.[2] During the summers and vacations of the Medical School, Alsberg devoted his time to research in physiology with Professor F. S. Lee, and in biological chemistry with Dr. P. A. Levene, now of the Rockefeller Institute of Medical Research, then associate in chemistry at the Pathological Institute of the New York State Hospitals.

In July, 1900, after graduating from the Columbia Medical School, Alsberg went to Strassburg, Germany, for post-graduate work, where he studied pharmacology under Schmiedeberg, physiological chemistry under Hofmeister, and clinical medicine under Naunyn. Here he also was associated with E. S. Faust, then Privatdozent at the Pharmacological Institute, now professor of pharmacology at Würzburg; also with Wolfgang Heubner, and others. During the succeeding two years his time was devoted almost exclusively to biological chemistry, notably pharmacology, but he continued his interest in clinical and general biological matters, for the study of which there were unusually good opportunities at the University of Strassburg. During this time he conducted special investigations into the structure and biological signifi-

[1] C. L. Alsberg was prepared for college by tutors and at the Mt. Morris Latin School, entering Columbia College in 1892. He received the degree of A.B. in 1896.

[2] The Department of Physiological Chemistry in the Columbia Medical School was founded in 1898–99, during Alsberg's third year there. At that time only one course in physiological chemistry was offered and that was *required* of "second year men" in medicine. Alsberg's early interest in physiological chemistry was shown by the fact that, while a "*third* year man" in good standing at the Medical School, he took, *as an elective,* the newly established course in that subject for second year men—something no other third year medical student attempted, then or since. The records show that in spite of this heavy addition to his regular work, Alsberg stood among the very highest in physiological chemistry and in the entire medical course. [Ed.]

cance of the nucleic acids. He went to Berlin in 1901 where he spent a year in chemistry with Emil Fischer and in plant physiology with Kny. One vacation was spent at Frankfurt a/M with Ehrlich, Weigert, Edinger and C. von Noorden, in studies especially of the side-chain theory and other conceptions of immunity; another vacation was devoted to clinical medicine with Kuttner, Piorkowski and others.

In the fall of 1902 Alsberg returned to this country, to accept the position of assistant in physiological chemistry at the Harvard Medical School. In 1905 he was advanced to instructor in biological chemistry and put in charge of the organization of the Department of Biological Chemistry at the new Harvard Medical School. From 1906 to 1908 he was in charge there (jointly with L. J. Henderson) of the teaching and research in biological chemistry. From 1907 to 1908 he conducted, in addition, special investigations for the U. S. Bureau of Fisheries, at Woods Hole, Mass.

While at Harvard, Alsberg not only organized and developed an efficient and unusual department for undergraduate teaching, but also, as head of the department, put on a firm basis, for the first time in that institution, a system of graduate instruction and research in biological chemistry.

Alsberg's success as a teacher, both of undergraduates and graduates, has been appreciated by all who have come in contact with him. In fact, it has been recognized by many that his gift in this direction is so pronounced that they have repeatedly urged him to devote himself exclusively to teaching. But the strong spirit of research, coupled with his broad biological interests, would not permit him to confine himself to teaching, and when, in 1908, the position of chemical biologist, in charge of the Poisonous-Plant Laboratory of the Bureau of Plant Industry in the U. S. Department of Agriculture, at Washington, was offered to him, he accepted it with the belief that, by freeing himself from the enticing but time-consuming occupation of teaching, he might accomplish more in research. This conclusion has been amply justified by the results of his investigation of poisonous plants, notably the loco weed, and the biochemistry of various moulds. In this connection, it may be

recalled that the investigations of spoilt corn by Alsberg and his co-workers have revolutionized the methods of testing corn for its fitness as food.

Alsberg was secretary of the Section on Physiological Chemistry of the International Congress of Arts and Sciences, St. Louis Exposition (1905); also secretary and member of the Council of the Boston Society of Medical Sciences. He is Chairman of the Division of Biological Chemistry of the American Chemical Society, Fellow of the American Association for the Advancement of Science, and member of the following societies: American Chemical Society, American Society of Biological Chemists, American Physiological Society, Society for Experimental Biology and Medicine, Society for Pharmacology and Experimental Therapeutics, American Pharmaceutical Association, Washington Academy of Science, American Medical Association, American Association for Cancer Research, Corporation of the Marine Biological Laboratory at Wood's Hole, Massachusetts. He is one of the assistant editors of *Chemical Abstracts*.

Alsberg's appointment, by President Taft, as chief of the Bureau of Chemistry to succeed Dr. Harvey W. Wiley, has received the endorsement of all who know him. With his training and natural equipments, with his record of achievements in research and in practical chemistry, and with his professional standing as a scientist, it seems assured that the Bureau of Chemistry will continue to develop along the best and most approved lines of modern chemical science.

A list of Alsberg's most important papers is appended:

1901. P. A. Levene and C. L. Alsberg: Zur Chemie der Paranucleinsäure; *Zeitschrift für physiologische Chemie,* 31, 543.

1904. C. L. Alsberg: Beiträge zur Kenntnis der Nucleinsäure; *Archiv für experimentelle Pathologie und Pharmakologie,* 51, 239.— C. L. Alsberg: The influence of cholic acid upon the excretion of sulphur in the urine; *Journal of Medical Research,* 13, 105.

1905. C. L. Alsberg and Otto Folin: Protein metabolism in cystinuria; *American Journal of Physiology,* 14, 54.

1906. P. A. Levene and C. L. Alsberg: The cleavage products of vitellin; *Journal of Biological Chemistry,* 2, 127.

1907. C. L. ALSBERG: On the occurrence of oxidative ferments in a melanotic tumor of the liver; *Journal of Medical Research,* 16, 117.—R. FITZ, C. L. ALSBERG and L. J. HENDERSON: Concerning the excretion of phosphoric acid during experimental acidosis in rabbits; *American Journal of Physiology,* 18, 113.—P. A. LEVENE and C. L. ALSBERG: Über die Hydrolyse der Proteine mittels verdünnter Schwefelsäure; *Biochemische Zeitschrift,* 4, 312.

1908. C. L. ALSBERG: Beiträge zur Kenntniss der Guajak-Reaktion; *Archiv für experimentelle Pathologie und Pharmakologie,* Supplement-Band ("Schmiedeberg-Festschrift"), p. 39.—C. L. ALSBERG and E. D. CLARK: On a globulin from the egg-yolk of the spiny dog-fish, *Squalus acanthias* L.; *Journal of Biological Chemistry,* 5, 243.—C. L. ALSBERG and E. D. CLARK: The blood clot of *Limulus polyphemus; Ibid.,* 5, 323.

1909. C. L. ALSBERG: Agricultural aspects of the pellagra problem in the United States; *New York Medical Journal,* July 10.—C. L. ALSBERG: The formation of gluconic acid by the olive-tubercle organism and the function of oxidation in some micro-organisms; *Proceedings of the Society for Experimental Biology and Medicine,* 6, 83.—C. L. ALSBERG: The globulins of the egg-yolk of Selachians; *Proceedings of the American Society of Biological Chemists,* 1, 160, and *Journal of Biological Chemistry,* 6, p. xiii.—C. L. ALSBERG and C. HEDBLOM: Soluble chitin; *Proceedings of the American Society of Biological Chemists,* 1, 192, and *Journal of Biological Chemistry,* 6, p. xlv.—C. L. ALSBERG and C. A. HEDBLOM: Soluble chitin from *Limulus polyphemus* and its peculiar osmotic behavior; *Journal of Biological Chemistry,* 6, 483.

1910. C. L. ALSBERG: Recent work in biological chemistry; *Journal of the American Chemical Society,* 32, 704.—O. F. BLACK and C. L. ALSBERG: The determination of the deterioration of maize with incidental reference to pellagra. *Bulletin 199,* Bureau of Plant Industry, U. S. Department of Agriculture.—C. L. ALSBERG: Note on the use of chitin in dialysis; *Proceedings of the American Society of Biological Chemists,* 1, 225, and *Journal of Biological Chemistry,* 7, p. xii.—C. L. ALSBERG and E. D. CLARK: The hemocyanin of *Limulus polyphemus; Journal of Biological Chemistry,* 8, 1.

1911. C. L. ALSBERG: The toxic action of *Amianthium muscaetoxicum;*

Proceedings of the Society for Pharmacology and Experimental Therapeutics, *Journal of Pharmacology and Experimental Therapeutics,* 3, 473.—C. L. ALSBERG: Mechanisms of cell activity; *Science,* 34 (n. s.), 97.—C. L. ALSBERG and O. F. BLACK: Biological and toxicological studies upon *Penicillium puberulum,* Bainier; *Proceedings of the Society for Experimental Biology and Medicine,* 9, 6, and Proceedings of the American Chemical Society, BIOCHEMICAL BULLETIN, 1, 103. —C. L. ALSBERG: The formation of *d*-gluconic acid by *Bacterium savastoni,* Smith; *Journal of Biological Chemistry,* 9, 1.—C. L. ALSBERG: Proceedings of the meeting of the section of biological chemistry of the American Chemical Society (Chairman's report); BIOCHEMICAL BULLETIN, 1, 94.—O. F. BLACK and C. L. ALSBERG: Observations on the deterioration of maize; *Ibid.,* 1, 130.

1912. C. L. ALSBERG and O. F. BLACK: Studies on barium feeding; *Proceedings of the Society for Experimental Biology and Medicine,* 9, 37.—C. L. ALSBERG and O. F. BLACK; Laboratory studies on the relation of barium to the loco-weed disease. *Bulletin* 246, Bureau of Plant Industry, U. S. Dept. of Agriculture.—C. L. ALSBERG and O. F. BLACK: Biochemical and toxicological studies on *Penicillium stoloniferum,* Thom; *Proceedings of the Eighth International Congress of Applied Chemistry,* 19, 15.

H. M. A.

A DIFFERENTIAL CHEMICAL STUDY OF GLUCOSES FROM A CASE OF PANCREATIC DIABETES[1]

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My new chemical method for the differential or fractional study of carbohydrates has been successfully applied to the study of the lactoses of milk,[2] and also of the glucoses in the urine of the last period of cachexia in a case of diabetes.[3] I have lately applied this method to the sugar in the urine of Louis Dufaut, a hospital patient for a year in ward IV of the National Hospital of Buenos Aires, where he was under the immediate treatment of my illustrious teacher and friend, Dr. Abel Ayerza.[4]

For nearly three years I have been engaged in the laborious task of endeavoring to fractionate the glucoses[5] in the urines of this patient, as I have already fractionated the lactoses of milk. I fractionated the products of condensation (or perhaps also of decomposition) of glucoses in urine that yielded a residue, after evaporation, of 83.76 grams per thousand parts of urine, a polaristrobometric deviation of 54° 06′ per thousand, a reduction corresponding to 75 grams of sugar per thousand, and a fermentation representing about 60.8 grams of sugar per thousand, but obtained only a *single* osa-

[1] Translated (and in part abstracted) by Dr. Max Kahn from the introduction to the author's paper, in French, in the *Revista de la Universidad de Buenos Aires,* 1912, xvii, pp. 108–221, a copy of which was forwarded by Professor Landolph for this purpose.

[2] Landolph, *Argentina Médica,* July 27 and August 3, 1907, and March 28, 1908.

[3] Landolph, *Revista de la Universidad de Buenos Aires,* 1906, xi, pp. 101, 153 and 232.

[4] The clinical history of this patient was published by the author in the *Revista de la Universidad de Buenos Aires,* 1912, xvii, p. 108.

[5] Professor Landolph believes that diabetic urine contains a number of GLUCOSES differing in their fermentability, optical properties, reducing powers and ability to yield osazones. [Trans.]

zone with a melting point of 189–190° C., equivalent to 41 grams of sugar per thousand. After hydrolysis[6] of a large quantity of urinary residue, the values for polariscopic deviation, reduction, and fermentation were about *ten units less* than the corresponding figures for non-hydrolyzed urine; and the quantity of sugar in the hydrolyzed urine, as represented by osazone, did not equal half the amount of sugar obtained from the original urine. These differences can be explained in several ways; but since "diabetic sugar," as I have already demonstrated, is a *collection* of several distinct chemical substances, it is highly probable that in such treatment some of these components are so modified that they no longer form osazones, although they retain their fermentability. *It is true that this explanation does not accord with conventional views, but, nowadays, the phenomena in chemistry and physics which do not agree with current theories are the ones that should be probed and investigated in the interest of truth.*

When a portion of evaporated, syrupy residue from my patient's urine was allowed to age, there was a marked change in the residue, due either to the nature of the syrup or to chemical decomposition in it. This was not surprising in view of the complex composition of diabetic urine, and the further fact that the original sugar may undergo a process of slow hydrolysis, or rather condensation, to form higher compounds like dextrins and analogous substances, which then yield, with phenylhydrazin, resinous *pseudoösazones* having very low melting points, such as I have isolated from gastric juices.[7]

Discussing, now, some of the details of my analytic data, I find that in the *second* treatment[8] down to the fourth extraction, the

[6] Hydrolysis was performed in the following way: 100 c.c. of urine, or urinary extract, were heated for seven hours on a water bath with 5 c.c. of hydrochloric acid solution (strength not stated). The liquid was then evaporated to a volume of 60 c.c.

[7] Landolph, *Revista de la Universidad de Buenos Aires,* October, November and December, 1910.

[8] Professor Landolph examined a number of urines from the same patient by several processes for the determination of the content of sugar. The urines were evaporated over a water bath to a syrupy consistency and the examinations of the separate urinary residues are called "Treatment" I, II, etc. After completion of the alcoholic extractions, a portion of each extract was hydrolyzed

residue,[9] though always quite abundant, decreased from 68.17 grams per thousand parts of urine in the first to 36.26 grams in the third. On comparing the figures obtained from the original urine with the figures for the extracts of its residues, I observed polariscopic deviations which were only one-fourth, one-fifth and one-tenth as great, respectively, as those for the untreated urine. The disappearance of the aldehydic function led me to suppose that there was condensation and not oxidation, since fermentation and reduction tests were still very marked and showed the presence of half or even more of the total dry residue. As regards the *characteristic* osazone, it was obtained only in small amounts with nearly, but not quite, the same melting point, *i. e.,* instead of melting at 189–190° C. it melted at 185–186° C.; whereas the *pseudoösazone* (with a melting point of 75–76° C.), which was resinous and alcohol-soluble, corresponded to 66.72 grams of sugar per thousand (33.36 grams of polymerized sugar).

Hydrolysis produced analogous changes in the urine, but here the *true osazone,* with a melting point of 174–175° C., was represented by the small quantity of 1.75 grams per thousand of urine (0.87 gram of glucose). This, therefore, was different from the osazone obtained from the non-hydrolyzed material. The *pseudoösazone* from the *hydrolyzed* solution amounted to 44.94 grams of sugar per thousand, or 22 units less than the *pseudoösazone* from the non-hydrolyzed solution (melting point of 95° C. instead of 75° C.). For the fourth, fifth and sixth alcoholic extracts of the *first* treatment, analogous figures were obtained.

In the first extract[10] of the *second* treatment, the degree of the reduction was maintained for each observation, while the fermentability of the non-hydrolyzed solution became very feeble. Here the principal osazone was *pseudoösazone* (with a melting point of 112° C.) amounting to 38 grams per thousand of urine (17.5 grams

with dilute hydrochloric acid solution and the "*sugars*" in the hydrolyzed and non-hydrolyzed portions fractionated with basic lead acetate and mercuric nitrate. [Trans.]

[9] After the urine was evaporated over a water bath to a syrupy consistency, the residues were successively extracted with absolute alcohol. These "treatments" are called "extraction" 1, 2, etc.

[10] After extraction with alcohol, 24.167 grams of extract were dissolved in 500 c.c. of water. This was divided into two parts. The first part was precipitated with basic lead acetate, the second with mercuric nitrate.

of glucose). The *true osazone,* with a melting point of 177–178° C., amounted to only 1.74 grams per thousand of urine (0.87 gram of glucose). After hydrolysis[11] we obtained in three different fractions (12.20, 10.62 and 2.96) a total of 25.78 grams of osazones per thousand of urine (14.39 grams of glucose). The melting points were respectively 170°, 177° and 157–159° C. It is singular that here no *pseudoösazones* were obtained.

For the second extract[12] of the *second* treatment, we obtained, from the non-hydrolyzed solution, osazone with a melting point of 95–97° C., amounting to 11 grams per thousand of urine (5.5 grams of sugar). Here too, after hydrolysis, we obtained two fractions of *true osazone* (melting points of 172–173 and 180–181° C.) amounting to 19.75 grams per thousand of urine (9.87 grams of sugar) but again no *pseudoösazone.*

For the third alcoholic extract of the *second* treatment, we obtained practically no fermentation, and only *pseudoösazones.* In the *hydrolyzed* solution, however, fermentation was pronounced; and we obtained a mixture of *true osazones* (melting points of 180–190° and 187–188° C.) amounting to 10.06 grams per thousand of urine (5.03 grams of sugar—5.40 grams of fermentable sugar). The *pseudoösazone* with a melting point of 100° C. and amounting to 6.83 grams per thousand of urine (3.42 grams of sugar), corresponded, perhaps, to the difference between 7.42 grams of sugar per thousand of urine (polariscopic determination) and 5.40 grams per thousand of urine (estimated by fermentation), *i. e.,* 2.02 grams.

For the fourth alcoholic extract of the *second* treatment, we obtained from both the non-hydrolyzed and hydrolyzed solutions, mixtures of *true* and *pseudoösazones* in approximately equal proportions.

In the alcoholic extracts of the *third* treatment, fermentation in general was always pronounced, corresponding to an increase in the amounts of *true osazones* and *pseudoösazones;* but, with this

[11] Hydrolysis was conducted by heating the extract with hydrochloric acid solution over a water bath. See footnote 6.

[12] The residue remaining after the first alcoholic extract was again extracted with alcohol, and also treated with mercuric nitrate and basic lead acetate. See footnotes 8 and 9.

anomaly, that in the sixth and seventh extracts (*third* treatment) the weights of the sugars, as represented by the amounts of *pseudoösazones,* were three or four times greater than the weights of the total dry residues.

As regards the *fourth, fifth* and *sixth* treatments,[13] we obtained neither polariscopic deviation, reduction nor fermentation, but we did obtain *pseudoösazones.*

We also noticed that for the fifth alcoholic extract of the *second* treatment the total dry residue obtained upon hydrolysis was always greater than the total dry residue of the non-hydrolyzed portion, evidently due to the absorption of oxygen from the air (which perhaps also provoked a condensation or resinification of phenylhydrazine).

In order to obtain an approximately correct idea of the action of basic lead acetate or mercuric nitrate upon the urinary glucoses, I treated each of the first and second extracts of the *second* treatment with basic lead acetate in one portion and mercuric nitrate in another. *The lead salt precipitated nearly all of the optically active sugar from the non-hydrolyzed solution, while the mercuric nitrate did not have any effect* in this regard, but both reagents diminished the quantity of the reducing sugars.

From the first fraction of the *second* treatment, basic lead acetate removed all the sugar that yielded *pseudoösazones,* and left only the sugar which formed *true osazones*—the latter in diminished quantities, at least in the first and second crystallizations. Treatment with mercuric nitrate yielded only traces of *true osazones* but, in the second crystallization, a marked amount of *pseudoösazones* was obtained.

From another portion of the second alcoholic extract of the *second* treatment, basic lead acetate again precipitated all the active sugars, while mercuric nitrate did not affect them. With mercuric nitrate, only *pseudoösazones* were obtained.

[13] Procedures similar to those previously indicated were adopted in the study of "treatments" IV, V and VI: The urine was evaporated in each case, the residue extracted with alcohol several times, and each of the extracts divided into two portions. One portion was hydrolyzed, the other was not. The hydrolyzed and non-hydrolyzed portions were individually treated in different portions with basic lead acetate and mercuric nitrate. See footnote 8.

These observations show that the isolation and identification of urinary sugars is a very complicated process. One must work with large quantities of material in order to be able to differentiate, recrystallize and purify all the fractions. I am now engaged in an extension of this work.

THE DETECTION OF ACETO-ACETIC ACID BY SODIUM NITROPRUSSID AND AMMONIA[1]

V. J. HARDING AND R. F. RUTTAN

(Chemical Laboratory, McGill University, Montreal, Canada)

The use of sodium nitroprussid and ammonia, followed by the addition of an acid insufficient in amount to completely neutralize the ammonia, was first suggested by Le Nobel as a method of detecting small quantities of acetone. This test is based on the original test of Legal but, as the two tests differ in result, it is proposed to call the test depending on the use of ammonia, the Le Nobel test, and to reserve the term Legal test exclusively for the action of sodium nitroprussid and potassium (or sodium) hydroxid followed by acidification.

The two tests differ in the following points: (1) The Le Nobel test gives with acetone a much bluer shade of purple and (2) is an extremely delicate test for aceto-acetic acid.

The usual way, in clinical work, of applying the Le Nobel test is to first acidify the urine with acetic acid, add a few drops of a dilute solution of sodium nitroprussid and then overlay the solution with concentrated aqueous ammonium hydroxid solution. On applying this test, the authors discovered several anomalies which can be summarized as follows:

(*A*) Acetone in water and when added to urine, making concentrations similar to those occurring in cases of acetonuria, gives the test very faintly and only after long standing—by no means as distinctly as the natural cases.

(*B*) If some samples of urine which give a marked response to the Le Nobel test be distilled with acids, the test given by the distillate (where the acetone is presumably ten to twenty times more concentrated than in the original urine) is very much less marked.

[1] Abstract of paper published in the *Bio-Chemical Journal*, 1912, vi, p. 445 (Oct.).

If such a urine (*B*) first be boiled under a reflux condenser in presence of a little acetic, oxalic or sulfuric acid, and the Le Nobel test applied, the test gives either a negative result or the response is much diminished in intensity. As these urines contained aceto-acetic acid, which would be destroyed by heat, it was evident that the previous presence of this acid in the urine could account for the anomalies observed. That this was so was established in the following way.

The urine was acidified with oxalic acid, saturated with sodium chlorid and rendered acetone-free by aspiration for an hour with a current of air, as in the Folin method of estimating acetone. At the end of that time a determination of free acetone, by the Folin method, showed that none was present, although the residual urine responded to the Le Nobel test with undiminished intensity, and the test became negative when the liquid was boiled for fifteen minutes under a reflux condenser.

Aceto-acetic acid, however, is stated in the literature to give a faint reddish-brown or orange-red coloration with sodium nitroprussid and ammonia—unchanged by the addition of acid. To determine this point, a solution of aceto-acetic acid was made by hydrolyzing ethyl aceto-acetate with the theoretical quantity of potassium hydroxid in the cold for twenty-four hours. This hydrolyzed solution was found to respond to the Le Nobel test exactly as the urine of an acidosis patient. The test became negative on boiling the solution under a reflux condenser, and was unaffected by the removal of the free acetone.

In consequence of these facts the authors have no hesitation in saying that aceto-acetic acid of itself responds to the Le Nobel test and that, in the great majority of cases, a positive result when the Le Nobel test is applied to a urine indicates aceto-acetic acid and not acetone. On comparing the delicacy of the Le Nobel test for aceto-acetic acid in urine, with the ferric chlorid test, the authors found that the former will just detect about one part of aceto-acetic acid in 30,000 parts of urine, while the latter fails at 1 part in 7,000. The limit of detection of aceto-acetic acid in water by the Le Nobel test is over 1 part in 80,000.

ORTHO-TOLIDIN AS AN INDICATOR FOR OCCULT BLOOD

R. F. RUTTAN AND R. H. M. HARDISTY

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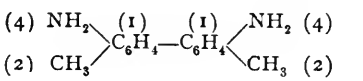
The authors have lately called attention to the advantages of *o*-tolidin over benzidin and phenolphthalin as a clinical reagent for the detection of occult blood.[1]

The properties and derivatives of *o*-tolidin,



were first described by one of the writers in 1886.[2] This substance was compared with guaiacum, benzidin and phenolphthalin in aqueous solutions of blood and in solutions containing urine, stomach contents, and feces. It was found to be a very delicate reagent for the detection of blood in aqueous solution, and to have some important advantages over the other clinical reagents when used in the detection of blood in excretions and secretions. The reagents were made up as follows: Guaiacum, 1 in 25 methylated spirits; benzidin and *o*-tolidin, in solutions of similar strength, in glacial acetic acid; phenolphthalin, prepared as recommended by Kastle.[3]

The solutions to be tested were made up from a 0.1 per cent. solution of crystalline hemoglobin in water. The hydrogen peroxide solution employed was made up to approximately 3 per cent. from Merck's perhydrol. In testing, 1 c.c. of the reagent, 1 c.c. of the solution to be tested and 1 c.c. of diluted perhydrol were employed.

In aqueous solution, as the average of ten tests, it was found that guaiacum detected blood, 1 in 50,000; benzidin detected blood, 1 in 700,000; *o*-tolidin detected blood, 1 in 7,000,000; phenolphthalin detected blood, 1 in 10,000,000, or even in greater dilutions.

[1] Ruttan and Hardisty: *Canadian Medical Association Journal*, Nov., 1912.

[2] Ruttan: *Proceedings of the British Association for the Advancement of Science*, 1886.

[3] Kastle: *Bulletin 51*, Hygienic Laboratory, Washington, D. C.

Guaiacum and benzidin, when positive, gave prompt reactions but in very dilute solutions the color faded quickly. *o*-Tolidin developed the greenish-blue, or deep blue, more slowly but the color persisted for some time, even several hours.

The results of the comparative tests are briefly summarized below.

(1) *In urine:* Guaiacum and benzidin detected blood, 1 in 6000; benzidin was slightly the more sensitive reagent; *o*-tolidin detected 1 in 24,000; phenolphthalin, less than 1 in 2,000.

(2) *In feces:* Feces of patients on a meat-free diet for seven to ten days were used and a 2 per cent. emulsion prepared. Guaiacum detected blood, 1 in 10,000; benzidin and *o*-tolidin, 1 in 100,000, the tolidin reaction being slightly slower but persisting—the benzidin color fading quickly in very dilute solutions; phenolphthalin gave reactions only when dilutions did not exceed 1 in 2,000.

(3) *In stomach contents:* Stomach contents after ordinary test-meals were employed. One c.c. of stomach contents was added to the reagent before the diluted blood solution was introduced. Guaiacum detected 1 in 5,000; benzidin and *o*-tolidin, 1 in 30,000; phenolphthalin, even after the acidity of the stomach contents had been neutralized before applying the solution, was less delicate than guaiacum.

Experiments were conducted to determine the keeping properties of the reagents. Although benzidin and *o*-tolidin are about equal in delicacy for blood in feces and stomach contents, the delicacy of the benzidin reagent diminishes 50 per cent. in 24–36 hours, while *o*-tolidin will remain unchanged in delicacy for from three to four weeks.

o-Tolidin is as sensitive a reagent for occult blood in stomach contents and feces as benzidin. Its action is less inhibited by urine than any of the other reagents. Its solution in acetic acid can be kept for one month without its delicacy being reduced. After that its value decreases slowly. Benzidin[4] solutions in acetic acid cannot be kept twenty-four hours without very serious deterioration in delicacy; some preparations decreasing over 50 per cent.

[4] Three different products were compared.

SYNTHETICAL PROPERTIES OF EMULSIN

VERNON K. KRIEBLE

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In a recent communication[1] the writer described an emulsin which produced lævo-mandelonitrile when allowed to act for three and one-half hours on an amygdalin solution. Those experiments were conducted during the spring of 1910. Much to our surprise when the research was continued in October, 1912, it was found that, under the conditions previously described, the nitrile produced was dextro active. This seems to explain the fact that the author's results differed from those of Feist, Rosenthaler, and Auld, who found dextro-nitrile. Their samples of emulsin were evidently much older than the one used by the author for his first determinations.

It seems very probable that there are two synthetic enzymes in a fresh sample of emulsin, one of which synthesizes dextro-mandelonitrile from benzaldehyde and hydrocyanic acid, while the other synthesizes a lævo-nitrile. The one synthesizing the dextro-nitrile is evidently more stable.

Fresh emulsin was extracted from bitter and from sweet almonds. It was found that the sample from sweet almonds, when allowed to act on amygdalin for three and one-half hours, produced lævo-nitrile while the one from the bitter almonds was dextro active.

The detailed experimental results will appear very shortly in one of the chemical journals.

[1] Krieble: *Journal of the American Chemical Society*, 1912, xxxiv, p. 716.

ON THE OCCURRENCE OF NICOTINIC ACID IN RICE BRAN

U. SUZUKI AND S. MATSUNAGA

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One kilo of fat-free rice bran was extracted with hot alcohol (80–85 per cent.). The alcoholic extract was greatly concentrated by evaporation, diluted with water, and shaken with ether for the removal of fat, etc. The residual aqueous liquid, after evaporation of the ether, was treated with sulfuric acid (total, 3 per cent.) and precipitated with phosphotungstic acid. After barium decomposition of the precipitate, in the customary manner, about 1 gram of nicotinic acid (picrate) was isolated. The free acid, and the copper as well as the platinic-chlorid double salts, were also prepared and identified. Analytic data are appended.

A SUMMARY OF THE ANALYTIC PERCENTAGE DATA

Picrate, $C_6H_5NO_2 \cdot C_6H_3N_3O_7$

	C	H	N	Picric acid	Cu	Pt
Calculated	40.91	2.27	15.91	65.06	....	....
Found	40.45	2.41	16.50	65.50	....	....
	40.68	2.51	16.19	....	....	....
Free acid, $C_6H_5NO_2$						
Calculated	58.54	4.07	11.38	....	....	....
Found	58.36	4.32	11.80	....	....	....
Copper salt, $(C_6H_4NO_2)_2Cu$						
Calculated	....	....	9.13	....	20.68	....
Found	....	....	9.06	....	20.94	....
Platinum-chlorid double salt, $(C_6H_5NO_2 \cdot HCl)_2PtCl_4$						
Calculated	....	....	....	....	....	29.72
Found	....	....	....	....	....	30.00

This appears to be the first time that nicotinic acid has been detected in vegetable matter, although Jahns,[1] and Schulze and Frankfurt[2] have found trigonellin (the methyl-betain compound of nicotinic acid) in plants, and Schreiner and Shorey[3] have identified, in humus soils, picolin carboxylic acid (a homolog of nicotinic acid).

[1] Jahns: *Ber. d. deut. chem. Gesell.*, 1885, xviii, p. 2518; 1887, xx, p. 2840.

[2] Schulze and Frankfurt: *Ibid.*, 1894, xxvii, p. 769; BIOCHEMICAL BULLETIN, 1912, ii, p. 18.

[3] Schreiner and Shorey: *Bull.* 53, U. S. Dept. of Agric., p. 28 (1909).

A STUDY OF THE INFLUENCE OF CANCER EXTRACTS ON THE GROWTH OF LUPIN SEEDLINGS[1]

JACOB ROSENBLOOM

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Introduction. One of the peculiar effects of cancer is the resultant cachexia. There have been many efforts to find, in cancer tissue, a poison that might account for the characteristic cachexia. It has been claimed that the cachexia is due to pressure by the growing tumor on the blood vessels and consequent interference with adjacent circulation, with development of areas of necrosis, autolysis, and production of hemolytic and toxic substances.

Rülf[2] considers that proteases are important factors in the causation of cancer cachexia. Bard[3] found that blood is rapidly hemolyzed in hemorrhagic carcinomatous exudates in serous cavities, which is not the case in exudates under other conditions. Kullmann[4] observed that extracts of carcinoma contain hemolytic substances that are active *in vivo* and *in vitro,* soluble in alcohol and water, and toxic for all varieties of corpuscles. Micheli and Donati[5] also found hemolytic substances in eight of sixteen tumors, of which five hemolyzed all varieties of corpuscles and three acted on some varieties only. They thought the hemolytic substances result from autolysis of the tumors, as it is well known that certain hemolytic substances occur among the products of autolysis of normal tissues.

[1] This paper presents the results of a preliminary study that was begun, at Dr. Gies' suggestion, as a part of the plan of biochemical research described in *Studies in cancer and allied subjects, conducted under the auspices of the George Crocker Special Research Fund,* 1912, iii, p. 153 (in press). The plan includes a study of the effect of cancer extracts on cells of all kinds, including cancer cells.

[2] Rülf: *Zeit. f. Krebsforsch.,* 1906, iv, p. 417.

[3] Bard: *La semaine med.,* 1901, xxi, p. 201.

[4] Kullmann: *Zeit. f. klin. Med.,* 1904, liii, p. 293.

[5] Micheli and Donati: *Riforma med.,* 1903, xix, p. 1037.

Müller[6] claimed, from the results of a study of nitrogenous metabolism in cancer patients, that in cachexia of cancer there is toxogenic destruction of protoplasm independent of nutrition; *i. e.,* a specific toxic effect of cancerous tissue. Müller's results have been confirmed by other workers[7] but cumulative research has shown that the cases with normal protein catabolism exceed in number those with increased protein catabolism.[8]

According to the prevailing opinion cancer cachexia is not specific, but is the same as the cachexia of other conditions. It has been impossible to show the occurrence in cancer tissue of any substance that would account for the cachexia of this disease.[9]

In the study described below, we ascertained some of the effects of extracts of cancer tissue on the growth of lupin seedlings, in the hope that this procedure for the detection of toxic substances might yield significant results.

Experimental. *Preparation of lupin seedlings.* Lupin seeds were soaked in water overnight. Seeds of the same size were then selected and planted in wet moss. After three or four days the seedlings were taken from the moss, the coat of each removed, and the sprout rinsed with distilled water. The root was carefully measured on a millimeter scale. The seedlings were then fastened on glass rods drawn out at one end to form a sharp pointed L and suspended in perforated cork covers over 400 c.c. Jena beakers, each containing 200 c.c. of water and 5 c.c. of boiled or unboiled cancer extract prepared as described below. "Control" seedlings were suspended in distilled water. The glass rods were so adjusted that the roots were immersed in the liquid but the cotyledons were not in contact with it. Four seedlings were suspended in each beaker. At intervals of 20 hours all the seedling roots were measured.[10]

[6] Müller: *Zeit. f. klin. Med.,* 1889, xvi, p. 496.

[7] *Char. Annal.,* 1891, xvi, p. 138; *Arch. prov. de Med.,* 1899, March; *Arch. f. Verdauungskr.,* 1899, v, p. 540; *Riv. ven. d. sci. Med.,* 1899, xvi, p. 31; *Zeit. f klin. Med.,* 1897, xxxiii, p. 385.

[8] *Zeit. f. Krebsforsch.,* 1904, i, p. 199: *Salkowski Festschrift,* Berlin, 1904, p. 75; *Fifth Ann. Rep't Cancer Lab.,* New York State Dep't of Health, 1903–1904.

[9] Blumenthal: *Salkowski Festschrift,* Berlin, 1904.

[10] True and Gies: *Bulletin of the Torrey Botanical Club,* 1903, xxx, p. 390; Rose: Biochemical Bulletin, 1911, i, p. 428.

DATA SHOWING EFFECTS OF EXTRACTS OF CANCEROUS AND NORMAL TISSUES ON THE GROWTH OF LUPIN SEEDLINGS

I. *Extract of Bone Sarcoma*

Lupin seedlings	Rate of growth per plant, in millimeters			
	Unboiled extract		Boiled extract	
	1st 20 hr.	2d 20 hr.	1st 20 hr.	2d 20 hr.
A	14	19	18	30
B	12	28	13	38
C	20	22	15	24
D	17	40	16	30
Average	16	27	16	30
Control (average)	5	8	6	8

II. *Extract of Fibroma of Uterus*

A	8	12	6	10
B	6	12	8	10
C	8	10	8	12
D	6	8	8	16
Average	7	10.5	7.5	12
Control (average)	8	10	8	11

III (*a*). *Extract of a Carcinoma of the Breast*

A	18	9	12	8
B	12	7	12	6
C	15	8	9	8
D	15	8	15	8
Average	15	8	12	7.5
Control (average)	17	7	16	8

III (*b*). *Extract of Normal Breast Tissue Near the Cancer*[11]

A	20	8	26	6
B	18	6	17	8
C	24	8	24	8
D	22	6	25	7
Average	21	7	23	7.3

III. (*c*). *Extract of Pectoral Muscle Removed at Operation*[11]

A	14	7	26	9
B	26	6	18	9
C	14	11	26	12
D	24	8	24	10
Average	19.5	8	23.5	10

Preparation of cancer extracts. Fresh cancerous tissue, direct from the operating room, was minced, then triturated with sand and water, and the thin mixture frequently shaken for about an hour.

[11] "Control" figures are given in section III (*a*).

The liquid was strained through gauze, then filtered. Portions of this filtered extract (boiled or unboiled) were used in the manner indicated above.

Data pertaining to growth. The summary on page 231 presents the results of this study.

General conclusion. The extracts failed to inhibit growth of the seedlings. The observed acceleration of growth was probably due to inorganic salts in the extracts. It is possible, of course, that deleterious action by cancer toxins was neutralized or overcome by the stimulating power of associated nutrient substances. This particular point requires special investigation.

THE BIOCHEMISTRY OF THE FEMALE GENITALIA[1]

3. A quantitative study of certain enzymes of the ovary, uterus, and bladder, of pregnant and non-pregnant sheep

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(Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York)

Introduction. In this study we used the pregnant and non-pregnant ovaries and uteri, and also the bladder, in order to compare our genital results with those for an organ with a supposedly non-dynamic function. We desire to express our thanks to Dr. Robert T. Frank for his interest, and for his kindness in placing at our disposal the genital material employed.

Methods. 1. PREPARATION OF EXTRACTS. (*A*) Five grams of finely divided fresh material, washed free from blood and thoroughly triturated with sand, were treated with 100 c.c. of water and allowed to stand for 24 hours, under toluene, with frequent shakings. At the end of this time the extract was filtered through muslin, made up to 100 c.c., and aliquot portions used for the enzyme tests. (*B*) Glycerol extracts were made in the same manner.

2. ESTIMATION OF ENZYMES. In each case, a control test was made with boiled extract. *Lipase.* A mixture of 10 c.c. of the extract, 0.5 c.c. of neutral ethyl butyrate and 1 c.c. of toluene was placed in a bottle and allowed to digest at 40° C. After 24 hours $n/20$ sodium hydroxide solution was used to determine the acidity, with phenolphthalein as the indicator. From this amount was subtracted the "control" acidity (10 c.c. of extract and 1 c.c. of toluene).

Amylase. To 10 c.c. of 1 per cent. freshly prepared starch

[1] The first paper in this series (a general review of the subject) has been accepted for publication in a later issue of the BIOCHEMICAL BULLETIN. The second paper appeared in the January issue of the *Journal of Biological Chemistry*, 1913, xiii, p. 511. See also BIOCHEMICAL BULLETIN, 1911, i, p. 115.

[2] Mr. Lefkovics died shortly after the completion of this work. See BIOCHEMICAL BULLETIN, 1912, i, p. 573.

paste were added 10 c.c. of extract and 1 c.c. of toluene, and the mixture allowed to digest at 40° C., until duplicates no longer became blue with iodin solution. At this point the bottles containing the digestive mixtures were placed in boiling water to stop the digestions simultaneously. The contents of each bottle were then made up to 50 c.c. and run from a burette into boiling Fehling solution, acetic acid and potassium ferrocyanid being used to determine the end point. When the amount of sugar in the digestive mixture was less than that required completely to reduce the copper, a standard glucose solution was employed for that purpose.

Acid- and alkali-proteases. Ten grams of gelatin were dissolved in 100 c.c. of warm 1 per cent. solution of sodium fluorid colored with methyl violet. This solution was drawn into glass tubes 1 mm. in diameter and the filled tubes quickly placed in cold water to congeal the gelatin. The tubes were then cut into lengths of 2–3 cm. Ten c.c. of extract were placed in a small bottle closed with a perforated cork through which the gelatin tubes could be inserted; 1 c.c. of toluene was added and the digestions kept at room temperature for 48 hours. In the estimation of acid-protease (pepsin) the mixture was made acid with 0.2 per cent. hydrochloric acid solution and for alkali-protease (trypsin) they were rendered alkaline with 0.5 per cent. sodium carbonate solution.

TABLE SHOWING ENZYME VALUES OF PREGNANT AND NON-PREGNANT OVARY, UTERUS AND BLADDER OF SHEEP

A. Non-pregnant condition

Organ	Aqueous extract				Glycerol extract			
	Lipase, c.c.	Amylase, mg.	Acid-protease, mm.	Alkali-protease, mm.	Lipase, c.c.	Amylase, mg.	Acid-protease, mm.	Alkali-protease, mm.
Ovary	0.65	5	2	2	0.65	6	6	0.25
Uterine mucosa	3.05	10	1	1	1.95	5	3	4
Bladder mucosa	1.3	10	1.5	0	1.35	15	3	0

B. Pregnant condition

Ovary	1.3	10	2	7	2.0	7	7	0.5
Uterine mucosa	8.35	25.5	1	7	8.3	12	3	5
Bladder mucosa	1.1	7.5	1.5	0	1.35	6	4	0

The accompanying table presents the results obtained in this study. The lipase values are given in terms of the amount of $n/20$

sodium hydroxid solution necessary to neutralize the acidity developed by 1 gram of tissue. The amylase values are given in terms of the amount of maltose in mg. formed per gram of tissue. The acid- and alkali-protease values are given in terms of the number of millimeters of gelatin digested in a tube by 1 gram of tissue.

The results show that lipase and amylase were most abundant in both the ovaries and uterine mucosae of pregnant animals. Pregnancy had no quantitative effect on the acid-protease (pepsin), but alkali-protease was increased in both the ovary and uterine mucosa. The bladder extracts contained lipase, amylase, and acid-protease (pepsin), but no alkali-protease (trypsin).

THE BIOCHEMISTRY OF THE FEMALE GENITALIA[1]

4. On the absence of certain enzymes from the human chorion[2]

JACOB ROSENBLOOM

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During pregnancy the chorion frondosum unites with the decidua serotina to form the placenta. The enzymes of the placenta[3] have often been studied, but I am unable to find any record of a study of the enzymes of a human chorion. Through the kindness of Dr. Robert T. Frank, of New York, the writer received a fresh human chorion for such an investigation.

The available chorion, which weighed 10 grams after it had been washed free from all blood by means of a small amount of water, was finely minced and two portions, 5 grams each, were taken for the preparation of extracts, which were made as follows: (1) The material was triturated with sand, 200 c.c. of water added, and the mixture allowed to stand with frequent shakings for 24 hours under toluene. At the end of that time, the extract was filtered through muslin, and the filtrate used in the tests for various enzymes. (2) A glycerol extract was made in the same way. In testing for enzymes, control portions were always taken, which were boiled before their addition to the solutions or suspensions of substrate.[4]

The accompanying table presents the data obtained in this study. The data show that both glycerol and aqueous extracts of a human

[1] See the first footnote of the preceding paper in this issue of the BIOCHEMICAL BULLETIN.

[2] The analytic work was done in the Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York.

[3] Frank: *Surgery, Gynecology and Obstetrics*, 1912, xv, p. 561.

[4] See the description of methods in the paper preceding this one.

chorion were free from amylase, sucrase, maltase, lactase, lipase, peptidase, ereptase, acid-protease and alkali-protease.

Table showing results of enzyme tests in glycerol and aqueous extracts of a human chorion

Enzyme	Substrate	Glycerol extract	Aqueous extract
Amylase	Starch	Absent	Absent
Sucrase	Sucrose	Absent	Absent
Lactase	Lactose	Absent	Absent
Maltase	Maltose	Absent	Absent
Lipase	Ethyl butyrate	Absent	Absent
Peptidase	Glycyltryptophan	Absent	Absent
Ereptase	Witte peptone	Absent	Absent
Acid-protease	Gelatin and fibrin[5]	Absent	Absent
Alkali-protease	Gelatin and fibrin[6]	Absent	Absent

It would seem, from the results of this study, either that the enzymes of the placenta are formed at a comparatively late period or that the decidua serotina furnishes the enzymes which subsequently appear in the placenta. Possibly the presence of blood in the placenta accounts for the occurrence of certain enzymes that have been detected in the placenta.

[5] Extract rendered acid with 0.2 per cent. hydrochloric acid solution.
[6] Extract rendered alkaline with 0.5 per cent. sodium carbonate solution.

A DEPARTMENT OF BIOCHEMICAL RESEARCH AT VINELAND, NEW JERSEY[1]

AMOS W. PETERS

To the Training School at Vineland, N. J., belongs the credit for the first establishment anywhere in the world of a biochemical laboratory as one means of investigation of the problem of feeble-mindedness in children. To the writer of this article has fallen the honor as well as the heavy duty of testing what are the possibilities of biochemical research in the field of feeble-mindedness. The large problem which this unfortunate affliction of a considerable portion of humanity presents to organized society is becoming daily more evident, as its economic burden and its social consequences force themselves on public attention. Research on the problem is a crying need not simply from the humanitarian standpoint, but also as an economic necessity. The care and treatment of these cases and the governmental management of this problem, including its amelioriment and prevention, will in the future rest on the basis of data obtained by scientific research. At present we are proceeding on a very small amount of such data and we are just discovering, after some preliminary efforts made in the psychological direction, how extensive and manysided this problem is. What assurance have we that our present method of dealing with the problem is in rational accord with the nature and origin of the condition? Our procedures are in the stage of costly empiricism and in the very infancy of scientific investigation. It is therefore an important step forward when this institution ventures to add to its present psychological method of investigation that of the rapidly growing and fundamental science of biochemistry. The need for this additional method of attack, and the tendency of expert thought toward it, is well illustrated by the following quotation from the words of a leader in the study of problems of psychopathology, Dr. Southard:

[1] Reprinted from *The Training School,* 1912, ix, p. 70 (Sep.).

The majority of cases of mental diseases are, I am convinced by special studies, characterized by the occurrence of obvious brain lesions, *i. e.*, even in the present stage of science they possess a structural pathology. Do they therefore possess no functional pathology? Their possession of the two aspects is a truism. Should we not study both aspects?

Furthermore, suppose we learn that, whereas three quarters of our cases of mental disease exhibit obvious irrecoverable brain lesions, another quarter fails to show these. Suppose the methods of microscopic research should still fail to show in many cases essential or irreversible brain lesions, should we not stultify ourselves if we did not abandon *for the research campaign* both that psychopathology which has taught us the main course of our disease and the neuropathology which has proved usefully negative? Should we not repair at once to the chemistry of metabolism, the physiology of internal secretions, and the entire point of view of psychopathology? Discoveries in the latter fields, concrete and pertinent facts, would carry us back to the tissues and back to the processes of the nervous system, to neuropathology, structural and functional and to psychopathology, and enlighten many dark corners therein. *He who adheres to the classical problems as they lie within the teaching divisions of any science is not apt to change the face of that science.*[2]

It is the method of science to develop the ultimate truth with its numerous and involved qualifications, which are due to the infinite complexity of nature itself, by means of *hypotheses.* These are repeatedly set up and repeatedly confirmed or refuted and replaced by others of better construction in view of previous experience. Whether the hypothesis was exactly correct or not—ultimately tenable or untenable—becomes a matter of no practical significance. *The testing of hypotheses develops facts,* and facts, demonstrated and adequately qualified truths, are the precious heritage of the race from previous human endeavor. Now, then, the hypothesis which underlies the use of the biochemical method in this problem is that which postulates, simply, a relation between patho-

[2] Southard, E. E.: "Psychopathology and Neuropathology: The Problems of Teaching and Research Contrasted," *Amer. Jour. of Psychol.*, **23**: 230–235, 1912. Read by invitation in a symposium at a meeting of the American Psychological Association, December 28, 1911, at the Hospital for the Insane, Washington, D. C.

logical mental action on the one hand, and the physical condition of the brain and body on the other. We will not discuss this proposition—no, this hypothesis—with our readers. It is not worth while. We only wish gently to call their attention to it and to prevent them from shying at this subject on theoretical grounds. This, then, is our generalized hypothesis, and it is clear that finally our logical efforts will be directed toward the correlation of data, psychological and biological, taken in their widest sense. This part of our effort will be small, however, compared with the requirement for painstaking and persistent experimental determination of facts which are the real values we are seeking. In this connection it should be noticed that the present literature of chemical biology contains numerous concrete examples of investigations which have an evident relation to the problems of psychopathology viewed from the broad standpoint of Southard, as above quoted. In future numbers of the BIOCHEMICAL BULLETIN we shall, from time to time, present our readers with notes and criticisms on this literature.

It is important that the general aim of this biochemical effort should not be misunderstood, nor its results misinterpreted. The primary and only initial object is to contribute toward the *elucidation* of the conditions of psychopathological action by means of the biochemical method. The curing of tuberculosis was an entirely premature and abortive expenditure of effort before the elucidation of the cause and conditions of that disease. When once these conditions have been adequately determined, valuable applications of the new knowledge always follow, and sometimes with astonishing results. But now we are only in the beginning of the period of strenuous seeking after much needed information. We wish also to emphasize that we regard the biochemical as only one, but after the psychological the next in importance, of the methods that are available for determining conditions of abnormal mental action. We picture our final understanding of these conditions to be a composite and correlated result obtained by different methods, none of which alone would have ever yielded adequate knowledge.

Now we are asked just what, concretely, is the field of application of biochemistry to the problems of feeble-mindedness.

This question could be best answered by illustrations from the

literature of investigation along biochemical lines; but, as above stated, this we shall continue to present in future numbers of this BULLETIN. At present, before we have actually begun our own experimental work, we can give only an outline of the topics we plan to pursue to such extent as workers and material resources permit. The field is so rich as to tax the judgment in the selection of the first attacks, and we are well aware that we are outlining more than our present resources permit to be done in the near future. Publicity and hearty coöperation with other individuals and institutions is, of course, our policy. In the present article, however, and at the very beginning of our work, we are describing only the nature of the work to be done without specific detail regarding particular problems or methods.

Our primary line of effort to which the others are logically related is the study of the conditions of metabolism presented by the feeble minded of this institution. Very few studies of this nature have been made, and the material for them is here presented under favorable conditions for investigation. Promiscuous examinations or experiments will not be made. But at first typical and psychologically well-known and defined cases will be selected. For orientation they will at first be studied in their undisturbed condition before the experimental factor is introduced. By metabolism we understand, of course, the sum total of the chemical changes which a living organism continually performs within its tissues and upon the substances which it utilizes. The progress of biological science has made the term practically synonymous with the processes of life in so far as they are non-psychical. Under this head we intend to subject the idea of intoxication, whether endogenous (autointoxication) or exogenous, to a rather thorough testing, especially in its relation to psychopathological phenomena. Two other related topics with which we will be compelled to deal in this connection pertain to the subject of glandular secretions and that of lipoid or phosphorus metabolism. It is well known that the method of glandular feeding is extensively practiced in psychopathological cases and institutions. It appears that this is usually done in a promiscuous way with but little of the elements of control experiments or of adequate therapeutic indications. In our future notes and

criticisms on the literature, we shall treat this subject more fully. It seems a pity, from both the scientific and the humanitarian standpoint, that such potentially valuable experiments on human subjects should pass without an examination of their most important factor —that of the metabolism of the physiologically much affected subject.

Our second line of effort will be that of lipoid and brain chemistry. It will not be pursued extensively until we have obtained, from the observations of metabolism and the third line of effort described below, some indications of the directions in this large and inherently difficult field that it would be best to pursue. Contrary to the common impression, the present literature already shows the important and practical bearing of this little developed field of chemistry on the psychopathological problem.

A third kind of work which in the near future will become practically inevitable is the study of heredity, growth and development *from the particular angle of view of the psychopathologist.* It is well known how strongly the scientific and the public attention is now fixed upon the hereditary and congenital (if not hereditary) factors involved in the conditions of abnormal mental action. Without going into detail, we wish to emphasize the fact that the hereditary factor in this problem by no means removes it from the field of biochemical study, nor makes the pathological conditions any less amenable to elucidation by that method. In fact, the only real hope for the elucidation of the processes of reproduction and heredity seems, in the light of experiments already made, to lie in the direction of an intimate knowledge of the chemistry and physics of the protoplasmic basis of life.

Biochemical Laboratory, Training School,
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BIOCHEMISTRY IN NEW YORK TWENTY YEARS AGO[1]

E. E. SMITH

The status of biochemistry in New York City, in 1891, is well depicted by an incident that occurred in that year at the library of the New York Academy of Medicine. The writer had just come to the city and was seeking, for reference, a copy of Maly's *Jahresbericht für Thier-Chemie.* In reply to an inquiry, he was informed that such a work was not in the Academy library and would most likely be found at the Veterinary College. Chemistry was, indeed, well established at that time in the curriculum of the medical schools of the city, but it consisted largely of descriptive organic and inorganic chemistry, and found relatively scant application in physiology and pathology.[2]

To the younger graduates of Yale, the pioneer work of Chittenden was known, but it had not been implanted here. It did lead, however, to the inspiration of Dr. C. A. Herter, then beginning to specialize in neurology; and when he came to realize, as he soon did, how closely related was this field to the pathology of nutrition and determined to establish a laboratory for the investigation of this subject, he naturally turned to Prof. Chittenden for someone with technical training to undertake this work.

[1] For previous special contributions to the history of biological chemistry in New York see the BIOCHEMICAL BULLETIN, 1911, i, p. 245, and 1912, i, p. 377.

[2] "To appreciate the significance of all this, it should be remembered that, with the exception of the work in the pathological laboratories of the colleges, the work of the Board of Health, and the work done by Dr. S. J. Meltzer, there was practically no scientific investigation in medicine worthy of the name in New York City at that time (when the 'Laboratory of C. A. Herter' was created). What was true of New York was essentially true of the country at large. . . . Dr. Herter found the study of the nervous system so abounding in confusion that he soon turned his attention to chemical problems, especially those connected with pathological conditions. Among those intimately associated with him in this work have been E. E. Smith, A. J. Wakeman and, of late, H. D. Dakin." LUSK: *Science,* 1911, xxxiii, p. 846. [Ed.]

How little Dr. Herter appreciated the equipment that would be required is indicated by his suggestion that the work be conducted in the art studio of his brother, then absent in Europe. It did not require many months, however, to reveal to him something of the technical scope of the field to which he was to devote the two decades permitted him for the completion of his life work. At the outset, he desired adequate equipment; and when he returned from his summer rest, in 1891, he was enthusiastically appreciative of the well-equipped laboratory awaiting him in the basement of his residence. This, three years later, was transferred to his newly built home where the entire upper floor, 50 × 100 feet, was devoted to this special work. It was no unusual, though an unique, experience to house in his animal room, rabbits, dogs, monkeys, full grown hogs, and other animals in an array that would have astounded the uninformed passerby in this district of elegant homes.

It was not, however, the equipment that invites attention to Herter's early work nor was it the display he made of his devotion to this new field. Both were modest. What has lingered and always will remain in my memory of twenty years ago is the seriousness with which the work was undertaken. In later years, when his life was so filled with the success and magnitude of his work, it was to be expected that he would throw all that was in him into it; but that he should have devoted himself so largely to it when its value was uncertain, or at least not demonstrated, indicates the profound purpose that was leading him to undertake it. Not infrequently, when night had come and the day's work was done, we forgot ourselves in both discussing what we had attempted and planning what we hoped to do; finally awakening to a realization that we were neglecting the proper demands of our respective family circles.

Only a fraction of the work done at that period was ever published. The first paper, "Uric acid elimination in health and disease," was a record of investigations inspired by the extravagant claims of the English physician, Haig. We differed with him in many important conclusions. We did not find that uric acid formation was always constant and that elimination was determined by the degree of alkalinity of the blood, but found it to vary with the diet

in health and with conditions unknown to us in disease. Moreover, we did not recognize it as a causative factor in the many diseases to which this role was assigned by Haig, but rather regarded its increased elimination as a result of the morbid condition. Horbaczewsky's work did not come to our attention till after the publication of this first paper.

The study of epileptics led to the conclusion that, in some cases of so-called idiopathic epilepsy, the onset of the seizures was determined not by a uric acid accumulation, as claimed by Haig, but by a toxemia of gastro-intestinal origin. Indican, which had received scant attention from clinicians up to this time, was found to be a valuable index to the condition; as was also the elimination of phenol and ethereal sulphates. The occurrence of these products in undue quantity seemed to bear a direct relation to the onset of the seizures.

As was natural, there followed an elaborate study of the gastro-intestinal conditions in other diseases, especially those with marked neurotic manifestations; and the conclusion was reached that the neurotic exacerbations in many conditions were due to a gastro-intestinal toxemia. An entirely different line of study was the presence of lead and its distribution in cases of chronic lead poisoning. The results of these analyses were never published.

Investigations to which was devoted a very great amount of work and which covered a very wide scope, as well, were the studies of the causes of uremic intoxication. The many theories which had been elaborated to explain this condition were each in turn subjected to investigation, involving extensive animal experimentation as well as intricate chemical research. The work covered several years and the results were of very great interest to us, and certainly influenced Herter's later work, but they were never published.

During this period, there was a striking lack of activity in research in chemical pathology in New York; indeed, this was only the time of the awakening of general interest in the most active centers of medical science. Aside from Herter's work, only a single paper presented at the Academy of Medicine in that period comes to my mind; and that was so glaringly faulty that one hesitates to consider its sincerity.

Von Noorden's *Pathologie des Stoffwechsels,* which appeared

at this time, was as a beacon light on a dark night. I received my copy before Dr. Herter's attention was called to the work. He saw it on my table and, borrowing it, informed me shortly afterwards that if I really wanted a copy I had better send for another. True to his word, I never saw this first copy again and I doubt not that it rests now in his library well worn with eager study, which it received at that time. My second copy served a similar purpose in my own hands.

My personal relation with Dr. Herter was interrupted by the decision to study medicine. The modest beginnings of his work, which hardly interested more than a narrow circle of personal friends and admirers, grew to a proportion that brought him into national and, indeed, international prominence. A man of unusual personal charm and sincere purpose, he demonstrated how personal opportunity could find unselfish application to the benefit of his fellowmen in the field of applied medical science.

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IMMUNITY IN SOME OF ITS BIOCHEMICAL ASPECTS[1]

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(WITH PLATE 2)

Infection. One of the most interesting problems to all of us is that presented by disease, especially by what we call "infectious" disease. Under this term we mean disease produced by living organisms or their products. Among the organisms producing disease in man are bacteria, molds, yeasts, and protozoa, and we may conveniently speak of these collectively as germs.

The manner in which the various germs produce disease in man, their mode of entrance into the body, the part of the body attacked —all these differ considerably with the different germs. Some like the bacillus of diphtheria and the bacillus of tetanus (lockjaw) secrete very powerful poisons, and while the germs themselves do not penetrate deeply into the body tissues, their poison is absorbed and gives rise to severe symptoms. In the case of other germs, for example the tubercle bacillus, the organisms penetrate deeply into the body tissues and there multiply. In their growth they destroy the cells in which they lodge and, by their poisons, affect the entire body.

[1] Lecture delivered, by invitation, under the auspices of the Columbia University Biochemical Association, at the College of Physicians and Surgeons, November 16, 1912.

Most germs, for some obscure reason, affect by preference certain parts of the body. The typhoid bacillus usually lodges in the wall of the small intestine; the meningococcus prefers the lining membranes of the brain and spinal cord; the gonococcus is very prone to attack the mucous membrane of the genital organs and of the eye; the pneumococcus affects chiefly the respiratory organs; the diphtheria bacillus lodges in the throat and nasal passages; the malaria parasite lodges only in the red blood cells; and certain molds affect only the skin.

Immunity. NATURAL IMMUNITY. It is very well known, however, that certain infectious diseases occur naturally only among some of the lower animals and do not affect man, while conversely, others appear to attack only man. Among the latter may be mentioned typhoid fever, syphilis, gonorrhea. In speaking of the resistance evidently possessed by certain species we make use of the term *natural immunity*. Thus chickens and frogs possess a natural immunity against tetanus (lockjaw); dogs, a natural immunity against anthrax; goats, a natural immunity against tuberculosis; and man, a natural immunity against certain diseases of cattle. This natural immunity, however, is not always absolute. Chickens, for example, can be infected with tetanus if their bodies are chilled, and frogs can be made susceptible to tetanus by keeping them unduly warm.

ACQUIRED IMMUNITY. Another form of immunity is that observed in individuals who have had one attack of a particular infection; thereafter they are practically safe from a second attack. These individuals are said to possess an *acquired immunity*. This form of immunity is well illustrated in scarlet fever, measles, smallpox, yellow fever. Often this immunity lasts throughout the lifetime of the individual though there are exceptions.

In studying this form of immunity, Pasteur conceived the idea of artificially producing an attack of a given infection in order to protect the individual against another attack. He realized that it was necessary, however, to so control matters that the original attack should run a very mild course and not endanger the life of the individual. After considerable experimental work, Pasteur found that this could be accomplished by artificially weakening the

bacteria with which the original attack was produced. Subsequently Salmon and Smith, in this country, showed that it was not necessary to produce even a mild attack of the disease by injecting living germs, but that the injection of dead germs would produce an immunity against that particular infection.

Specificity of acquired immunity. Acquired immunity, whether caused by a previous natural attack of the disease, or artificially by the inoculation of living or dead germs, is always *strictly specific;* that is, the protection extends only to the particular disease which has previously occurred or against germs of the kind previously injected. An attack of scarlet fever protects only against scarlet fever but not against measles. Inoculating an individual with typhoid bacilli protects him only against typhoid fever, but not against dysentery, plague or cholera. This acquired immunity is often transmitted from mother to offspring, transmission being effected mainly, according to Famulener, through the colostrum.

ADDITIONAL NATURAL DEFENSES AGAINST DISEASE. Before examining into the nature of specific acquired immunity, let me call attention to certain important means by which the body is protected against infectious diseases in general. Many of these means are so commonplace that their significance is often overlooked.

The protection afforded by the unbroken skin is undoubtedly one of the most important means of defense. A similar protection, though less effective, is afforded by intact and healthy mucous membranes. The acid gastric juice undoubtedly destroys large numbers of swallowed germs. It has been found that fresh blood serum is able to kill a considerable number of germs, and this is therefore another mode of defense. The white blood cells (leucocytes) appear to be designed especially to destroy invading micröorganisms. These cells take hold of, or rather engulf, the germs and digest them. Still another mode of defense is seen in what takes place in abscesses. When these are examined, it is found that the body has built a wall of cells around the infected area, thus shutting off the germs and their poisonous products from the rest of the body. Finally, mention may be made of the collection of fluid, *i. e.,* of serum, as perhaps a means designed to dilute irritant poisons (pleurisy, peritonitis).

The means of protection we have just recited are all general in their action, that is, not directed specifically against only one particular infection. Let us now return to a consideration of the specific acquired immunity already mentioned.

Behring's discovery of antitoxin. Most of our knowledge concerning specific acquired immunity dates from Behring's discovery of the antitoxins of diphtheria and tetanus, in 1890.

Behring found that when an animal is injected with gradually increasing doses of toxin, *e. g.*, with diphtheria toxin, it is able, after a time, to withstand doses of the poison sufficient to kill hundreds of animals not so treated. He found that the blood serum of the treated animals contained something which neutralized the diphtheria poison, and rendered it harmless. This something he called an *antitoxin.* Investigation showed that the antitoxin was strictly specific, the antitoxin for diphtheria neutralized only the toxin of diphtheria, the antitoxin for tetanus, only that of tetanus.

Bacteriolysins and hemolysins (cytolysins). Another important advance was made in 1894 when Pfeiffer showed that, just as an animal injected with gradually increasing doses of toxin produces an antitoxin in its blood, so also, when injected with bacteria (cholera bacilli), it produces substances which kill and dissolve the injected micröorganisms. We have already said that fresh blood serum is able to kill a considerable number of bacteria, and that this probably constitutes one of the defenses of the body against bacterial invasion. When the animal is injected with gradually increasing amounts of bacteria, however, this destructive power increases very greatly, but only for the particular kind of bacterium used for injection. In other words, the action is strictly specific. If an animal is injected with cholera bacilli, the serum will, after a time, even in very small doses kill enormous numbers of cholera bacilli; tested against typhoid bacilli, or on other bacteria, its destructive effect is merely that of normal serum from an untreated animal. When the action of the serum is studied under the microscope, it is seen that the bacteria are actually broken up and dissolved. Hence such a serum is spoken of as a "*bacteriolysin.*" Since the bacteria are also killed by this action, we also use the term "*bactericidal*" in speaking of such a serum.

It has been found that this action may be developed against cells other than bacteria. When red blood cells are used for the injections, the serum acquires dissolving properties for these; and here again the action is strictly specific, so that when blood cells from a chicken are injected into an animal, the serum of the injected animal acquires increased solvent powers only for chicken blood cells, not for blood cells of other animals. Sera directed against blood cells are usually spoken of as *hemolysins*. The term *cytolysin* is used to embrace all these cell-dissolving sera.

Complement and immune body. Investigation has shown that the mode of action of these dissolving sera is somewhat complex, and consists of the joint action of two substances. It may be recalled that this dissolving action was observed in *fresh* serum. Serum which had stood for several days no longer possessed this property. The researches of Metchnikoff and Bordet showed that the full solvent power could be restored by the addition of a little fresh serum, even from a normal, untreated animal. Evidently, then, of the two substances concerned in this dissolving action, one is quite stable, and the other highly labile. The labile substance, derived from a normal untreated animal, is spoken of as the *complement;* it is not specific. The stable substance, present only in the serum of the treated animal, is called the *immune body;* it is highly specific. When an animal is repeatedly injected with gradually increasing doses of bacteria, or other cells, it responds by manufacturing large quantities of this "immune body" directed specifically against the injected cells. The complement is not increased in the process.

Agglutinins. When the serum of an animal which has been repeatedly injected with gradually increasing doses of bacteria is brought into contact with some of the bacteria, careful observation under the microscope reveals a very interesting series of changes. Thus, if typhoid bacilli are mixed with a specific antityphoid serum (obtained, let us say, from a rabbit previously injected with typhoid bacilli), one notices, first, that the motility of the bacilli becomes markedly diminished. This is followed by the gradual collection of the bacilli into clumps. At the end of an hour or two, in place of countless bacteria moving quickly through the field, one sees

merely several groups of absolutely immobile bacilli. If the reaction is feeble, the clumps are small, and one finds comparatively many isolated and, perhaps, also moving bacteria. This phenomenon is spoken of as *agglutination,* and the substance in the serum which brings this about is called *agglutinin.* The clumping thus brought about does not kill the bacteria; moreover, it makes no difference whether the serum is freshly drawn or has been kept for some time—it will agglutinate equally well; and it does not require the addition of fresh serum as do the bacteriolysins. Like the antitoxins and the bacteriolysins, the agglutinins are strictly specific, so that serum from an animal previously injected with typhoid bacilli will agglutinate only typhoid bacilli; one from an animal injected with dysentery bacilli, only such bacilli, etc.

Opsonins. We have already said that the white blood corpuscles (leucocytes) take up bacteria and destroy them. Wright, of England, showed that certain substances in blood serum have the power of increasing the appetite, as it were, of the leucocytes, and furthermore, that the amount of these substances can be increased by properly graduated injections of the appropriate bacteria. These substances he called *opsonins.* They are specific, just as are the antitoxins, the bacteriolysins, and the agglutinins; that is to say, when typhoid bacilli are injected into the body, only the opsonin for typhoid bacilli is affected; when staphylococci are employed, only the opsonin for such organisms is affected, etc.

Precipitins. If, instead of injecting bacteria or other cells, we inject an animal with solutions of albuminous material; for example, if we inject a rabbit with chicken-egg albumin, we find that the rabbit serum acquires the power to produce a precipitate when mixed with chicken-egg albumin. This action, too, is highly specific, so that if the serum is tested against the albumin from any other animal, *e. g.,* from a duck egg, no precipitate will be produced. If a rabbit is treated with human blood, the rabbit serum will produce a precipitate when mixed with human blood, but not when mixed with any other blood. The substance in the treated animal's serum is spoken of as a *precipitin.* This test, as you probably know, is used in criminal cases to determine whether or not certain stains are those of human blood or otherwise.

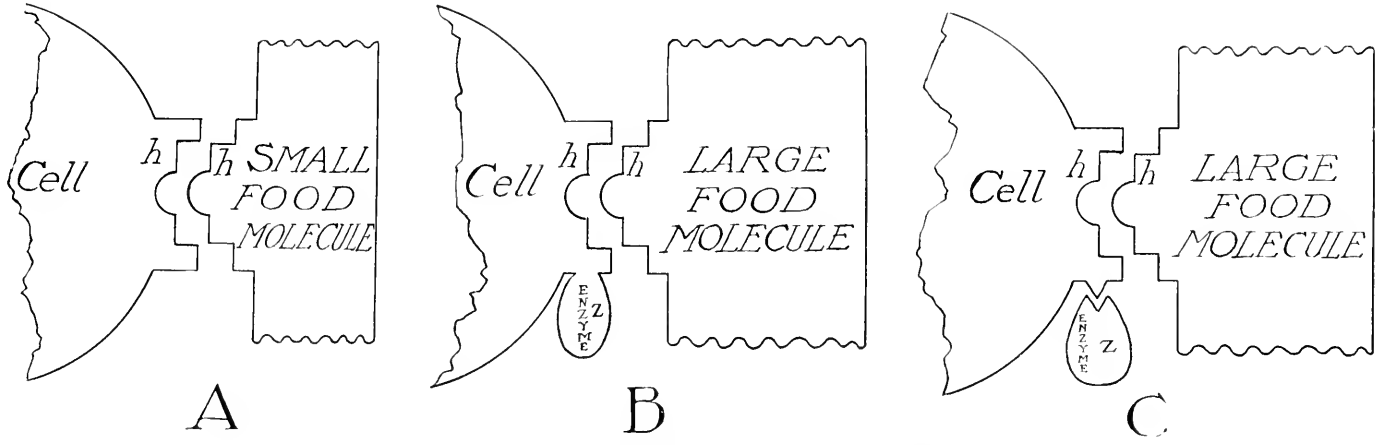
ANTI-ANTIBODIES. But even this list does not exhaust the list of "antibodies" which it is possible to produce. When enzymes are injected into an animal, the latter responds by producing anti-enzymes, and when certain "antibodies" are injected, *anti-anti-bodies* are produced.

Immunity regarded from the standpoint of cell nutrition. The whole subject of infection and immunity, and particularly the production of the antibodies just discussed, is best appreciated when regarded from the standpoint of nutrition; for what, after all, is this apparent conflict between bacteria and the animal body but the mutual attempt of each to use the other for food. Let it be noted that production of the various antibodies takes place only when the bacteria or other alien cells are introduced *parenterally, i. e.,* by ways other than the gastrointestinal tract. We may explain this by saying that when introduced by the gastrointestinal tract the molecules of the food stuffs (organic) are split up and rebuilt in such a way that the material requires no further extensive alteration in order to serve as food for the various cells of the body. In the animal body this breaking down and building up is delegated to certain specialized cells; in the primitive organisms, however, we must believe that each cell was required to break down and build up its own food. When parenterally situated cells of the higher animal are thus presented with the unprepared food which the parenteral introduction brings them, it may be assumed that they behave as does the primitive cell, and proceed to lay hold of and attempt to assimilate the injected material. With this introduction, we may pass at once to a consideration of Ehrlich's "side chain theory," which still offers the best explanation for the formation of the various antibodies. It is essentially a theory of cell nutrition.

EHRLICH'S "SIDE CHAIN" THEORY. According to Ehrlich's conception, every cell is armed with a large number of chemical groups whose function is to lay hold of nutriment and anchor this in the cell. These groups he calls *receptors* or *side chains.* Only such substances can serve as nutriment which can thus be bound chemically to the cell protoplasm. He believes that the receptors are of at least three different kinds, and speaks of receptors of the "first order," "second order" and "third order." These are best described with the aid of a diagram such as the accompanying one.

Receptors. In view of what has been said it is obvious that the simplest mechanism by which the cell can lay hold on food particles is a receptor which merely anchors food, leaving the digestion entirely to the cell proper. It may be assumed that this type of receptor suffices for comparatively small food molecules. When a larger and more complex food molecule presents itself, it may be assumed that a receptor would be required which not merely anchors but also acts on the food molecule to make it more readily assimilable. These two types are shown in *A* and *B* respectively (Plate 2). It will be noted that the receptor in *B* possesses an anchoring group (*h*) and an active group (*Z*) which acts on the food molecule. It is conceivable that an economy in structure could be effected in *B*, if, in place of the active group (*Z*), there were merely provision for the anchoring of an enzyme. The active group (*Z*) could then be dispensed with and the enzyme called upon only when a food molecule had been anchored by the receptor. Such an arrangement is shown in *C* (Plate 2).

Weigert's "over production" theory. At this point you may very properly inquire why we assume the existence of receptors of these types. To explain this, let us go back to the production of antitoxin in response to injections of toxin. It will be recalled that the toxin can be neutralized by the antitoxin. Moreover, and this is the important point, this action is strictly specific, so that, for example, diphtheria antitoxin neutralizes only diphtheria toxin; against any other toxin it is absolutely without effect. Since it can be satisfactorily shown that the antitoxin is not altered toxin, it is necessary to explain the production of antitoxin by the body cells. We have said above that only such substances can serve as nutriment for the cell which can be tied chemically to the cell protoplasm. Expressing this in terms of receptors, we would say that only such substances as possess groups fitting the receptors of the cell can be anchored to the cell. In thinking of these groups and the way in which they fit together, we must have stereochemical relations in mind. Ehrlich cites with approval a simile used by Emil Fischer, saying that the relation of the two groups must be that of lock and key. Granted, now, that certain food molecules have been anchored by fitting cell receptors, what follows? To explain this,



BOLDUAN: IMMUNITY IN SOME OF ITS BIOCHEMICAL ASPECTS.

Diagrammatic representation of receptors of three kinds.

Ehrlich makes use of an hypothesis advanced by Weigert in connection with regeneration. According to this, physiological function and structure depend upon an equilibrium of the tissues that is maintained by virtue of mutual restraint between their component cells. Destruction of a single integer or group of integers of a tissue or a cell removes a corresponding amount of restraint at the point injured, and therefore destroys equilibrium. This permits of the abnormal exhibition of bioplastic energies on the part of the remaining uninjured components, which activity may be viewed as a compensating hyperplasia. When such bioplastic activity is called into play there is always hypercompensation; that is, there is always more plastic material generated than is necessary to compensate for the loss. Thus far Weigert.

Ehrlich, in line with Weigert's over production theory, points out that, owing to the combination of toxin with receptors of the cell, the receptors are practically lost (at least temporarily) to the cell; that the cell or its fellows now produces new receptors to replace this loss; but that this production always goes so far as to make a surplus of receptors; that these receptors are thrown off by the cell, as unnecessary ballast so to speak, and then circulate in the blood as antitoxin. The same substance therefore, which, when part of the cell, combines with the anchoring group of the toxin, enabling this to act on the cell, when circulating free in the blood combines with and satisfies this anchoring group of the toxin, and prevents the poison from combining with and damaging the cells of the organism. It is obvious that this affords a complete explanation of specificity.

If we now go back to our diagrams (plate 2) we shall see that all of the antibodies discussed above fit readily into this scheme. So far as the antitoxins are concerned, these would be merely receptors of the first order, thrust off from the cell and circulating in the serum. Agglutinins and precipitins would belong to the second order; they have the active group as an integral part of the receptor. The hemolysins and bacteriolysins would be in the third order; fresh serum is active because it contains complement, but since the complement is very labile, the serum after a while contains only the immune body, *i. e.*, that part of the receptor which anchors the food molecule on the one hand and the ferment substance on the other.

Explanation of natural immunity. Ehrlich's views concerning the necessity for fitting receptors in order that a microörganism may attack the body cells afford a satisfactory explanation of the immunity possessed by certain animals against particular infections. Thus, it is obvious that the entire absence of receptors fitting a certain microörganism renders the body immune against infection by that microörganism. Moreover, the location of the receptors may be responsible for the relative immunity of an animal under natural conditions and its susceptibility when these conditions are changed. Thus, if receptors for a particular poison are present both in a vital tissue, like the brain, and in an indifferent tissue, like the muscles, it is clear that while an intracerebral injection of the poison might prove fatal, an intramuscular one might be almost without effect.

Anaphylaxis. Most of you are probably familiar with the work of Vaughan and Wheeler concerning the cleavage products of proteins, and recall that some of their products were highly poisonous. Certain observations of the past few years indicate that, in the parenteral digestion of proteins, similar cleavage products are produced. Historically this aspect of immunity may be said to date from 1906, from the studies undertaken by Ehrlich's pupil, Otto, and from experiments made about the same time in the U. S. Hygienic Laboratory by Rosenau and Anderson. In the course of the standardization of diphtheria antitoxin, it had been noted that guinea pigs, which had previously been injected with toxin-antitoxin mixtures, were often killed by a subsequent injection of horse serum. When the subject was studied it was found that, when an animal is injected with an alien protein, there develops after a time a specific hypersusceptibility for this protein. After a definite interval, if the animal is given a second injection of the same protein, violent symptoms occur, which may end fatally. The reaction is specific, so that animals sensitized, for example, to horse serum, manifest little or no hypersusceptibility to other sera. It is possible, however, to sensitize an animal to several proteins simultaneously. The sensitizing dose may be very small—even as little as one-millionth of a cubic centimeter of horse serum has sufficed to render a guinea-pig sensitive. A varying length of time must elapse after the sensitizing injection before the animal becomes fully

sensitive. In guinea-pigs treated with small doses of horse serum, from 12 to 14 days suffice; with large doses, the time required is longer, and may extend over weeks or even months. In any case, in order to produce severe symptoms, it is important that the second injection be large, say 5 to 10 c.c. in a guinea-pig. This phenomenon, spoken of as *anaphylaxis,* has come to occupy an important place in the theory of infection and immunity. What is the explanation of the phenomenon?

Enteral and parenteral introductions of protein contrasted. We know that the subcutaneous, intraperitoneal, or intravenous introduction of alien protein is followed by the formation of antibodies; at the same time it can readily be shown that no antibodies develop after the oral introduction of milk, eggs, or even of raw meat. In other words there is a marked contrast in the behavior of the body toward *enteral* and *parenteral* introductions of protein. In the former the protein is acted on by specialized cells, which, through their pepsin, trypsin and enterokinase, and erepsin, break down the protein molecule so that it loses its species identity. After this, absorption takes place, and with it there is a synthesis or rearrangement of the molecule whereby it is built up into protein specific to the body. Under normal conditions it is impossible to produce antibodies by feeding alien protein, though precipitins have been produced by overfeeding animals with large amounts of alien protein. When protein is introduced *parenterally* it gives rise to the formation of specific antibodies. In the case of the sensitized animals described above, the first injection causes the production of specific antibodies, among them specific cytolysins acting on the alien protein molecule. When the second injection comes, the alien protein is at once laid hold of by this antibody, protein cleavage results, and with it the liberation of poisonous cleavage products. These cleavage products cause the severe symptoms and even death that characterize anaphylaxis in guinea-pigs. That similar symptoms do not arise in the enteral digestion of protein would then be explained by saying that, in the specialized digestive apparatus, provision has been made either for preventing the formation of such poisonous cleavage products or for neutralizing them (conjugations?) before they can cause injury.

Significance of the period of incubation in anaphylaxis. An interesting result of these studies on anaphylaxis is the light they shed on the significance of the period of incubation, and also on the poisonous symptoms produced by bacteria from which no very poisonous substance can be extracted. Taking up first the latter point: It has been held that the various pathogenic bacteria, like the diphtheria and tetanus bacilli, either secrete toxins or, at least, contain such toxins bound up in their protoplasm. In the latter case, it was believed that these *endotoxins,* as they were termed, were set free during the destruction of bacteria in the body. From his studies on anaphylaxis, Friedberger concludes that it is entirely unnecessary to assume the existence of specific endotoxins in bacteria to account for the various symptoms seen in bacterial infections. By repeatedly injecting sensitized animals with minute doses of sheep or horse serum, he found it possible to produce all manner of fever curves at will, merely by varying the size of the dose and the interval between the injections. From this he concludes that the diversity of clinical symptoms of various infectious diseases can readily be explained, even on the assumption of but a single poison. He speaks of this as *anaphylatoxin,* and regards it as a cleavage product of protein of whatever origin introduced parenterally. Just as in enteral digestion, uniform cleavage products are formed from most diverse proteins, so, he believes, that in the parenteral protein decomposition leading to the formation of anaphylatoxin, a certain poison is uniformly produced. Whether or not, in addition to anaphylatoxin, there are other specific poisons for the various infectious diseases is immaterial; their existence (except in certain diseases) has not been proved, and the assumption of their existence is unnecessary. According to Friedberger, the assumption of a common anaphylatoxin is only apparently in contradiction to the well known law of specificity of infectious diseases. In the infectious diseases it is not the poison which is specific, but only the mode of its production. The production of anaphylatoxin requires the action of antibodies; the mere solution or disintegration of bacteria by other means does not suffice. In other words, a particular cleavage of the protein molecule is necessary.

Bearing of the period of incubation on intoxication by bacterial

infection in general. With this conception of the effects of parenteral protein cleavage, it is a simple matter to explain the significance of the period of incubation. For those of you who are not medical students, I will say that every infectious disease manifests itself only in a certain period of time after infection has taken place. Moreover, this interval is fairly constant. Thus, after a person has contracted typhoid fever, some ten to fifteen days elapse before symptoms develop. In measles, the incubation is regularly fifteen to eighteen days; in scarlet fever, regularly from three to five days, etc. Formerly this period was explained as the time necessary for the development of germs in sufficient number to produce symptoms. This explanation was unsatisfactory, because, in artificial infections, no matter how large the dose, it was never possible to shorten the incubation period below a certain minimum, and this minimum could not be explained. If, however, we regard infecting bacteria as protein introduced parenterally, we shall have no difficulty in explaining the incubation period as the time necessary for the body to develop antibodies which shall act on the bacteria and produce poisonous cleavage products. Even if we do not accept Friedberger's assumption of but a single anaphylatoxin, the same explanation holds for the liberation of *endotoxins.* In this connection, I ought to say that bacteria invading the body through the intestinal tract, *e. g.,* the typhoid bacilli, may still be regarded as introduced parenterally, because they pass the intestinal barrier and gain access to the other tissues of the body.

Modern chemotherapy according to Ehrlich. Before leaving the subject of infection and immunity, I should like to say a few words about the chemistry of the cell in relation to chemotherapy. I have already pointed out that Ehrlich holds that the action of a chemical substance on a given cell denotes the existence of definite chemical affinities between the substance and the cell. Applying this conception to the germicidal action of chemicals, he maintains that the latter must have a certain chemical affinity for the parasites in order to kill them. Substances having such affinities he terms *parasitotropic.* It is clear, however, that substances which can destroy parasites will also be poisonous for the animal body, *i. e.,* they will have chemical affinity for the tissues of the host. They are

therefore also *organotropic*. In the employment of chemical substances to combat infectious diseases, it follows that success can only be attained if the affinity of the chemical substances for the infecting parasite bears certain relations to their affinity for the infected body. Ehrlich's studies in this direction have, therefore, aimed to find poisonous substances whose parasitotropic affinity should be great in comparison to their organotropic affinity. In his studies on syphilis, he tested a very large number of substances, many of them combinations of arsenic. As each substance was tested it received a serial laboratory number, and finally, in "606," a substance was found which fitted the requirements to a high degree. This substance, *salvarsan,* has produced really marvelous results in the treatment of syphilis. This line of work appears very promising.

Chemical nature of antibodies. A closing word concerning the chemical nature of antibodies. Most of the studies have been made on diphtheria antitoxin, and although little is known concerning the constitution of this substance, it seems proable that it is protein in character. Certain it is that the antitoxin is associated with the globulins of the serum, and highly concentrated solutions of antitoxin have been prepared, by Gibson, by precipitating and then redissolving these globulins. Moreover, as Atkinson showed, the globulins increase markedly in the serum of immunized horses as the antitoxic strength of the serum increases.

The influence which the development of the field of immunity has had on biochemistry has been tremendous, for it has contributed not only new view points, but also entirely novel methods. Much has been learned about substances which no one had ever yet seen and which we know only through their action. That all this has been achieved is due mostly to one master mind, Paul Ehrlich. May he long continue to lead us!

A PLAN FOR THE ORGANIZATION OF THE AMERICAN BIOLOGICAL SOCIETY[1]

ALBERT P. MATHEWS

The present condition of the biological interests of the country may be called chaotic. There is no general organization and little cöoperation between various subdivisions of the science; there are a multitude of small societies and a large number of journals, few with any permanent support. This condition renders the science as a whole less effective in the community than it ought to be, and is expensive both of time and money. The time has come to effect some kind of coöperation of all biologists to secure the advantages which come from coöperation. These advantages could be obtained by the formation of a general society, to be called the AMERICAN BIOLOGICAL SOCIETY, along the lines of the American Chemical Society. (This Society might act as the Biological Section of the American Association for the Advancement of Science.)

Objects of the society: (1) To unite the biological interests of the country for purposes of education; mutual support; increased coöperation, defense and encouragement of scientific investigation; and to increase the influence of biological knowledge in the country; (2) to start and support a *Biological Abstract Journal;* (3) to provide for the permanent support of the biological journals of the country and to provide for new ones as necessity arises; (4) to

[1] This plan was proposed by Professor Mathews in 1908, in multigraphed circular form, to the members of the American Physiological Society. The plan was formally laid before the Council of the Physiological Society in December, 1908, in the hope that the Physiological Society would endorse the essential features of the suggestion. The author was appointed a committee of one to agitate the matter. Nothing further was done, however. The unsuccessful effort in December, 1911, to bring about an organization of a greater American Physiological Society, and the recent formation of the Federation of American Societies for Experimental Biology, give new interest to Professor Mathews' plan, which is published here in its original form, *at our request,* and with the permission of the author. See pages 269 and 271 of this issue of the BIOCHEMICAL BULLETIN. [Ed.]

diminish the cost, to the members of the society, of dues to societies and subscriptions for these journals.

Details of organization. MEMBERSHIP. All members of the present biological societies should be eligible for membership without further action and should become members on payment of the dues. Such societies are those of Anatomy, Physiology, Zoology, Botany, Experimental Medicine, Pharmacology when organized, Psychology, Biochemistry, Bacteriology, and so on. All persons sufficiently interested in the progress of biology to pay the dues of the society should be eligible for membership.

LOCAL SECTIONS. The constitution should provide for the formation of local sections in different cities, a certain per cent. of the dues of the members of such a local section to be repaid to the section for local expenses.

AFFILIATION OF PRESENT SOCIETIES. The present societies should ultimately organize as sections of the Biological Society, thus saving extra dues. Membership in these sections might be determined by the sections themselves.

DUES. Dues should be sufficient to provide that each member should receive the *Biological Abstract Journal,* and some or all of the other biological journals. How this may be arranged is shown beyond (page 265). The cost of the journals should be much lower to the members of the society than to outsiders.

Explanation of the proposed plan. The plan presented in the foregoing statements is virtually that adopted with such great success by the chemists of the country. A few years ago the chemists were in the position of the biologists today. There were several small societies; there was nominally a general organization dragging out an unprofitable existence. There were several journals badly supported. The American Chemical Society was organized and ultimately the smaller societies became convinced of the advantage of coöperation. Now, most of them have become sections of the general society. The growth of this society has been very rapid; and it has grown in vigor as well as in size. Two years ago[2] the society started a chemical abstract journal and it is not too much to say that this has done more for the chemical interests

[2] The reader is reminded that this was written in 1908. [Ed.]

of the country than any other step taken. *Chemical Abstracts* has welded the various divisions of the science together, and so great has its value proved to be, that the membership in the society has almost doubled since it was started.[2] The society publishes three journals, *Chemical Abstracts* (the abstract journal), the *Journal of the American Chemical Society,* and the *Journal of Industrial Chemistry,* which are distributed to all members of the society for the dues, $10 a year. *Chemical Abstracts* appears every two weeks; the other two are monthly journals.

Relation of the society to the naturalists. Two possibilities are open to us in forming the Biological Society: we could make use of the American Society of Naturalists, reorganize that and change it into a new society with new aims; or we might start a new society, leaving the "Naturalists" to fulfill some other useful function (such as that adopted in their recent reorganization). The name of the "Naturalists" is badly chosen for a general biological society, such as that proposed; and, since its partial resuscitation along its old lines might weaken our efforts (if the two societies should cover in any way the same field), it appears wiser to me to organize a new society, and to allow the "Naturalists" to have its aim changed to the one sketched in the plan of reorganization.

Discussion of the objects of the proposed biological society. THE IMPORTANCE OF A BIOLOGICAL ABSTRACT JOURNAL. (1) The objects in paragraph 1, page 261, are so desirable as not to need discussion. (2) The desirability of starting a *Biological Abstract Journal,* in English, has long been apparent. Funds alone have been lacking in the past to accomplish this object. The organization of this society would make it possible to issue such a journal. *This would do more to unify and stimulate biology than any move we could make.* (3) How the ends sought in objects 2, 3, and 4 (page 261) could be attained, will now be shown.

LIST OF THE PRESENT BIOLOGICAL JOURNALS AND THEIR ESTIMATED COST AND PRICE OF SUBSCRIPTION.[2] The figures submitted in this list are approximate only and are based on estimates supplied by various firms and individuals. The subscription list is a rough

[2] The reader is reminded that this was written in 1908. [Ed.]

estimate only. The cost is estimated on an edition of 500 copies.

Estimated cost of journals, containing tables and cuts, and printed on good paper with press work included; edition of 500 copies: 12-point (a good sized body type) $1.40 a page; 10-point (used for reviews) $1.80 a page; 8-point (bibliography) $2.11 a page; 8-point (tables) $3.40 a page.

Blank pages, and pages made up wholly of figures, $0.90. For half-tones, in the text, there is an extra charge of $1.00 each for makeready. For process-plates on coated paper: single plates, $4.00; double plates, $8.00.

An additional 1,000 copies would increase the cost only for inserts, the press work and the paper, and may be estimated at about $500 or $600 a year, on a journal of say 1,000 pages. The cost of a journal is thus seen to be almost wholly the cost of putting it on the press, or the cost of its first 500 copies.

Name of Journal.	*Subscription price per year on basis of present issues.*[2]	*Estimated cost of 500 copies.*
American Journal of Physiology	$15	$7,000
American Journal of Anatomy	5	3,000
Journal of Comparative Neurology	4	2,000
Journal of Morphology	9	3,000
Journal of Infectious Diseases	5	3,000
Journal of Experimental Medicine	5	2,500
Journal of Medical Research	8	4,000
Biological Bulletin	6	2,500
Journal of Biological Chemistry	8	3,500
Journal of Experimental Zoology	5	2,500
Anatomical Record	3	2,000
Psychological Review (bulletin and index)	5	3,000
Botanical Gazette	7	4,000
Total number, 13	$85	$42,000
Biological Abstract Journal		4,000
American Journal of Psychology	5	2,000

There are several other journals which might be added to this list and there are a few journals on the list which might be taken

[2] The reader is reminded that this was written in 1908. [Ed.]

care of by special institutes.[2] It is obvious, however, that the biologists have before them the problem of putting on a permanent foundation journals costing about $50,000 a year. This can best be done by making each journal self supporting, and this is only possible by increasing the number of subscribers.

How to increase the number of subscribers for the journals. At present it costs, let us say, $28 a year to subscribe for the *Journal of Physiology,* the *Journal of Biological Chemistry,* and the *Journal of Infectious Diseases.* Each of these journals probably has on the average a paid subscription list of something under 400.[2] Membership in the corresponding societies costs, in addition, about $2 a year for each society, or a total yearly expense of $34.

Now, if we could make a society of 2,000 members and charge each member $25 a year for all dues or, to be more liberal, let us say $30 a year, the society would have an annual income of $60,000; and for this sum, it could publish and supply to its members not three but thirteen journals without further cost. Moreover, each of these journals would have a large circulation, beneficial alike to the man who published in it and to the journal itself. Furthermore, the amount of the individual society expenses would be greatly reduced since, by proper organization, one or two paid secretaries would look after notices of meetings; bills for postage, announcements, programs, etc., would be less; and a saving would be effected all along the line, with a great gain in efficiency.

The income of the journals would also be augmented beyond the dues by the constant sale of back numbers, the sale of extra reprints and, in some cases, by legitimate advertising. Moreover, by keeping the present prices in effect for all non-members, nearly everyone would hasten to join the society; thus increasing our numbers and increasing the number of those among whom the expense would be divided, and making it possible, from time to time, to start new special journals with little increase in expense to the members. Furthermore, by maintaining the present prices to libraries and foreign subscribers, a considerable sum would be added to the treasury. For example, the present cost of these journals to subscribers is $83 a year. If there were a hundred subscribers at this price, it would add $8,300 to our income. Of course the total may easily be less

[2] The reader is reminded that this was written in 1908. [Ed.]

than this, but it will certainly amount to half that sum, since there must be fifty libraries subscribing at the old rate.

How to get the necessary 2000 members. As a nucleus of the society all members of the biological societies would probably join. There are 1,500 names in Cattell's *American Men of Science* who would be eligible and upon whose support we might confidently count. In addition probably 300 have joined the ranks of biology since that publication was issued or whose names were omitted through oversight. Let us say, at a liberal estimate, 1,800 all told. There probably could be found in addition 500 intelligent and public spirited physicians, and others sufficiently interested in biology, to join such a society with such great advantages in the matter of journals. These figures are maximum figures but they suffice to show that we could count on perhaps a thousand members at the start; and there is no doubt that the numbers would increase rapidly, just as they have done in the Chemical Society. We might also soon start a *Journal of Biological Industries,* or in other ways increase coöperation between the practical applications of biology and the science itself.

Other arguments might be presented, but these suffice to show the great advantages of coöperation and to make it evident that, in this way, we could attain these desirable objects: increase the influence of biology, increase coöperation; knit the science together, strengthen its practical applications; start a *Biological Abstract Journal;* provide for the support, and enlarge the usefulness, of our present journals, and provide for new ones as the need arises; and diminish the cost, to each one of us, of subscriptions and dues.

We should also accomplish more than this, for, by such an organization, we should be providing for the future, and organizing with the object of attaining certain well defined ideals. Whatever organization is attempted at this time should have in view the practical attainment of these ideals and should not be a mere repetition of what we have, with no definite plan and without foresight.

In view of the foregoing facts I move the adoption of the following: That the American Physiological Society expresses its approval of the objects sought in the plan presented for the formation of the AMERICAN BIOLOGICAL SOCIETY; and it recommends,

further, that the Society transmit to the other societies copies of this plan with the request that the plan be presented to the members of the societies; that each society appoint one delegate to meet members appointed by the other societies to act as a committee of organization of the AMERICAN BIOLOGICAL SOCIETY; and that such committee shall carefully examine into the feasibility of such an organization and, if possible, draft a constitution and report to the societies at their next annual meeting.

Suggestions for carrying out, practically, the journal part of the plan. (1) It will possibly be found that $30 or $20 a year is more than the majority of the Society feel able or willing to pay. Arrangements could be made whereby at a somewhat larger relative cost such members could subscribe to two, three, or half a dozen of the journals as they desired. Arrangements could be made with the journals whereby copies would be sent to the members of the Society at a reduced price, if a certain number of subscribers was received in this way. For example, the Society might offer the *Biological Abstract Journal,* and any two others, for $10 a year; the *Biological Abstract Journal,* and five others, for $20 a year; and the whole number, say, for $30 a year. In this way there would always be an incentive for the members, who could not at the start pay the full sum, to increase their subscriptions and thereby enable everyone to get his subscription at a reduced cost. It would not, however, be possible on this basis to give so much to the members as if all subscribed to all the journals, but still a great reduction of cost could be obtained. The object aimed at should be to increase as rapidly as possible the numbers of those taking the whole number of journals.

(2) The relation of the Society to the management of the journals would, of course, have to be worked out gradually. Several courses are open to the society. One is to leave the journals as they are under their present control and for the Society to make such arrangements with the journals as would be most advantageous to the members. This is the club-rate principle, the society buying so many copies at a reduced rate to distribute to its members. This arrangement might do as a temporary makeshift, to get started, but would probably be unsatisfactory in the long run, since it would not be permanent enough.

The Society might take over the financial responsibility of such journals as the Council of the Society deemed best; beginning, for example, with one or two with the largest circulation, adding the *Biological Abstract Journal,* and publishing the three for $10 or $12 a year, and distributing them to all its members. Then, as the journals wished and the Council and the Society decided, one after another of the other journals could be added until the whole list was included. This scheme would be feasible if we had a thousand members at the start. In any such arrangement the editorial boards of the journals would retain entire charge of the editorial management, so that the independence of the journals would be secured.

(3) If such an arrangement could be made with the Wistar Institute of Anatomy, it might become the publishing house for the Society, taking over the financial responsibility for additional journals, as the Institute has already done for several, and thus greatly extending the usefulness of the Institute. In this way the Society would aid the Institute in getting the journals on a firm basis by uniting in its support the biological interests of the country. At the start this plan might involve an increased outlay by the Wistar Institute, but, in the long run, the dues of the Society should suffice to maintain the journals. This plan would aid the Wistar Institute in doing the work it has undertaken.

(4) Provision could be made for the starting of new journals at any time, or for the support by the Society of those established by outsiders.

University of Chicago,
Chicago, Illinois.

ORGANIZATION OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY[1]

Comprising the American Physiological Society, the American Society of Biological Chemists, and the American Society for Pharmacology and Experimental Therapeutics

JOHN AUER

Among the most enjoyable features of the recent meetings at Cleveland (pages 271, 275, and 279) were the subscription dinners and smokers, held on the evenings of December 30 and 31, at the Colonial Hotel. These informal dinners were attended by the pharmacologists, physiologists and biochemists, and a pleasant flavoring of naturalists, zoologists and anatomists.

At the last of these dinners perhaps the most important development of the Cleveland sessions, so far as the pharmacological, physiological and biochemical societies are concerned, took place. At this dinner, delegates from the three societies, empowered to act, met in conference on the formation of an alliance which should more closely knit together the three societies *while yet jealously preserving the individuality of each component organization.* The delegates from the Physiological Society were Drs. Meltzer, Lee and Cannon; from the Biochemical Society, Drs. Lusk and Wells;[2] from the Pharmacological Society, Drs. Sollmann, Loevenhart and Auer.

Dr. Meltzer was elected temporary chairman and Dr. Cannon temporary secretary. The outcome of the proceedings of this conference committee can best be shown by a transcript of its minutes. The following motions were voted unanimously:

That a Federation of the three societies be hereby established.

[1] This account was presented, originally, as a part of Dr. Auer's report of the proceedings of the Society for Pharmacology and Experimental Therapeutics, page 279. [Ed.]

[2] Dr. Gies, the third delegate from the Biochemical Society (page 278), was unable to attend the Cleveland meetings because of the illness of his eldest son.

That the presidents and the secretaries of the constituent societies form the executive committee of the Federation.

That the chairmanship of the executive committee be held in turn by the presidents of the constituent societies who shall succeed one another annually in the order of seniority of the constituent societies (Physiological, Biochemical, Pharmacological).

That the secretary of the society whose president is chairman shall be the secretary of the executive committee.

That the secretaries of the three societies shall consult in preparing the programs of the annual meeting, and that, so far as practicable, and with the authors' consent, papers be so distributed as to be read to the society in which they properly belong.

That the programs of the three societies be published by the secretary of the Federation under one cover and that the expense of publication be shared *pro rata* by the societies according to the number of members.

That the official title of the new organization be the "Federation of American Societies for Experimental Biology: Comprising the American Physiological Society, the American Society of Biological Chemists, and the American Society for Pharmacology and Experimental Therapeutics."

That a common meeting place of the Federation with the societies of Anatomists, Zoologists and Naturalists is desirable but not mandatory.

That, in the name of the Federation, the International Physiological Congress be invited to meet in the United States in 1916.

That the present conference committee delegate all its powers to the executive committee of the Federation.

The first meeting of the new Federation will be held in December, 1913, in Philadelphia.

Rockefeller Institute for Medical Research,
New York City.

ANNUAL MEETINGS OF THE ORGANIZATIONS COMPRISING THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY[1]

PROCEEDINGS REPORTED BY THE SECRETARIES,

JOSEPH ERLANGER, A. N. RICHARDS, AND JOHN AUER

I. THE AMERICAN PHYSIOLOGICAL SOCIETY

Joseph Erlanger[2]

The Society held its twenty-fifth annual meeting in the Medical Building of Western Reserve University, Cleveland, Ohio, December 29, 1912 to January 1, 1913. Sixty-nine members were in attendance. Two executive sessions and six scientific sessions were held, two of the latter being joint sessions, one each with the American Society of Biological Chemists and Section K of the American Association for the Advancement of Science. The joint session with the American Society of Biological Chemists was opened with exercises in memory of the late Waldemar Koch. After the members of the Society had arisen as a token of respect to the memory of Doctor Koch, Prof. A. P. Mathews delivered a memorial address.

Papers and demonstrations. The titles of the papers and demonstrations, fifty-two in all, which were *read and discussed,* with the names of the authors, are appended:

S. Simpson: The rate of growth in the dog.—*G. N. Stewart:* Further observations on the blood-flow in man.—*J. A. E. Eyster* and *W. J. Meek:* Experiments on the sinus region of the mammalian heart.—*G. C. Robinson* (by invitation) and *J. Auer:* Cardiac anaphylaxis as shown by the string galvanometer.—*W. T. Porter:* The functional relations of cells in nerve centers.—*R. S. Lillie:* Correlation between the anti-stimulating action and the anti-cyto-

[1] See report on the recent organization of the Federation, page 269.

[2] Acting Secretary, vice Dr. A. J. Carlson, unavoidably absent.

lytic action of anesthetics.—*E. B. Meigs:* Studies in the general physiology of smooth muscle.—*W. P. Lombard:* The tickle sense.—*O. Folin, W. B. Cannon,* and *W. Denis* (by invitation) : A new colorimetric method for the determination of epinephrin.—*J. Auer* and *S. J. Meltzer:* The splanchnic as a depressor nerve.—*F. R. Miller:* The salivary secretion centers in the medulla.—*M. Dresbach* (by invitation): A bloodless method of recording blood pressure in animals.—*W. T. Porter:* A new electrical clock.—*S. P. Beebe:* A new form of apparatus for artificial respiration.—*A. D. Hirschfelder:* Some new apparatus.—*R. S. Hoslins:* Relation of fatigue metabolites to epinephrin efficiency.—*D. R. Hooker:* Perfusion of the respiratory center in frogs; the influence of calcium and potassium on the respiratory rhythm.—*A. Hunter:* The nitrogen excretion of normal and of thyroidectomized sheep.—*A. L. Tatum* (by invitation) : Studies in experimental cretinism with suggestions as to a biological test for thyroid secretion.—*R. Gesell* (by invitation) : The relation of pulse pressure to renal secretion.—*C. Brooks* and *A. B. Luckhardt:* The arterial blood pressure during vomiting.—*T. Sollmann* and *J. D. Pilcher* (by invitation) : The effects of aortic compression on the circulation.—*E. G. Grey* (by invitation) and *A. D. Hirschfelder:* Clinical observations upon the carbon dioxide percentage of alveolar air.—*C. W. Greene* and *W. Y. Skaer* (by invitation) : On the fat contents of the mammalian gastric glands in relation to the stages of digestion.—*S. Tashiro* (by invitation): The chemical change in nervous tissue during excitation.—*I. F. Zucker* (by invitation) : The pressor property of shed blood.—*H. Cushing, L. H. Weed* (by invitation) and *C. Jacobsen:* Further studies on the role of the pituitary gland in carbohydrate metabolism, with special reference to the autonomic control of the posterior lobe secretion.—*S. A. Matthews* and *D. D. Lewis* (by invitation) : The *pars intermedia;* its place in *Diabetes insipidus.*—*Lydia M. Degner* (by invitation) and *A. E. Livingston* (by invitation) : Effects of thyroidectomy and castration, respectively, on the pituitary in the rabbit.—*P. W. Cobb* and *L. R. Geisler* (by invitation) : The influence on foveal vision of the brightness of surroundings.—*D. E. Jackson:* Some observations on the peripheral action of certain drugs.—*G. L. Kite* (by invitation) : The relative permeability of the surface and

the interior portions of the cytoplasm of animal and plant cells.—*J. D. Pilcher* (by invitation): The excretion of nitrogen subsequent to ligation of successive branches of the renal arteries.—*W. E. Burge:* The uniform rate of destruction of ptyalin and pepsin by the electric current.—*G. H. Whipple:* Hematogenous jaundice and its relation to the liver.—*S. J. Meltzer:* Is the pulsation of the anterior lymph hearts responsible for the action of some drugs in cardiectomized frogs?—*H. McGuigan:* The synergic action of morphin and strychnin.

Joint programs. With Section K (Physiology and Experimental Medicine) of the American Association for the Advancement of Science: page 277; with the American Society of Biological Chemists: page 275.

The following ten papers were *read by title:*—*C. D. Snyder:* The influence of temperature on the mammalian heart.—*A. J. Carlson:* Some observations on the physiology of the empty stomach and esophagus in man and dog.—*H. C. Bradley:* The problem of enzyme synthesis.—*G. W. Crile:* The relation between the physical state of the brain cells and brain functions; experimental and clinical.—*Y. Henderson* and *C. T. Flynn* (by invitation): Oligemia in acute disease.—*H. McGuigan:* The secondary depression by epinephrin; the rate of destruction of the pressor and the hyperglycemic actions of epinephrin.—*W. B. Wherry* (by invitation): On the transformation of amoebae into flagellates and vice versa.—*P. E. Howe* (by invitation) and *P. B. Hawk:* The influence of fasting on the creatine content of muscle.—*C. D. Snyder:* A study of the electromyograms.—*A. J. Carlson:* The correlation of the physiological states of the thyroid of the fetus and of the mother.

New members: *G. C. Robinson,* Rockefeller Institute for Medical Research.—*J. D. Pilcher, P. J. Hanzlik, R. S. Pearce,* Western Reserve Medical School.—*E. C. Schneider,* Colorado College.—*A. H. Ryan,* University of Pittsburgh.—*M. Dresbach,* Cornell University.—*G. Bachmann,* Atlanta, Ga.—*H. G. Barbour,* Yale Medical School.—*W. DeB. MacNider,* University of North Carolina.—*A R. Moore,* University of California.—*H. B. Williams,* Columbia University—*V. H. K. Moorhouse,* Washington University.

The federation. At this meeting considerable progress was

made toward the formation of a close federation of the American Physiological Society, the American Society of Biological Chemists and the American Society for Pharmacology and Experimental Therapeutics. The Society expressed its desire to enter into such a federation, and a committee was appointed to confer with similar committees of the sister societies with a view to bringing about such a federation. The committee was granted power to make the arrangements for the next annual meeting. This committee was also directed to confer with a similar committee of the American Society of Naturalists to consider the advisability of establishing closer relations with that society (page 278).

Future programs. With regard to the measures of remedying the threatening congestion of programs that were referred to the Council at the last annual meeting, it was decided that should the federation of the three societies be accomplished (page 269), the secretaries of the federated societies be empowered to attempt the equalization of the programs of the three societies by placing papers on the program of the society to which its subject is most closely related. It was also decided to place at the end of the program papers presented by non-members, and, in the event of congestion of the program, to read these by title.

Officers-elect. The following officers were elected for the year 1913:

PRESIDENT—*S. J. Meltzer;* SECRETARY—*A. J. Carlson;* TREASURER—*Joseph Erlanger.*

ADDITIONAL MEMBERS OF THE COUNCIL—*W. B. Cannon* and *F. S. Lee.*

EDITORIAL COMMITTEE ON THE PUBLICATION OF THE AMERICAN JOURNAL OF PHYSIOLOGY FOR 1913—*W. T. Porter, A. J. Carlson, Joseph Erlanger, W. H. Howell, F. S. Lee, Graham Lusk, S. J. Meltzer.* (Appointed by the president.)

Local entertainment. The local Committee on Entertainment, following the plan that was first tried last year at Baltimore by the members and friends of the Society, again agreed to dispense with all private entertainment, and to substitute for it informal subscription dinners followed by smokers each evening while the Society was in session. These functions were open to all members

and guests of the Societies of the Experimental Biological Sciences. It was again demonstrated that this method of entertainment, by bringing all of the members together under conditions permitting informal discussion and exchange of ideas, adds greatly to the pleasure and value of the meeting.

Abstracts of the papers. The abstracts of the papers will be published in the February number of the *American Journal of Physiology.*

Washington University Medical School.

II. THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Alfred N. Richards

The sessions of the seventh annual meeting of the American Society of Biological Chemists were held in the medical buildings of Western Reserve University, Cleveland, Ohio, December 30, 1912-January 1, 1913. The scientific programs, which were of exceptional interest, are appended.

First session. December 30, 9 a. m. PRESIDING OFFICER: *President A. B. Macallum.*

A. B. Macallum: Presidential address, on The energy of muscular contraction; thermodynamic or chemodynamic?—*M. H. Givens* and *A. H. Hunter:* The excretion of pure catabolites in sundry types of mammalia.—*O. Folin* and *W. Denis:* The occurrence of uric acid in blood.—*L. J. Henderson* and *W. W. Palmer:* Studies of the excretion of acid.—*A. E. Taylor* and *A. I. Ringer:* On the utilization of ammonia nitrogen in the protein metabolism.—*W. McK. Marriott:* The determination of acetone substances in blood and tissues by micro methods.—**H. McGuigan* and *F. C. Becht:* The compression of the lung by inert gases.—*J. Rosenbloom:* A new method for drying tissues and fluids.—*W. N. Berg:* Surface tension in muscle contraction.

Second session. December 31, 9.00 a. m. JOINT SESSION WITH THE AMERICAN PHYSIOLOGICAL SOCIETY. PRESIDING OFFICERS: *President A. B. Macallum* and *President S. J. Meltzer.*

* Read by title.

A. P. Mathews: Memorial address on Waldemar Koch.—*L. Loeb:* The influence of pregnancy on the cyclic changes in the uterus.—*G. Lusk:* Metabolism of a dwarf.—*H. S. Gasser* (by invitation) and *A. S. Loevenhart:* The mechanism of stimulation by oxygen want.—*T. B. Osborne* and *L. B. Mendel:* Feeding experiments relating to the nutritive value of the proteins of maize.—*A. I. Ringer:* The fate of fatty acids in diabetic organisms.—*A. B. Macallum* and *W. R. Campbell:* On the secretion of pure acid by the kidney (with demonstration).—*D. Marine:* Hypertrophy and hyperplasia of the parathyroid in birds.—*G. H. Whipple:* Intestinal obstruction; study of a toxic substance present in the intestinal mucosa.—*E. V. McCollum:* The influence of the plane of protein intake on nitrogenous retention in the pig.

Third session. December 31, 2.00 p. m. PRESIDING OFFICER: *President A. B. Macallum.*

W. Salant* and *J. B. Rieger:* Further observations on the influence of caffein on creatin and creatinin metabolism.—H. H. Bunzel:* Quantitative oxidase measurements.—**H. S. Reed:* The regulating function of amylase in the fungus, *Glomerella.*—*A. P. Mathews:* A new method of determining valence based on molecular cohesion.—*H. G. Wells:* The entrance of chemical substances into diseased tissues.—*H. C. Bradley:* The problem of enzyme synthesis.—*R. T. Woodyatt* (by invitation): Certain 3-carbon atom complexes in metabolism.—**E. G. Hastings* and *E. B. Hart:* The presence of a lactic acid producing enzyme in *Bact. lactici acidi.*—*E. V. McCollum* and *Marguerite Davis:* The influence of the composition and amount of the mineral content of the ration on growth.—*H. C. Bradley:* Connective tissues of *Limulus.*—*S. Tashiro* (by invitation): A new method for the detection of minute amounts of carbon dioxid.—**L. H. Davis* and *A. D. Emmett:* A study of the chemical changes in meats during the process of drying by the vacuum method.—**H. T. Leo* and *P. E. Howe:* Muscle creatin; dialysis of creatin from dog muscle.—**N. Stadtmüller, M. Kahn* and *J. Rosenbloom:* Studies on sulfur metabolism; the urinary sulfur partition in various diseases.—**E. V. McCollum* and *H. Steenbock:* The metabolic end-products of the lipoid nitrogen of egg yolk.

* Read by title.

Fourth session. January 1, 2.30 p. m. JOINT SESSION WITH SECTION K OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, AND THE AMERICAN PHYSIOLOGICAL SOCIETY. PRESIDING OFFICER: *Professor J. J. R. Macleod.*

SYMPOSIUM. Some recent applications of physical chemistry in biology—(*A*) *A. B. Macallum:* Surface tension; (*B*) *L. J. Henderson:* The control of neutrality in the animal body; (*C*) *A. S. Loevenhart:* The physical chemistry of enzyme action.

Memorial addresses. In the presidential address at the opening of the first session, President Macallum made extended reference to the life, character, and achievements of the late Waldemar Koch, a charter member of the Society. At the opening of the joint session with the American Physiological Society, Prof. A. P. Mathews delivered a memorial address on Professor Koch.

New members: *Louis Baumann,* University Hospital, Iowa City; *F. J. Birchard,* U. S. Department of Agriculture; *Samuel Bookman,* Mt. Sinai Hospital, New York; *E. D. Clark,* Cornell University Medical College; *H. J. Corper,* University of Chicago; *A. A. Epstein,* Mt. Sinai Hospital, New York; *M. S. Fine,* N. Y. Post Graduate Medical School; *Isidor Greenwald,* Montefiore Home, New York; *W. H. Howell,* Johns Hopkins Medical School; *N. W. Janney,* The Herter Laboratory, New York; *I. S. Kleiner,* Rockefeller Institute for Medical Research; *P. A. Kober,* Roosevelt Hospital, New York; *F. C. Koch,* University of Chicago; *Leo Kristeller,* Berlin, Germany; *F. B. La Forge,* Rockefeller Institute for Medical Research; *A. P. Lothrop,* Columbia University; *W. DeB. MacNider,* University of North Carolina; *A. I. Ringer,* University of Pennsylvania; *W. C. Rose,* University of Pennsylvania; *E. C. Schneider,* Colorado College; *Harry Steenbock,* University of Wisconsin; *R. T. Woodyatt,* University of Chicago.

Officers-elect. The following officers were elected for the year 1913:

PRESIDENT—*A. B. Macallum;* VICE-PRESIDENT—*Graham Lusk;* SECRETARY—*Philip A. Shaffer;* TREASURER—*Donald D. Van Slyke.*

ADDITIONAL MEMBERS OF THE COUNCIL—*H. P. Armsby, Lafayette B. Mendel, H. Gideon Wells.*

NOMINATING COMMITTEE—*Carl L. Alsberg, H. D. Dakin, P. B.*

Hawk, Reid Hunt, Walter Jones, T. B. Osborne, A. N. Richards, H. C. Sherman, F. P. Underhill.

SPECIAL COMMITTEES. President Macallum appointed William J. Gies, Graham Lusk and H. Gideon Wells a committee to confer with similar committees from the American Physiological Society and the American Pharmacological Society concerning the formation of a federation of the three societies having for its object "the establishment of a stable connection between the three societies, for the purpose of fixing the time and place of the annual meetings, the arranging of joint sessions whenever possible, and in general to establish, officially, closer scientific and social affiliations between the sister societies, while retaining their individual independence." This committee was further empowered to act upon such matters connected with the proposed federation as should require decision before the next annual meeting of the Society, and was also authorized to confer with representatives of the American Society of Naturalists concerning closer affiliation with that Society (p. 274).

The Committee appointed at the sixth annual meeting to prepare a report concerning the nomenclature of the lipoids reported progress. Professor Leathes, a former member of the committee, was appointed chairman to succeed the late Professor Koch, Dr. E. K. Dunham was appointed to the vacancy created by Professor Koch's death, and Dr. P. A. Levene to the vacancy created by Dr. Jacques Loeb's resignation. The members of the reconstructed committee are J. B. Leathes, chairman, H. D. Dakin, E. K. Dunham, William J. Gies and P. A. Levene.

Vote of thanks. A unanimous vote of thanks was extended by the Society to Professors Macleod, Sollmann, Stewart and Pearce, and to the members of the "Local Committee," for the hospitality which the Society enjoyed.

Attendance. The following members were present at one or more of the sessions: J. J. Abel, Samuel Amberg, S. P. Beebe, W. N. Berg, W. R. Bloor, H. C. Bradley, H. J. Corper, Otto Folin, W. E. Garrey, H. D. Haskins, Shinkishi Hatai, R. A. Hatcher, P. B. Hawk, L. J. Henderson, A. H. Hunter, J. B. Leathes, A. S. Loevenhart, Graham Lusk, A. B. Macallum, J. J. R. Macleod, W. DeB. MacNider, W. McK. Marriott, A. P. Mathews, H. A.

Mattill, E. V. McCollum, F. H. McCrudden, L. B. Mendel, V. C. Myers, H. S. Raper, A. N. Richards, A. I. Ringer, E. W. Rockwood, Jacob Rosenbloom, L. G. Rowntree, Torald Sollmann, H. C. Wells, R. T. Woodyatt.

Abstracts of the papers. Abstracts of the papers will be published in the March number of the *Journal of Biological Chemistry*.

University of Pennsylvania.

III. THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS.

John Auer

The fourth annual meeting of the Society was held in the Medical Building of Western Reserve University, Cleveland, Ohio, on December 30 and 31, 1912. The scientific programs are appended:

First session. December 30, 9.00 a. m.—**W. Salant:* The influence of temperature on the toxicity of caffein.—**W. Salant:* Further observations on the influence of caffein on the circulation.—*S. P. Beebe* and *Eleanor Van Alstyne:* The effect of high protein diet on the growth of transplantable tumors of the white rat.—*L. B. Mendel* and *R. L. Kahn:* The physiological action of some methyl purins.—*J. A. E. Eyster* and *W. J. Meek:* The action of certain drugs on the electrocardiogram.—*P. J. Hanzlik* (by invitation): The intestinal absorption of alcohol.—*P. J. Hanzlik* (by invitation): The "toxic dose" of salicylates according to clinical statistics.—*W. H. Brown* and *A. S. Loevenhart:* The effect of hematin upon the circulation and respiration.—*W. DeB. MacNider:* The effect of anesthetics on the output of urine in uranium nephritis.—*G. B. Roth:* The physiological assay of aconitin.

Second session. December 30, 2.00 p. m. *L. G. Rowntree* and *R. Fitz:* Renal function in experimental passive congestion.—*R. Fitz* and *L. G. Rowntree:* The effect of temporary occlusion of renal circulation on renal function.—*W. W. Ford:* Observations on three poisonous fungi not previously described.—*J. D. Pilcher:* The protective action of lipoids against hemolysis.—*H. G. Barbour* (by invitation): The action of histamin upon surviving arteries.—*G. W.*

* Read by title.

Crile and *J. B. Austin:* Nitrous oxide sleep compared with normal sleep; brain cell studies.—*W. T. Porter* and *J. H. Pratt:* The action of diphtheria toxin on the vasomotor centre.—*H. Noguchi* and *J. Bronfenbrenner:* The effects of certain disinfectants and therapeutic preparations upon the cultivated spirochetes.—*F. M. Surface* (by invitation): The effect of surplus cow serum on complement fixation with infectious abortion.—**I. Adler* and *C. L. Alsberg:* Studies upon the long continued administration of adrenalin and nicotin.—**C. L. Alsberg:* The hemolytic power of various plants.

Third session. December 31, 9.00 a. m.—**Y. Henderson:* Demonstration of a carbonator for quantitative carbon-dioxide therapy.—*P. Lewis:* Further observations on the relations of vital stains to the tubercle.—*T. S. Githens* and *S. J. Meltzer:* On the course of the toxic effects of ether and chloroform under intratracheal insufflation.—*T. S. Githens:* On the influence of decerebration upon morphin tetanus in frogs.—*I. S. Kleiner* (by invitation): On the effect of sodium bicarbonate and sodium chlorid upon the convulsions produced by heroin and strychnin.—*J. Auer* and *S. J. Meltzer:* The influence of pituitrin upon the depressor action of the vagus nerve in cats.—*B. T. Terry:* The influence of heat upon the toxicity for trypanosomes of blood containing transformed atoxyl. —*B. T. Terry:* Variations in the toxicity of transformed atoxyl for trypanosomes, caused by altering the number of organisms.

Officers-elect. The following officers were elected for 1913:

PRESIDENT—*Torald Sollmann;* SECRETARY—*John Auer;* TREASURER—*A. S. Loevenhart.*

NEW MEMBERS OF THE COUNCIL—*J. J. Abel* and *Wm. DeB. MacNider.*

MEMBERSHIP COMMITTEE—*C. W. Edmunds* was reelected to serve three years, and the place made vacant by Dr. Sollmann's election to the presidency was filled by the election of *Reid Hunt.*

New members. Among the candidates for membership under investigation by the Membership Committee, the following were favorably reported to the Council, recommended for election, and elected by the Society: *H. G. Barbour,* Yale University; *Clyde Brooks,* University of Pittsburgh; *Cary Eggleston,* Cornell Uni-

* Read by title.

versity Medical College; *P. J. Hanzlik,* Western Reserve University; *D. E. Jackson,* Washington University; *I. S. Kleiner,* Rockefeller Institute for Medical Research; *O. H. Plant,* University of Pennsylvania; *A. H. Ryan,* University of Pittsburgh; *F. P. Underhill,* Yale University.

Attendance. The following members were present at one or more sessions of this meeting: J. J. Abel, Samuel Amberg, John Auer, S. P. Beebe, E. D. Brown, G. W. Crile, J. A. E. Eyster, W. W. Ford, T. S. Githens, C. W. Greene, Worth Hale, R. A. Hatcher, V. E. Henderson, A. W. Hewlett, A. D. Hirschfelder, D. R. Hooker, D. R. Joseph, P. A. Lewis, A. S. Loevenhart, W. DeB. MacNider, S. J. Meltzer, L. B. Mendel, J. D. Pilcher, J. H. Pratt, A. N. Richards, G. B. Roth, L. G. Rowntree, Torald Sollmann, G. N. Stewart, B. R. Terry.

Vote of thanks. At the last meeting the Society passed a vote of thanks to the Western Reserve University for the hospitality extended and to the "Local Committee," Drs. Macleod, Sollmann and Pearce, for their thorough arrangement of all the details which made the Cleveland meeting so pleasant.

Abstracts of the papers. Abstracts of the papers will be published in the March number of the *Journal of Pharmacology and Experimental Therapeutics.*

[The report on the organization of the FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY, which appears on pages 269–70, was taken bodily from Dr. Auer's account of the proceedings of the Pharmacological Society. (Ed.)]

Rockefeller Institute for Medical Research.

MEETING OF THE AMERICAN SOCIETY OF ANIMAL NUTRITION

(American Society of Animal Production)

PROCEEDINGS REPORTED BY

LEWIS W. FETZER

The annual meeting of the American Society of Animal Nutrition was held at Chicago, Ill., on November 30, 1912. The address of the president, H. J. Waters, of the Kansas State Agricultural College, at Manhattan, Kansas, dealt with a report on a "Study of the effects of different proteins and ash constituents on the growth of pigs."

Professor E. W. Morse, of the Office of Experiment Stations, U. S. Department of Agriculture, read a paper entitled "Suggestions concerning the planning and reporting of feeding trials," in which he pointed out some needed improvements in planning feeding tests so that the results obtained could be interpreted by modern biometrical and statistical methods, and that the work as a whole could be so systematized and coördinated that the results of each investigation could be compared with those obtained by others. At present the work in this direction is so uncorrelated that a compilation of results on any uniform basis is out of the question.

The standing committee on methods of reporting results of feeding experiments made a special report, which contained recommendations urging uniform methods of reporting data obtained in such experiments. The recommendations include a summary of the opinions expressed by a large number of investigators in response to questions previously propounded to members of the Society. The recommendations were adopted.

It was voted to enlarge the scope of the work of this society, to include all animal husbandry interests—problems connected with the breeding, judging and management of live stock. This is to be in addition to investigations in regard to nutritive value of feeds

and other problems pertaining to animal nutrition. The name of the Society was accordingly changed to "The American Society of Animal Production."

The officers elected for the coming year are as follows: PRESIDENT, *C. F. Curtiss* (Iowa State Agricultural College); VICE PRESIDENT, *E. B. Forbes* (Ohio State Agricultural Experiment Station); SECRETARY-TREASURER, *D. H. Otis* (University of Wisconsin); COMMITTEE ON EXPERIMENTATION, *H. J. Waters* (Kansas State Agricultural College) and *E. B. Forbes* (Ohio State Agricultural Experiment Station).

A meeting of the Society will be held at the time of the Panama-Pacific International Exposition, at San Francisco in 1915.

Office of Experiment Stations,
U. S. Department of Agriculture.

EIGHTH SCIENTIFIC MEETING OF THE COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION AT THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK, DEC. 6, 1912*

PROCEEDINGS REPORTED BY THE SECRETARY,
ALFRED P. LOTHROP

The *eighth scientific session* of the Columbia University Biochemical Association was held at the Columbia Medical School, at 4 p. m., on December 6, 1912.[1] Abstracts of the papers are presented here (pages 285–96) in two groups: (I) *Abstracts of papers on research by non-resident members*[2] and (II) *abstracts of papers from the Columbia Biochemical Department and affiliated laboratories.* The appended summary facilitates reference to the abstracts (45–62).[3]

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS

I

ALLAN C. EUSTIS. Biochemical reasons why free purgation is necessary in combating acidosis of diabetes; results of clinico-chemical observations. (45)

A. J. GOLDFARB. Studies on the effects of salinity changes upon regeneration. (46)

I. GREENWALD. The procedure of Salomon and Saxl as a diagnostic test for carcinoma. (47)

J. ARTHUR HARRIS AND ROSS AIKEN GORTNER. On the relationship between the weight of the sugar beet and the composition of its juice. (48)

PAUL E. HOWE (WITH H. C. BIDDLE). Fasting Studies. XI. Note on the composition of the muscle of fasting dogs. (49)

MAX MORSE. The role of phagocytes in the involuting tail of amphibian larvæ. (50)

* Scientific meetings are held regularly on the first Fridays of December, February and April, and on the first Monday in June.

[1] Proceedings of the sixth meeting were published in the last number of the BIOCHEMICAL BULLETIN, 1912, ii, p. 156. Proceedings of the seventh meeting are published on page 322 of this issue.

[2] Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the research was conducted.

[3] For abstracts 1–44 see BIOCHEMICAL BULLETIN, 1912, ii, p. 156.

I

MAX MORSE. Laboratory hints. (51)

JACOB ROSENBLOOM. The diffusion of iodo-eosin from ether through rubber into ether. (52)

JACOB ROSENBLOOM. The absence of certain enzymes from the human chorion. (53)

II

WALTER H. EDDY. The preparation of histon from flounder sperm. (54)

THUISCO A. ERPF-LEFKOVICS AND JACOB ROSENBLOOM. A quantitative study of certain enzymes of the ovary, uterus and bladder, of pregnant and non-pregnant sheep. (55)

NELLIS B. FOSTER. Pathological deviations in the chemistry of uremic blood. (56)

NELLIS B. FOSTER. Effect of phlorhizin on a dog with Eck fistula. (57)

FREDERIC G. GOODRIDGE AND NELLIS B. FOSTER. The relation of uricolysis to sub-oxidation. (58)

WILLIAM J. GIES. A demonstration of some of the tinctorial properties of pigments produced from thymol by ammonium hydroxid. (59)

B. HOROWITZ. Experiments on pigments produced from thymol by the action of ammonia. (60)

W. M. KRAUS. Influence of uranium nephritis on the excretion of creatinin, uric acid and chlorids, and the effect of creatinin injections during uranium nephritis. (61)

CHARLES WEISMAN. On the question of protein in expired air. (62)

I. ABSTRACTS OF PAPERS ON RESEARCH BY NON-RESIDENT MEMBERS[*]

45. Biochemical reasons why free purgation is necessary in combating acidosis of diabetes; results of clinico-chemical observations. ALLAN C. EUSTIS. (*Laboratory of Clinical Medicine, Medical Department, Tulane University, New Orleans, La.*) Observations were made upon twelve patients in the von Noorden clinic in Vienna and upon four patients in the private practice of the writer during the past year. Tests for indican were made by Salkowski's method, the same amount of urine being used for each test, and the grade of the color imparted by the indigo was recorded +, ++, +++, etc. The ammonia determinations were made according to Folin's method. In each case there was a marked degree of acetonuria, also a large percentage of ammonia nitrogen in the urine, and a high indican index.

According to the experiments of Dale, when *p*-hydroxyphenyl

[*] Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the research was conducted.

ethylamin is perfused through the liver, it yields phenyl acetic acid. As the amin is a product of intestinal putrefaction of tyrosin, and assuming that some such cleavage takes place with other aromatic amins of intestinal putrefaction, their formation should be markedly hindered by free purgation, with consequent reduction of acidemia. In the cases observed there was a drop in the proportions of ammonia, acetone, and indican when free purgation was instituted in conjunction with a low protein diet.

In two cases of fatal acidemia it was impossible to obtain free purgation owing to intestinal paresis, while in those cases in which free purgation was obtained, prompt relief from acidemia was noted.

46. Studies on the effects of salinity changes upon regeneration. A. J. GOLDFARB. (*Marine Biological Laboratory of the Carnegie Institution, Dry Tortugas, Florida, and the Biological Laboratories of the College of the City of New York.*) This investigation was made with the idea of ascertaining to what extent changes in density of sea water affected an organism, *Cassiopea xamanacha,* normally subject to relatively great changes in density of the sea. It was furthermore intended to compare the results, on one hand, with those upon the hydroid, *Eudendrium,* which lives in more dilute and more static densities, and on the other, with the classic results of Loeb on the *Tubularia* of Messina. Much care was devoted to reducing the number of variable factors, or in rendering them uniform, or eliminating them altogether, such as, variations in respiration due to varying volume, surface and depth of the solutions, variations due to differences in size of the medusæ, to level of amputation, to cyclical variations in density, to limited numbers (323 arms were used), etc.

The results are not easily summarized. Normal and supernormal regeneration occurred in normal sea water and in sea water diluted from 5 to 15 per cent. In the gradient series, regeneration was at first gradually, then more rapidly, reduced until a 50 per cent. solution was reached, in which regeneration was inhibited altogether. The medusæ however lived in this and in the 45 per cent. solutions. The other half of the curve was strikingly different, in that regeneration fell off very rapidly, ceasing completely in 125 or 133 per cent. solutions. The whole curve was strikingly different in character from the one described by Loeb.

It is particularly noteworthy that the curve for the *Eudendrium* is intermediate in character between *Tubularia* and *Cassiopea*. It seems altogether certain that Loeb's curve can no longer serve as a type, expressive of the behavior of organisms under varying conditions of density of the sea water, and it is doubtful whether the phenomenon can be expressed in a simple curve based on two variable factors. It appears also probable that the relatively high concentration in which *Cassiopea* normally lives may be associated with the high optimum density for regeneration in this animal. It is also probable that the minimal effects of increasing dilution may be an adaptive response to the extreme dilutions to which *Cassiopea* is normally subject.

47. The procedure of Salomon and Saxl as a diagnostic test for carcinoma. I. GREENWALD. (*Chemical Laboratory of the Montefiore Home, New York City.*) The procedure of Salomon and Saxl,[5] proposed as a test for carcinoma, was tried in a number of urines. All were positive. The precipitates obtained were filtered off, ignited, and weighed, with the results shown in the appended summary. Total sulfur was also determined. There was no apparent relation between the amount of sulfur precipitated by the Salomon-Saxl procedure, either absolute or relative to the total sulfur, and the presence or absence of carcinoma.

Types of cases	Number of urines	Number of cases	Sulfur precipitated in the test:	
			As $BaSO_4$ average, mg.	Relation to total sulfur, per cent.
Normal	8	6	9.5	1.21
Pathological	5	5	7.2	1.55
Carcinoma	9	8	6.6	1.41
Sarcoma	2	2	7.7	1.23

48. On the relationship between the weight of the sugar beet and the composition of its juice. J. ARTHUR HARRIS AND ROSS AIKEN GORTNER. (*Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, L. I.*) Although the literature pertaining to work on the sugar beet is very voluminous, but little attention has been paid to the relationships

[5] Salomon and Saxl: *Wiener klinische Wochenschrift,* 1911, xxiv, p. 449; *Deutsche medizinische Wochenschrift,* 1912, xxxviii, p. 53.

that may exist between the weight of the root of the beet and the chemical composition of its juice. We have compiled such data from various federal and state bulletins, and have examined them by calculating the intensity of such relationships on the — 1 to + 1 scale of the coefficient of correlation. We have also written the regression equations showing the absolute change in solids, sugar, or purity, associated with a unit change in the weight of the beet.[6]

We find that composition and purity are very closely correlated with the weight of the beet; as the weight increases, total solids, purity and sucrose fall rapidly. The following is a representative summary showing the rate of fall on the relative scale of — 1 to + 1 of the coefficient of correlation, and the rate on an absolute scale by the second term of the regression equation, where w = weight, s = sucrose, p = purity and t = total solids.

Data pertaining to 475 Beets[7]

$r_{wt} = -0.497 \pm 0.023$	$t = 20.119 - 0.096\ w$
$r_{ws} = -0.576 \pm 0.021$	$s = 17.644 - 0.122\ w$
$r_{wp} = -0.474 \pm 0.024$	$p = 88.516 - 0.273\ w$

Inasmuch as our results show the necessity of taking into account the weight of the individual beets in all studies on composition, and because of the bearing of our data on the beet sugar industry, we shall publish them in full in the *Journal of Industrial and Engineering Chemistry* (1913, v, p. 192).

49. Fasting studies. XI. Note on the composition of the muscle of fasting dogs. PAUL E. HOWE (WITH H. C. BIDDLE). (*Laboratory of Physiological Chemistry, University of Illinois.*) To be published in full in the April number of the BIOCHEMICAL BULLETIN.

50. The rôle of phagocytes in the involuting tail of amphibian larvæ. MAX MORSE. (*Boardman Laboratories, Trinity College, Hartford, Conn.*) Barfurth, Metchnikoff, Mercier, and others have sought, in the phagocytes, the principal factor in the absorption of tissues. This has been held in question mainly by Looss, who believes that a chemical dissolution is at the basis of the

[6] Harris: *American Naturalist*, 1910, xliv, p. 693.

[7] Nevada Data. Wilson: Bull. 32, Nev. Agri. Exper. Sta., 1896.

process and that this has no primary relation to the activity of the phagocytes.

It is conceivable that if phagocytosis is the principal factor, the blood-counts (differential and with the hemacytometer) would show both an increase in the total number of leucocytes and a difference between numbers of polymorphonuclear forms, in the bloods of a larva in which the process of metamorphosis has not begun and of a transforming individual. On account of the great difficulty in identifying the various forms of leucocytes in hemacytometer preparations, this method of counting was not adopted, but the counts were made upon smears, stained with Wright's stain. Three sets of individuals were used: (1) Larvæ in which the appendages had not appeared and hence no absorption of the tail had begun; (2) individuals in which the process of absorption had progressed to some extent and (3) those in which the absorption had been completed for a number of months, *i. e.*, adult frogs. Twenty-eight specimens were used and the *percentage* results are as follows:

	Polymorph	Basoph	Eosinoph	Large M	Small M
Individuals absorbing the tail.....	9.8	4.2	6.5	36.1	42.4
Non-absorbing	8.6	4.7	7.0	20.6	59.0
Adults.....	18.3	6.2	0.4	13.2	61.2

The polymorphonuclear type runs slightly more numerous in the individuals undergoing metamorphosis than in individuals before the process has begun, but they are found in much larger quantities in adults than in either of the other two groups. As Friedsohn and Neumann have shown, however, it is impossible to distinguish young polymorphonuclear leucocytes in the blood of amphibian larvæ from young erythrocytes and other forms of leucocytes, since all of the corpuscles originate from cells similar in appearance in all cases. Hence, the number of large nucleated forms doubtless includes young polymorphonuclear leucocytes; and if this were so, it would be expected that the number of the large ones would be smaller in adults, which is the case as seen in the above column of values for large mononuclear leucocytes. For these reasons, it is doubtful if there is any decided difference in the number of polymorphonuclear leucocytes in any stage of frog development; and therefore it is

doubtful whether these bodies play an important rôle in the absorption of the tail of the tadpole.

With regard to the basophiles and eosinophiles, it will be seen that they occur in smaller numbers in individuals undergoing metamorphosis than in young tadpoles and, so far as the basophiles are concerned, they are fewer in number in larvæ than in adults. The reverse is the case with the eosinophiles, being found in small numbers in the adult. The small mononuclear leucocytes occur in smaller numbers in the "absorbing" animals than in either of the other types. They are regarded by the investigators mentioned above as the young, indifferent, forms of the several types of leucocytes and probably, also, of the erythrocytes, but since they occur in larger numbers in the blood of the adult, this view is not borne out by the results of the present investigation.

It may be concluded, then, from this set of data, that leucocytes do not play an important rôle in protein and other transfer in the involuting tadpole tail nor do they initiate the process.

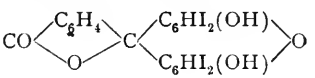
51. Laboratory hints. MAX MORSE. (*Boardman Laboratories, Trinity College, Hartford, Conn.*)

AN ULTRA-FILTER. Colloids may be filtered to advantage by coating the surface of an alundum filter-disc, placed in a Buchner funnel, with a thin collodion solution and applying the funnel to a pump, or the house vacuum. The first drainage through the filter will be coarse, the finer suspensions filling up the interstices of the filter disc; afterwards, fine particles filter.

A CONVENIENT HOT-WATER BOTTLE HOLDER. Select a Florence flask with a neck whose sides are as nearly parallel as possible for a distance and wrap half a yard of quarter-inch twine around the neck, spirally; then wrap electrician's tape or surgeon's tape around the whole, again spirally, thus keeping the twine from unwinding. The heat does not soften the rubber of the tape sufficiently to cause it to leave the twine. If in place of the twine, small round leather belting be used, the apparatus is perfectly satisfactory for all time. The belting may be obtained in any sewing-machine shop or in a belting supply house at small cost.

52. The diffusion of iodo-eosin from ether through rubber into ether. JACOB ROSENBLOOM. (*Laboratory of Biological*

Chemistry of the University of Pittsburgh.) Boas and I[8] have shown that various cholesterol esters diffuse from ether solution through rubber into ether; cholesterol-stearate, for example, with a molecular weight of 652.51, diffuses very readily. Recently I found that the acid form of iodo-eosin diffuses very quickly under similar conditions. This substance, with a molecular weight of 836, has the composition indicated by the appended structural formula:



53. The absence of certain enzymes from the human chorion. JACOB ROSENBLOOM. (*Laboratory of Biological Chemistry of the University of Pittsburgh.*) Published in full in this issue of the BIOCHEMICAL BULLETIN, page 236. See also abstract 55.

II. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEMICAL DEPARTMENT AND AFFILIATED LABORATORIES

54. The preparation of histon from flounder sperm. WALTER H. EDDY. Testes, obtained from *cold storage* flounders and preserved in alcohol, were ground and extracted with 0.8 per cent. hydrochloric acid solution. The histon was obtained from the acid extract after the manner of Kossel and Kutscher, and purified by washing with water, re-dissolving in 0.8 per cent. hydrochloric acid solution and reprecipitating with ammonia, several times. Like ammonia-precipitated *thymus* histon, this histon was insoluble in water and the hydrochloric acid solution, after dialysis to neutrality, gave all the characteristic histon tests. The "ammonia precipitate," washed with water, alcohol and ether, and dried to constant weight at 105° C., contained 18.08 per cent. of nitrogen (one determination only, because of lack of pure material). Like that of *thymus* histon, the hydrochloric acid extract of the histon from flounder testes yielded, when saturated with sodium chlorid, a precipitate which was soluble in water. Such a solution gave the characteristic ammonia and alkaloidal tests, but was not precipitated

[8] Rosenbloom: BIOCHEMICAL BULLETIN, 1912, ii, p. 67; Boas and Rosenbloom: *Proc. Soc. Exp. Biol. and Med.*, 1911, viii, p. 132.

by cold nitric acid solution. Histon from *fresh* testes is in course of preparation for comparisons with the product from cold storage material. We are also investigating the presence of histon in flounder roe.

55. A quantitative study of certain enzymes of the ovary, uterus, and bladder, of pregnant and non-pregnant sheep. THUISCO A. ERPF-LEFKOVICS AND JACOB ROSENBLOOM. Published in full in this issue of the BIOCHEMICAL BULLETIN, page 233. See abstract 53.

56. Pathological deviations in the chemistry of uremic blood.[9] NELLIS B. FOSTER. In general it was found that there is a considerable increase in the amount of non-protein nitrogen in the blood in severe cases of nephritis. However this is not an invariable rule, as very severe cases sometimes show an approximately normal figure; and a high non-protein nitrogen value has been noted in other diseases, such as valvular heart cases and pneumonia. The results must be interpreted in the light of the whole clinical picture. When the total non-protein nitrogen is 1 gram or over, per liter, the prognosis is probably to be regarded as extremely grave. The *percentage* of non-protein nitrogen that occurs as urea is extremely variable, and seems to bear no constant relation to the *total* non-protein nitrogen. The data are so lacking in concordance that it must be left to further investigation, now in progress, to disclose in uremic blood chemical substances which either qualitatively or quantitatively present a constant divergence from normal.

57. Effect of phlorhizin on a dog with Eck fistula. NELLIS B. FOSTER. It has been stated by Rosenfeld that the administration of phlorhizin to dogs with Eck fistula does not induce glucosuria. Such a result would have so much bearing upon our ideas of the mode of action of phlorhizin that the matter required further study. One gram of phlorhizin in olive oil emulsion was given to a dog with an Eck fistula. Glucose was found in the urine, in considerable quantity, for nine days subsequent to the phlorhizin administration.

58. The relation of uricolysis to suboxidation.[10] FREDERIC G. GOODRIDGE AND NELLIS B. FOSTER. In order to investigate the

[9] Foster: *Archives of Internal Medicine*, 1912, x, p. 414.

[10] Goodridge and Foster: *Ibid.*, 1912, x, p. 585.

subject of diminished oxidation in its relation to uric acid excretion, we studied cases of poisoning by illuminating gas (people) and by potassium cyanide (dogs). The results show that retardations of the oxidizing processes, either by deprivation of oxygen (gas) or by interference with cellular functions (cyanide), were not followed by increased excretion of uric acid. It appears improbable, therefore, that uric acid destruction in the body is a simple oxidizing process.

59. A demonstration of some of the tinctorial properties of pigments produced from thymol by ammonium hydroxid. WILLIAM J. GIES. The phenomena described in a previous communication on this subject[11] were demonstrated, as an introduction to the succeeding communication by Mr. Horowitz. Ammonium hydroxid produces from thymol a *blue* pigment (or mixture of pigments?). Ether extracts the blue material from the alkalin liquid, but in ether solution the pigment is *red*. After evaporation of the ether from such an extract, a purplish oily product remains. This residue yields a purplish solution in alcohol, which becomes *blue* when it is rendered *slightly* alkalin. Filter paper, soaked in such a blue, alkalin, alcoholic solution, and then dried at room temperature, assumes a *bright red* color as the alcohol disappears. Treated with alcohol, such red filter paper, particularly if slightly moist, becomes *bright green*. Interesting probabilities suggested by these results, and the possible relationship of these color phenomena to the pigments in the *Monardas*[12] and other plants, will be investigated.

60. Experiments on pigments produced from thymol by the action of ammonia. BENJAMIN HOROWITZ. Professor Gies has found that thymol, in contact with ammonia, develops a blue color.[13] Under his direction I have been making a study of this phenomenon.

The question early arose as to whether ammonia and thymol alone are sufficient for the formation of the pigment. Certain ob-

[11] Gies: BIOCHEMICAL BULLETIN, 1912, ii, p. 171.

[12] Wakeman: *Bulletin of the University of Wisconsin,* No. 448; Science series, 1911, iv, p. 81.

[13] Gies: BIOCHEMICAL BULLETIN, 1912, ii, p. 171. See also the preceding abstract, above.

servations by Liebermann[14] (whose work on colored compounds produced from phenols has been of great importance), as well as the many uses of oxidizing agents in the production of phenol pigments, suggested that atmospheric oxygen takes an active part in the process. This supposition was confirmed. A current of hydrogen passed through an aqueous ammonia-thymol mixture inhibited the formation of pigment. Nascent hydrogen (formed by the addition of Zn dust or sodium amalgam) acted similarly. On the other hand, the addition of a few drops of hydrogen peroxid greatly accelerated the production of pigment by oxidation. A rise has been noticed in an aqueous ammonia-thymol mixture inverted over water, indicating the absorption of oxygen.

The addition of Zn dust to the blue solution in an open vessel caused the color to disappear, except near the surface, where blue always remained, showing the influence of atmospheric oxygen. Ether was added to ascertain whether the reduced substance could be extracted by it and whether the chromogen thus removed by ether would yield pigment in the presence of oxygen. The bottle was now *tightly stoppered* and allowed to stand. Within 24 hours the red ether layer (the blue pigment yields a *red* solution in ether) had become *colorless*. On releasing the stopper *the ether solution became colored again*. This was repeated many times with different samples but always with the same result. Attempts have since been made to isolate the reduced product by evaporating the ether solution in a current of hydrogen, but so far without success. The work is in progress.

61. Influence of uranium nephritis on the excretion of creatinin, uric acid and chlorids, and the effect of creatinin injections during uranium nephritis. W. M. KRAUS. In *acute* uranium nephritis in dogs, creatinin was excreted in decreased amounts; uric acid, in increased amounts. In *subacute* uranium nephritis, creatinin was eliminated in decreased amounts; uric acid and chlorids, in increased amounts (2 weeks). Creatinin, injected in *normal* dogs, appears to be excreted "in toto." Creatinin, injected during *acute* uranium nephritis, is not wholly eliminated. Such an injection causes decreased output of endogenous creatinin,

[14] Liebermann: *Ber. d. d. Chem. Gesell.*, 1874, vii, p. 247; 1875, viii, p. 1649.

also uric acid, chlorids and water; and death may ensue. Creatinin, injected during *subacute* uranium nephritis, is excreted "in toto" and apparently does not affect the excretion of endogenous creatinin, uric acid, chlorids or water.

Two of the animals with *acute* nephritis died after injection of creatinin, in marked contrast with the apparent lack of effect of creatinin injections in the dogs with *subacute* nephritis. In *acute* nephritis, where creatinin (endogenous and injected) and all the other substances named above were excreted in decreased amounts, there was apparently a partial arrest of renal function. In the *subacute* nephritis this did not occur. In *acute* nephritis there is probably not only insufficient intact tubular epithelium to carry the additional burden, viz., the injected creatinin, but this additional burden aggravates the preexisting condition. In *subacute* nephritis, on the other hand, regeneration occurs, thus increasing the functional possibilities of the kidney, so that injected creatinin can be excreted "in toto."

Fever increases creatinin excretion. It is also known that certain infections, *e. g.*, diphtheria, cholera, pneumonia and colon infections, cause tubular nephritides. If, instead of *adding* hypercreatininemia to a nephritis which is predominatingly tubular (as described for the above experiments with uranium nephritis), there should be hypercreatininemia *followed by* tubular nephritis, it is probable that a similar reaction would result, namely, a uremia-like toxemia, ending perhaps in death.

Creatinin has been taken only as an example of urinary substances normally excreted by the kidney tubules. It seems probable that other normal substances, which are increased in fever and excreted by the tubules, would act in a similar way, *i. e.*, to overtax an already overfunctioning kidney in a condition aggravated by a tubular nephritis. This suggestion as to the production of uremia does not concern the substances directly causing it, merely the mechanism of their retention.

62. On the question of protein in expired air. CHARLES WEISMAN. Rosenau and Amoss[15] recently published a paper in which they stated that expired air contains volatile protein. Their

[15] Rosenau and Amoss: *Journal of Medical Research,* 1911, xxv, p. 35.

conclusions were dependent on anaphylactic phenomena that appeared to be obtained with condensations from expired air. At Dr. Gies' suggestion I am repeating their experiments with a view of applying the findings to problems in ventilation and disease. Six repetitions of the experiments by Rosenau and Amoss have been made thus far, with negative results in each instance.

We believe that Rosenau and Amoss failed to conduct adequate control experiments, both on the toxic effects of blood serum and on their anaphylactic tests. Their choice of sites (heart, brain) for the injections is open to criticism. Injections into the *heart* may produce lesions of the heart or lungs as well as pericardial injury and hemorrhage; and, after injury to the bundle of Hiss, resultant symptoms like the Stokes-Adams syndrome, with consequent difficulty of respiration, may simulate anaphylactic effects. Besides, in such injections, there is no assurance that the entire amount of injected fluid goes into the heart. As for injections into the *brain,* Rosenau and Amoss themselves say: "When the second injection was placed under the dura, through the optic foramen, the results were sometimes clouded by the appearance of symptoms which were interpreted to be the result of central irritation." In our work, the second injection was made intravenously (external jugular vein).

The conclusion by Rosenau and Amoss, that expired air contains protein ("volatile"), appears to be unwarranted. The experiments are in progress with the coöperation of Drs. J. Bronfenbrenner and S. Gitlow.

[The proceedings of the February and April meetings of the Biochemical Association will be published in the April number of the BIOCHEMICAL BULLETIN.]

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New York.

FOLIA MICROBIOLOGICA

Perhaps it will be of some interest for readers of the BIOCHEMICAL BULLETIN to learn that the recently founded *Nederlandsche Vereeniging voor Microbiologie* publishes a quarterly journal entitled *Folia Microbiologica.* The editors are: Professors *Beijerinck* (Delft), *Klein* (Groningen), *Poels* (Rotterdam), and *Sleeswijk* (Delft). The subscription price of *Folia Microbiologica* is Five Dollars ($5.00) a year. The first volume is now complete and contains the following papers:

Numbers 1 and 2: *Beijerinck,* Mutation bei Mikroben; *Klein,* Ueber die biologische Analyse des Kaseinantiserums; *Jacobsen,* Die Kulturbedingungen von *Hæmatococcus pluvialis.*

Number 3: *Söhngen,* Ueber fettspaltende Mikroben und deren Einfluss auf Mölkereiprodukte und Margarine; *Ross Van Lennep,* L'influence des substances fixes sur l'anaérobiose dans les milieux de culture liquides; *Fresemann Viëtor,* Ueber die proteolytische und antiproteolytische, resp. antitryptische, Wirkung des menschlichen Blutserums; *Reeser,* Complement fixation of different sera prepared at the State Serum Institute, Rotterdam; *Boeseken* und *Waterman,* Ueber die Wirkung der Borsäure und einiger anderen Verbindungen auf die Entwickelung von *Penicillium glaucum* und *Aspergillus niger.*

Number 4: *Eijkman,* Untersuchungen über die Reaktionsgeschwindigkeit der Mikroörganismen; *Beijerinck,* Die durch Bakterien aus Rohrzucker erzeugten schleimigen Wandstoffe; *Van Calcar,* Ueber die Kenntnis des anaphylaktischen Zustandes des tierischen und menschlichen Organismus; *Waterman,* Beitrag zur Kenntnis der Kohlenstoffnahrung von *Aspergillus niger; Jacobsen,* Die Oxydation von elementarem Schwefel durch Bakterien.

The editorial introductory notice concludes with these words: "If foreign authors should wish the fruits of their researches to appear in our columns, they would be most heartily welcomed. The Latin title of our journal indicates that this new publication has not sprung from chauvinism. We entertain very earnest convictions on the '*internationalism of science.*'"

C. A. PEKELHARING

University of Utrecht,
Holland.

BIOCHEMICAL BIBLIOGRAPHY AND INDEX

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Biochemical literature is becoming so abundant and comprehensive, and so detailed and miscellaneous, that earnest efforts to assimilate a considerable part of it are apt to induce severe attacks of indigestion. Ways and means for rapid and accurate sifting and classification of subjects, experimental data, conclusions, and theories, gain importance with the increase of activity, and the growth of interest, in biochemistry. Appropriate year books, reviews, Zentralblätter, and especially the Department of Biological Chemistry in *Chemical Abstracts,* acquire cumulative usefulness as biochemical research develops in quantity, variety and extent.

The writer finds it very desirable to maintain a running, quarterly, card index of the titles of the papers in the leading biochemical journals. This index is particularly valuable during the intervals between the publication of indices of volumes of abstracts and reviews. In the belief that it may be helpful to others, a copy of a portion of the index is presented below. Sections of the bibliography and index will be printed regularly in the BIOCHEMICAL BULLETIN, if the writer's opinion on its probable general utility proves to be correct.

In the appended bibliography, titles are shortened in a free and easy manner, redundant words are ignored, common words are abbreviated, surnames of collaborators are connected by hyphens, and punctuation marks are omitted, for the sake of condensation. Volume numerals are given in Roman, and the Arabic numerals immediately following them, or placed at the beginning of sections, designate respective issues of the volume; numerals separated by a slanted line indicate month and day of issue. The numeral at the *end* of each item is that of the initial page of the corresponding paper. The numeral at the *beginning* of each item indicates sequence in the

bibliography. The latter numerals are used in the index of subjects at the end (page 305). Abbreviations of words in the index are similar to those in the bibliography. The system of notation in the index, although a space-saving device, makes it easy to refer to any title in the bibliography. The index is a compass not an encyclopedia; its purpose is achieved if it acts as a convenient guide to the sources of information—if it helps the reader speedily to locate and follow the main currents through the literature.

Biochemical bibliography and index: 1912; fourth quarter (Sept.–Dec.); JOURNALS: *Biochemische Zeitschrift, Zeitschrift für physiologische Chemie, Journal of Biological Chemistry, Bio-Chemical Journal, Biochemical Bulletin.*

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[1] See explanation on page 298.

BIOCHEMICAL NEWS, NOTES AND COMMENT

I. GENERAL

Necrology. *David Axenfeld,* professor of physiology at Perugia.—*Carl Binz,* professor of pharmacology at Bonn.—*Edward Curtis,* emeritus professor of materia medica and therapeutics at Columbia University.—*Elie de Cyon,* some time professor of physiology at the Academy of Sciences of St. Petersburg, lately of Paris.—*Wilhelm Ebstein,* professor of internal medicine at Göttingen.—*Humphrey O. Jones,* professor of chemistry at Cambridge.—*Oswald Kohts,* professor emeritus of diseases of children at Strassburg.—*Ewen McIntyre,* for many years president of the N. Y. College of Pharmacy.—*J. W. Mallett,* professor emeritus of chemistry at the University of Virginia.—*Hermann Munk,* professor emeritus of physiology at the veterinary college in Berlin.—*Clarence V. Murphy,* bacteriologist and medical chemist at the Mass. State Sanatorium, Rutland.—*Aimé Pagnoul,* formerly director of the Agricultural Station at Pas-de-Calais.—*Heinrich Ritthausen,* professor emeritus of agricultural chemistry at Königsberg.—*Preston B. Rose,* formerly assistant professor of physiological chemistry and toxicology, and lecturer on renal diseases, at the University of Michigan.—*O. T. Williams,* lecturer on pharmacology and demonstrator of biochemistry at the University of Liverpool.

In memoriam. LORD LISTER. The Lister memorials comprise a "Lister International Memorial Fund," from which will be drawn from time to time a Lister international award for the most notable contribution to surgery in any part of the world, and which will also support fellowships and studentships in surgical research; a monument in London; a memorial tablet in Westminster Abbey;

a monument in Glasgow; and the preservation of the ward in the old building which is now being torn down to make way for a new building of the Royal Infirmary. This ward will be arranged as it was in Lister's time, furnished with contemporary articles and provided with exhibits showing the work that Lister did, and with articles of a personal nature associated with the man in his work. Contributions may be made to any of the memorials, and may be sent to Dr. W. W. Keen, 1729 Chestnut Street, Philadelphia. Each contributor is asked to designate the particular memorial to which he wishes his contribution to be applied (p. 189).

PAUL C. FREER. The July issue of the *Philippine Journal of Science* was a memorial to the late Dr. Paul C. Freer, director of the Bureau of Science of the Philippine Islands, dean of the College of Medicine and professor of chemistry at the University of the Philippines, founder and editor of the *Philippine Journal of Science* (p. 189).

Honors. NOBEL PRIZES were presented by the King of Sweden at a banquet in Stockholm, on December 10. Those to whom awards had been made were present, including Dr. Alexis Carrel, of the Rockefeller Institute for Medical Research (p. 190).—The Nobel prize for chemistry has been divided between Professors Grignard, of Nancy, and Sabattier, of Toulouse.—Professor Sabattier has given his portion of the Nobel prize in chemistry to the building fund of the Toulouse Institute of Chemistry.—A reception was given in honor of Dr. Alexis Carrel, at New York University, on November 16, when President Taft, the French ambassador, and others, delivered congratulatory addresses and Dr. Carrel responded.

ORDER OF MERIT. Dr. Paul Ehrlich, of Frankfort, and Dr. Emil Warburg, president of the "Reichsanstalt" at Charlottenberg, have been made members of the Bavarian-Maximilian Order, which is the highest Bavarian decoration for scientific services.

CORRESPONDING MEMBER. Prof. F. E. Lloyd, of McGill University, has been elected a corresponding member of the Centro de Sciencias, Letras, e Artes, Campinas, S. Paulo, Brazil, in recognition of his work on the desert rubber plant, guayule.

HONORARY MEMBER. At its meeting on December 3 the Acad-

emy of Medicine, of Paris, elected Professor Delezenne of the Pasteur Institute an honorary member of the section on anatomy and physiology.

ANNIVERSARY CELEBRATIONS. On October 22 Prof. *A. Kossel,* of Heidelberg, celebrated the twenty-fifth anniversary of his professorship.—The twenty-fifth anniversary of Prof. *Charles Richet's* appointment to the chair of physiology in the Faculty of Medicine, of Paris, was celebrated on December 22. Professor Chauveau presided at the celebration and presented Dr. Richet with a Festschrift to which three score scholars had contributed from different countries, among them being Pavloff, Kossel, Verworn, Sherrington, Chauveau and Bouchard. After the presentation, addresses of congratulation were made by Professors Landouzy, Dastre, Gley, Langlois, and others.

COMPLIMENTARY DINNERS. Dr. *Jacques Loeb* was the guest of honor at the second annual dinner of the Columbia University Biochemical Association, at the Chemists' Club, N. Y., Nov. 6 (p. 322). —Prof. *R. H. Chittenden* will be the guest of his many pupils and friends at a dinner at Delmonico's, N. Y., on March 1.

AWARDS OF MEDALS. *By the Royal Society:* The *Davy medal* to Prof. Otto Wallach, of Göttingen, for his researches on the chemistry of the essential oils and the cycloölefines; the *Buchanan medal,* to Col. Wm. C. Gorgas, of the U. S. Army, chief sanitary officer of the Panama Canal zone.—*By the Prussian government:* The gold medal for science to Dr. Walther Nernst, professor of chemistry at Berlin.—*By the Swedish Medical Society:* The *Retzius medal* to Dr. J. N. Langley, professor of physiology at Cambridge, for his work on the nervous system.

PRIZES. The *Gedge prize* of Cambridge University has been awarded to Mr. A. V. Hill, of Trinity College, for his essay on the heat production of amphibian muscle and of cold-blooded animals. —*Prize for work on diabetes:* The medical society of Carlsbad has offered $1,000 for the best work or works on "Treatment of diabetes mellitus, with special reference to balneotherapy." Competition is open to physicians of all countries, and any language may be used. All communications should be addressed to the Vereinigung Karlsbäder Aertze, Carlsbad, Austria. The jury consists of

Professors von Jaksch of Prague, Lüthje of Kiel, Ortner of Vienna, Schmidt of Innsbruck, and Dr. Ganz. Essays must be received by Dec. 31, 1913.

Retirements, resignations, declinations, appointments. RETIREMENTS: Dr. *Franz Pfaff,* professor of pharmacology and therapeutics, Harvard Medical School.—Captain *R. W. Silvester,* for twenty years president of Maryland Agricultural College. (He has been made president emeritus and librarian of the institution.)—Dr. *G. R. Kraus,* professor of botany at Würzburg.

DECLINATIONS. Prof. *Andrew Boss,* in charge of the department of farm management of the department of agriculture, University of Minnesota, has declined an offer of the position of director of the new government demonstration farms and trial gardens, at Mandan, N. D.—Prof. *E. M. Freeman,* chief of the division of plant pathology and assistant dean and secretary of the faculty of the college of agriculture, University of Minnesota, has declined an offer of the position of chief pathologist of the Kew Botanical Gardens. The position carries a salary of $4,700.

APPOINTMENTS have lately been announced, as follows:[1]

Alabama Polytechnic Institute: Dr. *Joseph S. Caldwell* (University of Chicago), professor of botany.

Albany Medical College: Dr. *Ralph E. Myers* (Harvard Medical School), instructor in pharmacology.

Australian Institute of Tropical Medicine (Townsville): Dr. *Young* (Lister Institute of Preventive Medicine), biochemist.

British army medical advisory board: Dr. *Leonard Hill,* civilian physiologist.

Cambridge University: Dr. *W. B. Hardy,* university lecturer in physiology.

Carnegie Institution, Nutrition Laboratory: Dr. *Sergius Morgulis* (Sheldon fellow, Harvard University, 1911–12, recently investigator in the laboratory of Professor Zuntz, Berlin), associate in animal metabolism.

College of Physicians and Surgeons (Baltimore): Dr. *Bartgis McGlone,* associate in physiology and embryology.

Cornell College of Agriculture: Mr. *M. J. Prucha,* assistant professor of plant physiology (promotion).

[1] In this summary of appointments, institutions from which *resignations* occurred are named in parenthesis. See also pages 321 and 324.

Georgetown University: Dr. *L. W. Fetzer* (U. S. Department of Agriculture), associate professor of pathological chemistry.

Guy's Hospital Medical School: Dr. *S. Martin Lowry,* lecturer on chemistry.

Harvard University: Dr. *R. P. Strong* (director of the Government Biological Laboratory at Manila, professor of tropical medicine in the Philippine Medical School), head of the newly established department of tropical medicine.

Industrial: Dr. *H. J. Wheeler* (Agricultural Experiment Station, Rhode Island State College), manager of the agricultural service bureau of the American Agricultural Chemical Company (Boston and New York).

Johns Hopkins Medical School: Dr. *B. B. Turner,* assistant in pharmacology.

Maryland Agricultural College: Prof. *T. H. Spence* (vice-president), acting president.

Montefiore Home (New York City): Dr. *H. D. Dakin,* consulting chemist; Dr. *Nelson W. Janney,* chemist; Dr. *Isaac Levin* (Columbia University), director of the department of cancer research.

N. Y. College of Pharmacy: Dr. *George C. Diekman,* associate dean.

N. Y. University and Bellevue Hospital Medical College: Dr. *A. O. Gettler,* associate professor of chemistry (promotion).

Oxford University: Dr. *W. H. Perkin* (University of Manchester), Waynflete professor of organic chemistry.

Rhode Island Agricultural Experiment Station: Dr. *Burt L. Hartwell* (Rhode Island State College), director, vice Dr. H. J. Wheeler, resigned.

St. Louis University: Dr. *P. M. Carrington* (U. S. Marine Hospital Service), professor of hygiene.

Siam: Mr. *W. B. Freeman,* of Denver, director of the public system of irrigation and drainage.

State University of Kentucky: Dr. *Joseph H. Kastle,* director of the Agricultural Experiment Station and dean of the College of Agriculture.

U. S. Dep't of Agriculture: Dr. *Carl L. Alsberg* (Bureau of Plant Industry), chief of the Bureau of Chemistry (pages 211 and 329).—Dr. *L. A. Clinton* (Conn. Agricultural Experiment Station, Storrs), director of farm-management investigations for the North Atlantic states. —Dr. *W. D. Bigelow,* member of the board of food and drug inspection, vice Dr. R. E. Doolittle, resigned.

U. S. Bureau of Mines: Dr. *Reid Hunt,* member of the commission on the hygiene and danger conditions in mines.

University of California: Dr. *J. W. Gilmore* (College of Hawaii), head of the department of agronomy, College of Agriculture.—Dr. *H. J. Webber* (Cornell College of Agriculture), director of the Citrus Experiment Station and dean of the Graduate School of Tropical Agriculture.

University of Chicago: Appointments necessitated by the death of Prof. Waldemar Koch[2] —Dr. *Fred C. Koch,* instructor in physiological chemistry; Dr. *Shiro Tashiro,* assistant in physiological chemistry; Miss *Mathilde Koch,* research assistant in physiological chemistry; Dr. *G. L. Kite,* assistant in pharmacology. (Associate professor S. A. Matthews is conducting the course in pharmacology.)

University of Illinois: Dr. *C. W. Allee* (University of Chicago), instructor in plant physiology.

University of Kansas: Dr. *F. P. Chillengworth,* assistant professor of physiology.—Dr. *C. A. Shull* (University of Chicago), assistant professor of plant physiology.

Yale University: Dr. *F. P. Underhill,* professor of pathological chemistry in the Medical School.

Lectures. Middleton Goldsmith lectures of the N. Y. Pathological Society. Dr. *E. F. Bashford,* director of the Imperial Cancer Research Fund of England, delivered two lectures on "Cancer" at the N. Y. Academy of Medicine, on the evenings of October 2 and 4.

Lectures by visiting members of the 15th International Congress on Hygiene and Demography (p. 129). Prof. *Max Rubner,* Berlin: (N. Y. Academy of Medicine), *Wesley M. Carpenter lecture,* Oct. 3, The life of a cell; *Harvey lecture,* Oct. 5, Modern steam sterilization; (N. Y. University and Bellevue Hospital Medical College), *Herter lectures* (5), Energy problems in nutrition, Oct. 7–11.—Prof. *Carl von Noorden,* Vienna: (N. Y. Post-Graduate Medical School), lectures on New aspects of the pathologic treatment of diabetes, and Diagnosis and treatment of nephritis, October 29–31; (Johns Hopkins Hospital), The principles of treatment of diabetes mellitus, Nov. 2; (St. Louis Medical Society), Treatment of acetonuria, Sept. 30.—Prof. *Hermann*

[2] Biochemical Bulletin, 1912, i, pp. 372 and 522.

Strauss, Berlin: (N. Y. Post-Graduate Medical School), Gastric secretion from the therapeutic point of view, Oct. 14, and The method and purpose of dechlorination in nephritis, Oct. 15.

HUXLEY LECTURE. Dr. Simon Flexner delivered, at Charing Cross Hospital Medical College, on October 31, a Huxley lecture on Recent advances in science in relation to practical medicine.

MISCELLANEOUS ITEMS. Prof. *M. T. Bogert,* president of the Society of Chemical Industry, lectured before the McGill Chemical Society, Montreal, Dec. 16, on The classification of carbon compounds, and in the evening addressed the Montreal members of the Society of Chemical Industry at a banquet at Coopers Limited. On the following day he addressed the Toronto members of the society at a banquet at the Engineers' Club, Toronto, on A closer coöperation between the universities and chemical industries.

At McGill University the annual university lecture for the current year was delivered, Oct. 8, by Prof. *F. E. Lloyd,* on The artificial ripening of bitter fruits.

Prof. *J. J. R. MacLeod* recently delivered, at the University of London, eight lectures on Carbohydrate metabolism.

Endowments, funds and buildings. FUNDS AND ENDOWMENTS. The executors of the estate of George Crocker have filed their final accounting with the courts which shows that *Columbia University* has received from the estate $1,566,635 for the cancer research fund (p. 194).—Mr. Austen Chamberlain has received £48,000 towards the £100,000 which he is raising for the *London School of Tropical Medicine.*—Mr. George F. Baker, president of the First National Bank of New York. has given a large sum, reported to be $2,000,000, to effect an alliance between the *New York Hospital* and the *Cornell University Medical College.*—Dr. John C. Hemmeter, *University of Maryland,* made, at the celebration of academic day, November 12, a gift of $10,000 as a beginning on the endowment of the chair for experimental physiology.—An annual fund of $15,000, to support research in medicine at the *University of Toronto,* has been subscribed for five years by a few citizens of Toronto.

BUILDINGS AND EQUIPMENT. The cornerstone of the new dispensary building of the College of Medicine of *Syracuse University*

was laid on December 14.—A bronze bust of Dr. E. W. Hilgard, emeritus professor at the *University of California,* was recently unveiled in the foyer of the new agricultural hall when the building was dedicated. The occasion was also marked by the formal investiture of Prof. Thomas F. Hunt as dean of the department of agriculture.—Mr. Andrew Carnegie has offered to the *University of Paris* the last $20,000 necessary for equipping the new Institute of Chemistry in course of erection in the Rue Pierre Curie.

Societies, associations, etc. AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. A full account of the proceedings has been published in *Science,* issue of Jan. 10, p. 41.

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY. Proceedings are published in this volume, p. 271.

RUSH SOCIETY. This society, established through the initiative of the Medical Department of the University of Pennsylvania, was organized November 21. Its objects are similar to those of the Harvey Society (New York), namely, the diffusion, by lectures, of knowledge concerning recent advances in the medical and the general biological sciences, and public hygiene. The officers are: *Richard M. Pearce,* president; *Alfred Stengel,* vice-president; *William Pepper,* secretary-treasurer, and *A. E. Taylor, A. C. Abbott* and *H. H. Donaldson,* councilors.

SIXTH INTERNATIONAL CONGRESS FOR GENERAL AND MEDICAL ELECTROLOGY AND RADIOLOGY. This congress, held in Prague during the first week of November, was attended by 760 members. About 130 papers were read. An interesting account of the proceedings was published in the *Journal of the American Medical Association,* Dec. 14, p. 2169.

NEW HOSPITAL ASSOCIATION. Delegates from twenty-nine hospital dispensaries met recently at the N. Y. Academy of Medicine and organized an association to be known as the *Associated Out-Patient Clinics of the City of New York.* The association aims to coördinate the work of existing dispensaries and out-patient clinics, to eliminate unworthy applicants for treatment, and to promote proper standards of treatment, economy and efficiency in dispensary management.

New Orleans Academy of Science. The newly reorganized New Orleans Academy of Science held its first regular meeting on November 12. As now organized it consists of sixteen sections with a chairman for each section, among them Biology and Physiology, Gustav Mann, *chairman.*

The N. Y. Gastro-Enterological Society was founded, December 3, largely through the efforts of Dr. G. R. Lockwood. The object of the society, as the name implies, relates to the study and discussion of gastro-intestinal diseases. At the meeting for organization, Dr. Max Einhorn was elected president, Dr. G. R. Lockwood, vice president and Dr. Harold Barclay, secretary-treasurer, for 1912–13. The officers constitute the council. The *charter* members are Drs. Harold Barclay, George E. Brewer, H. S. Carter, Max Einhorn, Ellsworth Eliot, Wm. J. Gies, W. Van V. Hayes, Lucius W. Hotchkiss, Ludwig Kast, Edward Leaming, G. R. Lockwood, Wm. G. Lyle, Charles Peck, A. R. Stern. The society will meet at the homes of members, on the second Mondays of January, March, May and November. Harold Barclay, *Secretary* (68 West 56th Street, New York).

Officers-elect of biological organizations. American Society of Biological Chemists (p. 275).

American Society for Pharmacology and Experimental Therapeutics (p. 279).

American Physiological Society (p. 271).

American Association for the Advancement of Science: *President,* E. B. Wilson.

Society of American Bacteriologists: *President,* C.-E. A. Winslow; *vice-president,* Chas. E. Marshall; *secretary-treasurer,* A. Parker Hitchens; *council,* W. J. MacNeal, L. F. Rettger, D. H. Bergey, H. A. Harding; *delegate to council of American Association for the Advancement of Science,* S. E. Prescott.

American Society of Naturalists: *President,* Ross G. Harrison; *vice-president,* E. M. East; *secretary,* B. M. Davis; *treasurer,* J. Arthur Harris; *additional members of the executive committee,* A. P. Mathews and A. L. Treadwell.

American Phytopathological Society: *President,* F. C.

Stewart; *vice-president,* Haven Metcalf; *secretary-treasurer,* C. L. Shear; *councilor,* W. J. Morse.

BOTANICAL SOCIETY OF AMERICA: *President,* D. H. Campbell; *vice-president,* M. A. Howe; *treasurer,* Arthur Hollick; *secretary,* George T. Moore; *councilors,* G. F. Atkinson, R. A. Harper and William Trelease.

AMERICAN SOCIETY OF ZOOLOGISTS: *President,* Henry B. Ward. —*Eastern Branch: President,* Raymond Pearl; *vice-president,* Alexander Petrunkevitch; *secretary-treasurer,* Caswell Grave; *additional members of the executive committee,* C. E. McClung, R. G. Harrison (elected, 1910), and H. E. Jordan (elected, 1911).—*Central Branch:* The present officers continue until the next meeting of this branch (BIOCHEMICAL BULLETIN, 1912, i, p. 494).

SOCIETY OF CHEMICAL INDUSTRY: *President,* Marston T. Bogert.

Miscellaneous items. MEDALLION OF VAN'T HOFF. The Dutch sculptor, Pier Pander (Rome), has executed a bronze medallion of van't Hoff. Any one desiring to purchase a copy of it should address Prof. Ernst Cohen, van't Hoff Laboratorium, University, Utrecht, Holland. If 100 copies are sold, the price will be 6.50 Marks (5.50 Marks if 200 copies are sold). The medallion has been executed after a portrait relief in marble by Pier Pander.

CITRUS EXPERIMENT STATION. The University of California has for several years maintained four separate sub-stations in southern California. These will be united into an enlarged research station which will probably be located at Riverside. While this station will be designated the Citrus Experiment Station, after the dominant industry of southern California, the work will relate to all crops grown in that region. The coupling of the Graduate School of Tropical Agriculture with the Station for Agricultural Research will make it unique among our agricultural experiment stations.

CORONER'S CONSULTANTS. Coroner Peter M. Hoffman, of Cook County, Ill., has named Drs. John H. Long, Walter S. Haines, Ludvig Hektoen and John A. Wesener, and Chief Justice Harry Olsen of the Municipal Court, as his consulting staff. With the aid of this staff of consultants, and the establishment of a chemical laboratory, the coroner hopes to reduce the number of fatalities from poison-

ing which annually swell the list of deaths, registered as "suspicious," that require investigation by the coroner. The salary of the chemist in charge of the laboratory will be $2,500 per annum; there will be one assistant. Applications may be sent to the Coroner, Room 500, County Building, Chicago, Ill.

THE HARRIMAN RESEARCH LABORATORY, which operates a building on the grounds of Roosevelt Hospital (N. Y.), has been incorporated. It was established in 1910 and is maintained by Mrs. E. H. Harriman for the study of chemical problems connected with disease.

INTERNATIONAL BUREAU OF FOODSTUFFS. Delegates from the various governments represented at the international congress for the investigation of methods of analysis have established, in Paris, a permanent international bureau of analyses of foodstuffs.

NEW JOURNAL OF SCIENCE. The publishing house of Julius Springer, Berlin, announces the publication, beginning January 3, 1913, of a new weekly journal, *Die Naturwissenschaften,* which, according to the announcement, "für den deutschen Wissenschaftsbetrieb ungefähr das leisten soll, was die *'Nature'* für den englischen und die *'Science'* für den amerikanischen leisten." Each number will contain about 24 pages; the subscription price is 24 Marks. The *Naturwissenschaftliche Rundschau,* edited by Prof. W. Sklarek and published by Friedrich Vieweg und Sohn, which for twenty-seven years has maintained high scientific standards, will be merged in the new journal.

"PAWLOW." I note with interest Professor Halsted's protest[3] against the spelling of Lobachevski's name with a "w," a sort of scientific Wellerism which Teutonic influence has foisted upon the English language. Is it too much to hope that some day we may find American physiologists referring to Pavloff instead of to Pawlow, or is it true that in such mixed crosses, as the heredity experts would say, German pedantry is prepotent over common sense? *J. F. Abbott* (*Science,* 1912, xxxvi, p. 595).

ARTIFICIAL MILK PRODUCED FROM SOYA BEANS. An artificial milk manufactured from soya beans, which is said to contain "all the

[3] Halsted: *Science,* 1912, xxxv, p. 736.

elements" of the best milk and can be used for the same purposes, was recently shown to a gathering of scientists in London. The artificial milk is said to be more digestible than ordinary milk and its cream more nourishing. It can be used for all cooking purposes and good cheese can be made from it, but it will not yield butter. As it is germ-free it will keep longer than milk. The discovery is the work of three Germans who spent three years in perfecting it. The process of manufacture is simple and always produces the same result. The "milk" is not touched by hand or exposed to atmospheric influence until it is poured into bottles for delivery. The "milk" can be sold at 6 cents a quart, which is 2 cents cheaper than the cost of London milk, and the cheese at 6 cents a pound (*Journ. Amer. Med. Assn.*, 1912, lix, p. 1637).

Biochemistry in England. During the past year a movement toward the organization and closer social affiliation of those biologists and chemists who are interested in the investigation of problems common to the two branches of science has resulted in the formation of the Biochemical Club of England. . . . The movement cannot fail to lead to meetings which will be stimulating and full of interest, if one may judge by the success which has attended the similar organization, the American Society of Biological Chemists, since its organization six years ago. Chemical points of view are rapidly gaining a preeminent position in the biological and medical sciences. The closer association and coöperation of investigators in medicine with scientists who are attacking allied problems in other fields, such as agriculture, plant physiology and pathology, microbiology in its industrial applications, etc., is certain to afford advantages of mutual value. In the United States, compared with Germany for example, there has always been less tendency for the student of chemistry in medicine to hold aloof from the biochemist proper. The consideration of medical problems from a more strictly biological point of view is a timely attitude, and the new English organization with its broad affiliations is a commendable one (*Editorial: Journ. Amer. Med. Assn.*, 1912, lix, p. 1803).

Inventor of gelatin capsules. It has been incorrectly assumed that the apothecary Gross von Figely, of Vienna, in 1865, introduced gelatin as a vehicle for medicines. The *London Journal*

of Arts (August, 1848, page 42) contains an article which shows that the real inventor of gelatin capsules was James Murdoch, of London. In England he was granted a patent in May, 1848, entitled "An invention for preserving medicines, etc., in solid, liquid, or powdered form, protected from the air." The description follows. "The capsule consists of two parts, which fit together; one part forms a case to receive the substance to be preserved, and the other forms the cover, which fits tightly over the case; by simply moistening the edges, the capsule can be closed airtight. The most suitable form is a cylinder with hemispherical ends. They are made as follows: "Polished metallic rods, of the form and size of the case and cover, are dipped by pairs in a solution of gelatin, which is drawn off from the rods after drying. In order to facilitate the loosening of the capsules, the rod may be slightly smeared with oil or fat. Each rod must have an opening from end to end, to allow the air to escape after dipping in the gelatin. In addition to animal jellies, starch paste and other mucilaginous liquids can be used. For medicinal substances Iceland moss is best." *F. M. Feldhaus* (*Chemiker Zeitung,* No. 74, 1912).

The prevention of senility. Dr. Metchnikoff recently addressed a letter to a leading Hungarian daily paper in which he published the results of his latest investigations. His scientific discoveries, he says, have been so exaggerated in lay papers that he has resolved henceforth to write direct to the public. After mentioning his early theory that the length of life among animals varies inversely with the length of the large intestine, and his later theory that senility is the consequence of the effects of toxins (chiefly phenols) produced by the intestinal bacteria, Metchnikoff refers to experiments in which he actually succeeded in producing, in apes, senile degeneration by giving them for some time small doses of *p*-cresol. These were the fundamental investigations which led to the solution of the question as to how the action of the intestinal bacteria might be checked or diminished. The lactic acid bacillus has proved to be the best for this purpose. An obstacle to the work of the lactic acid bacilli has been their need of sugar, which does not reach the rectum in a usable form. Dr. Metchnikoff and Dr. Wollman, his pupil, have overcome this difficulty by cultivating bacteria that pro-

duce sugar from starch. It is now possible to supply a diet capable of supporting the bacillus that limits the action of the intestinal flora. Of course, concludes Metchnikoff, the struggle against senility is not concluded. Whether these discoveries will actually tend toward the lengthening of human life is a question of the future, but it cannot be denied that a beginning has been made, and we have reason to hope that from these investigations mankind may derive practical benefit (*Journ. Amer. Med. Assn.*, 1912, lix, p. 815).

Electrons. *Abstract of an address before the American Philosophical Society at Philadelphia, Nov. 1, by Sir William Ramsay.* The actual existence of electrons in motion has been conclusively demonstrated; the mass of an electron is not far from one 1830th of that of an atom of hydrogen; and as the mass of an atom of hydrogen is now known with fair accuracy, that of an electron is nearly 0.8×10^{-27} gram. Electrons in motion *are* negative electricity; they constitute a form of matter, which, at present, has more claim to the term "elementary" than have most of the "elements." Indeed, metals must be regarded as compound substances, of which one component consists of one or more electrons; these electrons are, as a rule, not very firmly attached, as is evident from the generally easy oxidation of most metals. Non-metals are also composed partly of electrons, not so easily detached. The "combination of elements with each other" consists in the shifting of one or more electrons from the more metallic to the less metallic element; no doubt it will some day be possible to give "structural formulae" to the elements, showing the relationship in position, or in directed motion, between the true elements and their attached electrons. The word "electricity" has a dual meaning; it may mean first, an assembly of electrons, stationary or in motion; or second, waves in the ether, produced by the stopping or starting of electrons in motion. The motion of electrons constitutes one factor of electrical energy; wave-motion in the ether can be used as a means of generating electrical energy, by employing the waves in making electrons move. Progress in man's command of natural forces has been made by learning how to direct and control the motion of masses—in other words, by acquiring a knowledge of mechanics; progress in the future will consist in acquiring the power to control

and direct the motions of electrons. This has already been largely achieved by electric contrivances: it is, however, only by the use of concrete ideas regarding the "material" used, viz., electricity, that the progress of invention and discovery can be hastened (*Science,* 1912, xxxvi, p. 684).

II. COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

1. General notes

Marriages. On October 2, Miss Rowena Farmer and Dr. Oscar M. Schloss.—On October 15, Miss Ethel P. Willcox and Dr. Harold E. Woodward.

Appointments. Columbia University: Dr. *Russell L. Cecil,* Proudfit fellow in medicine; Dr. *Wm. H. Woglom,* assistant professor engaged in cancer research (page 201).—Cornell University Medical College: Dr. *Stanley R. Benedict,* professor of chemistry (promotion).—Johns Hopkins Medical School: Dr. *Edwards A. Park* (Columbia University), assistant professor of pediatrics.—Massachusetts Agricultural College: Dr. *H. D. Goodale* (Carnegie Institution, Station for Experimental Evolution), research biologist, department of poultry husbandry.—Turck Research Laboratory (New York): Dr. *Anton R. Rose* (Columbia University), chemist.—University of Texas (Austin): *Mary E. Gearing* (Public Schools, Houston, Tex.), professor of domestic science; *Anna E. Richardson* (Agnes Scott College, Decatur, Ga.), assistant professor of domestic science.—U. S. Department of Agriculture, Bureau of Chemistry: *Carl L. Alsberg,* chief chemist (p. 211); *H. E. Buchbinder,* assistant chemist. (P. 324.)

New members and officers-elect of societies. American Society of Biological Chemists: Members, *Louis Baumann, Samuel Bookman, Ernest D. Clark, Isidor Greenwald, Alfred P. Lothrop;* Nominating Committee, *Carl L. Alsberg, P. B. Hawk* and *Alfred N. Richards;* Lipoid nomenclature committee, *Wm. J. Gies;* Committee on the organization of a federation of biological societies, *Wm. J. Gies* (p. 277).—American Society of Naturalists: *E. Newton Harvey.*—American Society of Zoologists: *E. Newton Harvey.*—Phi Lambda Upsilon: National president, *A. D. Emmett;* national secretary, *H. L. Fisher;* registrar, *George D. Beal.*—Society for

Clinical Serology and Hematology (New York): Secretary, *D. J. Kaliski.*—Nu Sigma Nu Alumni Association (New York): Executive Committee, *Wm. K. Terriberry;* Nominating Committee, *Ralph G. Stillman.*

2. Proceedings of the Association

Second annual dinner. The second annual dinner of the Association (and the first meeting of the Association for the academic year 1912–'13) was held at the Chemists' Club, 52 East 41st Street, on Wednesday evening, November 6.[4] About 125 members and their guests were present, the attendance being so large, in fact, that the main dining room of the Club was filled to capacity and it was necessary to use a room on the floor above for the accommodation of about 20 members.

The dinner was given in honor of Dr. Jacques Loeb, of the Rockefeller Institute for Medical Research. A delightful half hour's informal social gathering preceded the banquet. The president, Dr. Walter H. Eddy, was the toastmaster and appropriately introduced the speakers of the evening. Prof. Lafayette B. Mendel, of Yale University, spoke briefly on the characteristics of scientific men. Prof. Marston T. Bogert, of Columbia University, President of the Society of Chemical Industry, brought to the Association, and its guest of honor, the greetings of that Society. Profs. C.-E. A. Winslow, of the College of the City of New York, and Graham Lusk, of the Cornell University Medical College, also spoke informally and in a very entertaining way. The main address was delivered by Dr. Loeb, who gave a very interesting and instructive account of the results of some of his recent work on the permeability of cells.

Prof. Max Morse, of Trinity College, proposed that Dr. Loeb be elected an honorary member of the Association. The motion was seconded by Dr. E. Newton Harvey, of Princeton University, and unanimously carried by a rising vote.

The names of those present and the groupings at the tables are indicated on pages 323 and 324.

[4] An account of the *first* annual dinner, in honor of Prof. R. H. Chittenden, was published in the BIOCHEMICAL BULLETIN, 1911, i, pp. 334–339.

*Charles Baskerville
*Marston T. Bogert
Walter H. Eddy
*Jacques Loeb
*Graham Lusk
*S. J. Meltzer
*Lafayette B. Mendel
*C-E. A. Winslow

Ella Hazel Clark
Helen Gavin
*Marie L. Minor
Helen G. Russell
Emily C. Seaman
Mary B. Stark
Helen S. Watt
*Mary D. Womack

J. J. Bronfenbrenner
†J. G. M. Bullowa
A. J. Goldfarb
Benjamin Horowitz
Louis Hussakof
†Max Kahn
†William Weinberger
Charles Weisman

*Ch'lotte G. Bultman
Mary C. de Garmo
*†Mary B. Kirkbride
Jessie A. Moore
*Fairfax T. Proudfit
*Mil'd D. Schlesinger

Tula Lake Harkey
*Israel S. Kleiner
Alfred P. Lothrop
Anton R. Rose
*Mary D. S. Rose

*James Ewing
*Cyrus W. Field
Nellis B. Foster
F. G. Goodridge
*P. A. Levene
*William H. Park
*E. E. Smith
†Alexander Smith
*Karl Vogel

*John Auer
C. Stuart Gager
*Walter A. Jacobs
*F. H. McCrudden
H. O. Mosenthal
*Edgar W. Olive
*D. D. Van Slyke

*Jerome Alexander
*†J. P. Atkinson
*Edwin J. Banzhaf
Charles F. Bolduan
†Wm. B. Boyd
†F. T. Van Beuren
Harry Wessler
†H. B. Wilcox

B. C. Gruenberg
Max Morse
Raymond C. Osburn

*Louisa Bruckman
*Harriet C. Jacobson
Marguerite T. Lee
Helen McClure

Arthur Knudson
*†Albert Plaut
Edward Plaut
*Eugene Unna

*E. H. Bartley
*Walter A. Bastedo
*†S. P. Beebe
*Charles A. Doremus
*Henry C. Sherman
Matthew Steel
*Frederick H. Sykes
*H. T. Vulté

Ula M. Dow
Ada M. Field
Ruth S. Finch
*Edith C. Keefer
*Ethel MacMillan
*Alice E. Skinner
*Wilhelmina Spohr
Helen B. Thompson

*Ronald M. Ferry
T. Stuart Hart
Paul E. Howe
*Ewing H. Rand

Anna M. Connelly
*Mathilda L. Mayer
Elizabeth Rothermel
Ethel W. Wickwire

Harvey B. Clough
Samuel Gitlow
Fred W. Hartwell
C. A. Mathewson

Donald Gordon
John L. Kantor
Daniel R. Lucas
Ralph G. Stillman

* Guest.

† Detained or obliged to leave before the conclusion of the dinner.

*Orabel Chilton	Will H. Chapman	William J. Gies
*Helen Ide Gray	*Fred'k H. Morrison	Joseph S. Hepburn
Alice H. McKinney	*Harold E. Smith	Walter F. Hume
*Bessie G. Pond		Walter M. Kraus
	Ernst Boas	S. Kubushiro
S. R. Benedict	Ernest D. Clark	E. G. Miller, Jr.
R. A. Cooke	Harry L. Fisher	P. W. Punnett
*M. S. Fine	Ross A. Gortner	*T. B. Reed
*V. C. Myers	Isidor Greenwald	Grover Tracy
	E. Newton Harvey	
*†M. I. Falk	Michael Heidelberger	
*R. G. Reese	*John J. Kenny	
†Wm. W. Tracey	J. Buren Sidbury	
G. W. Vandegrift	Louis E. Wise	

Proceedings of the eighth scientific meeting. The second meeting of the Association for the academic year 1912–'13 was held at the Columbia Medical School, on Friday, Dec. 6, at 4.15 P. M., instead of the regular weekly departmental seminar. At Dr. Gies' suggestion the Association began, with this meeting, a series of quarterly sessions for the presentation of the results of research by its members. As presiding officer at the seminars, Dr. Gies outlined the plan and purpose of these scientific sessions and formally turned over the meeting to the Association. President Eddy then took the chair. The scientific program and abstracts of the papers are given on page 284.

The remaining meetings of this series, for the year 1912–'13, will be held on February 7, April 4 and June 2. Abstracts of the communications will be published in the succeeding issues of the BIOCHEMICAL BULLETIN.

ALFRED P. LOTHROP, *Secretary.*

3. Columbia Biochemical Department

Resignations and appointments. STAFF. The following further changes in the staff, for the year 1912–'13, were officially authorized during the quarter ending Dec. 31: Dr. *Jacob Rosenbloom,* associate, resigned to accept the assistant professorship of

* Guest.

† Detained or obliged to leave before the conclusion of the dinner.

biochemistry at the University of Pittsburgh.—Dr. *Herman O. Mosenthal,* instructor, appointed associate, vice Dr. Rosenbloom resigned.—Dr. *Max Kahn* appointed instructor, vice Dr. Mosenthal promoted.—Dr. *Clayton S. Smith,* instructor, resigned to accept an assistantship in pharmacology in the Bureau of Chemistry, U. S. Department of Agriculture, Washington.—Dr. *Louis E. Wise* appointed instructor, vice Dr. Smith resigned (page 203).

The retirement of Drs. Rosenbloom and Smith from the department as noted above, after active and very successful terms of service, occasioned deep regret among their associates at Columbia, whose hearty good wishes attend them in their new fields of usefulness. (See bibliography, below.)

STUDENTS. Arbuckle Sugar Co. (Brooklyn): *Abraham Gross,* research chemist.—Harriman Research Laboratory (Roosevelt Hospital, N. Y.): *Marston L. Hamlin,* research assistant.—Industrial School (New Bedford, Mass.): *Constance C. Hart* (Teachers College), assistant.—State Normal School (Truro, N. S.): *Blanche R. Harris* (Teachers College), assistant.—Texas (North) State Normal School: *Blanche E. Shaffer* (Teachers College), professor of home economics.—N. Y. University and Bellevue Hospital Medical College: *Percy W. Punnett,* assistant in chemistry.—University of Kentucky: *Mary E. Sweeny,* head of extension department.—University of Porto Rico; *L. A. Robinson,* professor of psychology.—Washington State College (Pullman); *Louise McDanell,* instructor in domestic science.—West High School (Rochester, N. Y.): *David F. Renshaw,* instructor in chemistry.

Dr. Rosenbloom's career. Jacob Rosenbloom was born in Braddock, Pa., on Feb. 25, 1884. His early education was received in the public schools and high school of North Braddock, Pa. At the end of a four-year course at the University of Pittsburgh he received the degree of B.S. in chemistry, in 1905. From 1905 to 1909 he was a student here at the College of Physicians and Surgeons, receiving the degrees of M.D. and Ph.D. in 1909. His major subject for the Ph.D. degree was biological chemistry, with Professor Gies.

Dr. Rosenbloom was assistant in this department for the year 1909–1910; associate (also assistant pathologist to the German

Hospital, N. Y.), during 1910–12. The summer of 1912 was spent at Johns Hopkins University in clinical medicine. Last October he received and accepted appointment as assistant professor of biochemistry in the University of Pittsburgh.

Dr. Rosenbloom is a Fellow of the American Association for the Advancement of Science, a member of the American Society of Biological Chemists, Society for Experimental Biology and Medicine, American Chemical Society, Chemists' Club of New York, Sigma Xi, International Psychoanalytic Association, and the Society for Biological Research of the University of Pittsburgh. He married, in June, 1911, Miss Merla Cohen of Baltimore, Md.

DR. ROSENBLOOM'S PUBLICATIONS. **1905.** A colorimetric method for the determination of tungsten; *Thesis* for the degree of B.S., Univ. of Pittsburgh.

1907. Some azolitmin compounds of mucoids, nucleoproteins and other proteins, with exhibition of products (with Wm. J. Gies); *Proc. Amer. Soc. Biol. Chem.*, i, p. 48; *Jour. Biol. Chem.*, iii, p. xxxix.

1909. A contribution to the study of the nature and origin of the Bence Jones protein, with bibliography; *Dissertation,* Columbia University. Pp. 64.

1910. On the effects and fate of injected connective tissue mucoid (with Wm. J. Gies); *Proc. Amer. Soc. Biol. Chem.*, i, p. 271; *Jour. Biol. Chem.*, vii, p. lviii.—Is the Bence Jones protein produced from osseoalbumoid?; *Ibid.*, p. 227 and p. xiv.—A study of the duodenal contents in man (with M. Einhorn); *Arch. Internal Med.*, vi, p. 666; *Int. Beitr. z. Path. u. Ther. d. Ernähr. Stoffw. u. Verd'krank.*, ii, p. 184.

1911. A histological and chemical study of the fatty matter of normal and cryptorchid testes (with F. M. Hanes); *Jour. Exp. Med.*, xiii, p. 355.—A study of the nitrogen metabolism in three cases of duodenal alimentation (with M. Einhorn); *Amer. Jour. Med. Sci.*, cxlii, p. 7; *Int. Beitr. z. Path. u. Ther. d. Ernähr. Stoffw. u. Verd'krank.*, iii, p. 5.—A new process for the purification of lipins, with demonstrations (with Wm. J. Gies); *Proc. Amer. Soc. Biol. Chem.*, ii, p. 8; *Jour. Biol. Chem.*, ix, p. xiv.—A demonstration of the osmotic pressure exerted by fat (with Wm. J. Gies); *Proc. Soc. Exp. Biol. and Med.*, viii, p. 71.—The effects of intraperitoneal injections of epinephrin on the partition of nitrogen in the urine of dogs (with W. Weinberger); *Ibid.*, p. 131.—Experiments on the diffusibility of cholesterol esters and

of lecithan compounds (with E. Boas); *Ibid.*, p. 132.—The importance of the colloidal nitrogen in the urine in the diagnosis of cancer (with M. Einhorn and M. Kahn); *Amer. Jour. of Gastro-Enter.*, i, p. 12; *Arch. f. Verdauungskrank.*, xvii, p. 557.—On the lipins of the heart muscle of the ox; *Science*, xxxiv, p. 221; BIOCHEM. BULL., i, p. 114.—The effect of pregnancy on the lipins of the ovary and corpus luteum of the cow; *Ibid.*, p. 222 and p. 115.—A proposed chemical classification of lipins, with a note on the intimate relation between cholesterols and bile salts (with Wm. J. Gies); *Ibid.*, p. 51.—Intracellular lipins; *Ibid.*, p. 75.—A review of the history of Bence Jones protein and multiple myeloma; *Ibid.*, p. 161.—The older theories of edema; *Ibid.*, p. 275.

1912. Osseoalbumoid as a possible precursor of Bence Jones protein; *Arch. Internal Med.*, ix, p. 236.—Spontaneously precipitated Bence Jones protein in urine; *Ibid.*, p. 255.—The glycyltryptophan and tryptophan tests for cancer of the stomach (with C. H. Sanford); *Ibid.*, p. 445.—A note on the distribution of chlorate in a woman fatally poisoned by potassium chlorate; BIOCHEM. BULL., i, p. 483.—A study of the diffusibility of lipins from ether through rubber membranes into ether; *Ibid.*, ii, p. 64.—The colloidal nitrogen in urine from a dog with a tumor of the breast (with M. Kahn); *Ibid.*, p. 87.—Effects of intraperitoneal injections of epinephrin on the partition of nitrogen in urine from a dog (with W. Weinberger); *Ibid.*, p. 123.—A quantitative study of the lipins of bile obtained from a patient with a biliary fistula; *Ibid.*, p. 182.—A disturbing factor in Lieben's and in Gunning's test for acetone in urine; *Jour. Amer. Med. Assn.*, lix, p. 445.—A report of some new chemical analyses of urinary calculi, with indications for treatment (with M. Kahn); *Ibid.*, lix, p. 2252.—The diffusion of iodoeosin from ether through rubber into ether; *Proc. Soc. Exp. Biol. and Med.*, x, p. 48.

[*Dr. Rosenbloom's papers in this issue* (*pp. 229, 233, 236,* and *290*) *were submitted for publication in December 1912.*]

Awards of degrees at Columbia. Mr. Anton R. Rose recently passed a public examination for the Ph.D. degree, thus completing the requirements for that degree in biological chemistry. His dissertation is entitled Biochemical studies of phyto-phosphates. —Miss Clara W. Hasslock and D. F. Renshaw completed on Oct. 10 the requirements for the degree of A.M.

Miscellaneous items. Professor Gies delivered a lecture in

the autumn series at the New York Botanical Garden, October 26, on The chemical production of albuminous substances in plants. On October 7 he addressed the Section on Research of the First District Dental Society of the State of New York, at the Academy of Medicine, on Recent developments in the study of dental caries. Dr. Lothrop followed with a paper on the work he has been doing in this connection on salivary mucin.[5]—Professor Gies was one of the organizers of the *New York Gastro-Enterological Society* (p. 315). He is Secretary of a Committee of Twenty-five, of Prof. R. H. Chittenden's pupils, in charge of a dinner to be given at Delmonico's on March 1 in honor of Professor Chittenden, and of a fund to be given to Yale in the name of Professor Chittenden.

[5] Gies: *Journal of the Allied (Dental) Societies,* 1912, vii, pp. 397 and 478; Lothrop: *Ibid.,* p. 410.

EDITORIALS

A year's experience in the conduct of the BIOCHEMICAL BULLETIN has induced us to change our plan of quarterly issue. The manuscript of future numbers will be sent to the printer on the first day of each natural quarter; the months of issue will be January, April, July and October; and the contents of each issue will pertain to the quarter preceding the month of issue. Volume II will close with the July number.

New plan of quarterly issue of the Bulletin

We congratulate President Taft and the country on the appointment of Dr. Carl L. Alsberg, in succession to Dr. Harvey W. Wiley, as Chief of the Bureau of Chemistry. Dr. Alsberg's training in chemistry, in general biology and in medicine has been unusually broad and deep. His chemical knowledge, his sanitary comprehension, his scientific wisdom, and his zeal as an investigator, have had exceptional fruitage throughout his entire professional career. Admired as a gentleman by all who know him and respected by his colleagues everywhere as a scientist of eminent capacity, Dr. Alsberg is also universally esteemed for his habitual fidelity to duty, his moral integrity and his high professional purpose. We look forward with great confidence to a career for Dr. Alsberg which will be distinguished by a patriotism, a zeal in public service, a personal courage, a common sense, a scientific exactness, an aggressiveness in the detection of violations of law, an executive capacity, that will be the delight of all his biochemical colleagues and the pride of his countrymen—and in this faith we tender him our felicitation and support.

Carl L. Alsberg

As we are about to close this issue of the BULLETIN, we learn that the following testimonial (as proposed by Prof. Graham Lusk), which was sent a few days ago (Jan. 21) to about 275 of Dr. Alsberg's fellow workers in the American Physiological Society and

the American Society of Biological Chemists, has already been signed by nearly all of them:

To Dr. Carl L. Alsberg: We who, like yourself, are active workers in the field of experimental biological science, congratulate the country and yourself on your appointment as Chief of the Bureau of Chemistry in the Department of Agriculture. We wish to express our confidence in your ability and integrity. We desire for you a successful administration which shall promote the public welfare, shall jealously guard the public health, and shall uphold the dignity of the science which you represent.

We are greatly indebted to Dr. Adler for the biographical and bibliographical facts, pertaining to Dr. Alsberg, on pages 211–216 of this issue.

Stock poisoning due to spoiled silage. Help!

During the past winter a number of cases of stock poisoning due, apparently, to the feeding of spoiled or moldy silage, were brought to our attention. At that time we were unable to give the matter due consideration. During the present winter, however, we shall be in a position to make some preliminary studies to ascertain the cause of toxicity in silage. Our work would be greatly facilitated if those readers of the BIOCHEMICAL BULLETIN who know of such cases of stock poisoning would bring this matter to the attention of the owners so that samples of the silage might be forwarded to the Chemical Section of the Iowa State College. The samples should be accompanied by full particulars regarding the apparent cause of spoilage and the symptoms exhibited by the animals to which the silage was fed. *Arthur W. Dox.*

Demand for biological chemists in the hospitals

Until recently the pathological work in our larger hospitals has been done by attending physicians. The growth of this special field, however, has made it impossible for any one to keep abreast with it and do anything else. The progress of medical science constantly tends toward what is most refined and subtle. As the microscope revealed new fields of research, so chemistry has opened previously unim-

agined paths for investigation. The directors of hospitals are alive to this fact, as is shown by the increasing demand for biological chemists to cooperate with pathologists in the investigation of disease. Up to now relatively few men have been adequately trained to be hospital chemists. What is required of them, and what will be demanded of them more and more, is not the making of routine analyses at the suggestion of some attending physician, who in all probability has but a vague idea of what he wants; but rather that, unguided, they shall be able to discern in any disease process a definite problem for investigation, and shall be competent to establish the conditions and conduct the details of suitable experimental research thereon. In order to do this with any degree of success, a hospital chemist must, in addition to his knowledge of biological chemistry, have a fairly good understanding of general pathology and bacteriology. It is not *necessary* that he be a physician, since technical familiarity with the clinical aspects of disease will not be required of him.

In the next decade, if not sooner, there will be a great demand for this type of highly trained biological chemist. The beginning of this demand is seen now in the growing number of biological chemists attached to the main hospitals of our larger cities. These chemists are on the same footing with the pathologists and the bacteriologists. Although at present their remuneration is not what it should be, this will be remedied as soon as their value to hospitals is clearly shown. This field of work should be particularly attractive to those who do not care for an academic career but who are devoted to biochemical research and averse to commercial chemistry.

Federation of American Societies for Experimental Biology

In the fall of 1911, when it was seriously proposed to merge the American Society of Biological Chemists into the American Physiological Society, we were among the many who objected to the plan on the ground that such a merger would be detrimental to biological chemistry as a science and as a profession—sufficient reason for dissenters, even if a merger were ordered, to maintain the existence of an independent American Biochemical Society.[1] In

[1] Editorial: Biochemical Bulletin, 1911, i, p. 364.

presenting these objections informally to our colleagues we emphasized, however, the desirability of more intimate affiliation between the leading biological societies, *in harmony with the policy of the American Society of Biological Chemists from its establishment,* and suggested the organization of a *"federation"* of independent societies for the attainment of that purpose and other mutually advantageous objects. There was much discussion but no decision.

The organization of the Biochemical Society of England, meanwhile, with all that its existence implies,[2] gave added weight to the objections that were raised against the assimilation of the American Society of Biological Chemists by the American Physiological Society.

During the past year the "federation" idea has won its way into unanimous acceptance, as is indicated by the account in this issue of the organization of the *Federation of American Societies for Experimental Biology* (page 269). The Federation is, in effect, an embryonic American Biological Society, the independent societies being its working divisions. The plan of federation has not weakened the independence or impaired the autonomy of any of the constituent societies.

We heartily commend to the attention of all our readers the Mathews *plan for the organization of the American Biological Society,* which is published in full in this issue (page 261). We believe that the logical development of the Federation would secure all the many desirable results at which Professor Mathews' excellent and far-reaching plan is aimed, *including the establishment and successful conduct of a Biological Abstract Journal.* We hope to present the views of some of our colleagues on this and related subjects in our July issue.

The knowledge of nature as it is—not as we imagine it to be—constitutes true science.—*Paracelsus.*

Electrons

Liability to error is the price we pay for forward movement.—*Sidgwick.*

The secret of all who make discoveries is to look upon nothing as impossible.—*von Liebig.*

[2] Halliburton: Biochemical Bulletin, 1912, i, p. 484; ii, p. 128; 1913, ii (this issue), p. 318.

BOOKS RECEIVED

The BIOCHEMICAL BULLETIN will promptly acknowledge, under this heading, the receipt of all publications that may be presented to it. From time to time, selections will be made for review on pages of the volume to be appropriately indicated here. Reviews will be matter-of-fact statements of the nature and contents of the publications under consideration, and will be intended *solely to guide possible purchasers.* The wishes or expectations of publishers or donors of volumes will be disregarded, when they are incompatible with our convictions regarding the interests of our colleagues. *The sizes of the printed pages are indicated, in inches, in the appended notices.*

Glycosuria and allied conditions. By P. J. Cammidge. Pp. 467—4 × 6¼; $4.50 net. Longmans, Green & Co., New York; Edward Arnold, London, 1913.

The chemical constitution of the proteins: Part II, Synthesis, etc. 2d ed. (One of the *Monographs on Biochemistry.*) By R. H. A. Plimmer, Univ. reader and ass't prof. of physiological chem., University Coll., London. Pp. 107—4⅜ × 7½; $1.20 net. Longmans, Green & Co., 1913.

Microscopy and the microscopical examination of drugs. By Chas. E. Gabel, microscopical food and drug analyst, Iowa State Dairy and Food Commission. Pp. 116—4 × 6⅝; $1.00. Kenyon Co., Des Moines, Ia., 1911.

Collected papers: Laboratory of physiological chemistry, Sheffield Scientific School, Yale University. 1911–1912. (35 reprints.)

Medical and surgical report of Bellevue and Allied Hospitals in the City of New York. By Van Horne Norrie, John A. Hartwell, A. Alexander Smith and Charles E. Nammack. Vol. iv, 1909–1910. (55 reprints.)

Report of the laboratories of the University of Buffalo, medical department; including the third Harrington lecture (Hektoen). No. 4, 1912. (8 reprints.)

Contributions from the physiological laboratory of the Medico-Chirurgical College, Phila. By Isaac Ott and John C. Scott. Part xix of Ott's contributions to physiology, 1912. (13 reprints.)

Report of the Pellagra Commission of the State of Illinois. Pp. 250—4¼ × 7. Nov., 1911.

Practical physiological chemistry. *A book designed for use in courses in practical physiological chemistry in schools of medicine and of science.* By Philip B. Hawk, professor of physiological chemistry and toxicology in the Jefferson Medical College of Philadelphia. Fourth edition, revised and enlarged. Pp. 475—4½ × 8; $2.50 net. P. Blakiston's Sons & Co., Philadelphia, 1912.

The protein element in nutrition. (One of the *International Medical Monographs.*) By Major D. McCay, professor of physiology, Medical College, Calcutta. Pp. 216—4 × 7, with 8 full page portraits of human subjects; $2.00 net. Longmans, Green and Co., New York; Edward Arnold, London, 1912.

Oxidations and reductions in the animal body. (One of the *Monographs on Biochemistry.*) By H. D. Dakin, The Herter Laboratory, New York. Pp. 135—4½ × 8; $1.40 net. Longmans, Green and Co., 1912.

Researches on cellulose. III (1905–1910). By C. F. Cross and E. J. Bevan. Pp. 173—3½ × 6; $2.50 net. Longmans, Green and Co., 1912.

An introduction to the study of the protozoa, with special reference to the parasitic forms. By E. A. Minchin, professor of protozoology in the University of London. Pp. 517—4 × 7½; $6.00 net. Longmans, Green and Co., New York; Edward Arnold, London, 1912.

OFFICERS OF THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY, 1912–1913*

OFFICIAL REGISTER, DEC. 31, 1912

WILLIAM J. GIES: *Professor and Chairman of the Staff;* Consulting chemist, New York Botanical Garden; Pathological chemist, Bellevue Hospital; Member of the Faculties of N. Y. Teachers College and N. Y. College of Pharmacy. [B.S., Gettysburg College, 1893 and M.S., 1896; Ph.B., Yale University, 1894 and Ph.D., 1897. Instructor, 1898–'02; adjunct professor, 1902–'05; professor, 1905–.]

PAUL E. HOWE: *Assistant Professor.* [B.S., University of Illinois, 1906; A.M., 1907 and Ph.D., 1910. Assistant professor, 1912–.]

NELLIS B. FOSTER: *Associate;* Associate Physician, New York Hospital; Chemist, St. Luke's Hospital. [B.S., Amherst College, 1898; M.D., Johns Hopkins University, 1902. Instructor, 1906–'08; associate, 1908–.]

WALTER H. EDDY: *Associate and Secretary of the Staff.* [B.S., Amherst College, 1898; A.M., Columbia, 1908 and Ph.D., 1909. Assistant, 1908–'10; associate, 1910–.]

ALFRED P. LOTHROP: *Associate and Departmental Registrar.* [A.B., Oberlin, 1906 and A.M., 1907; Ph.D., Columbia, 1909. Assistant, 1908–'09; instructor, 1909–'12; associate, 1912–.]

HERMAN O. MOSENTHAL: *Associate;* Assistant Attending Physician, Presbyterian Hospital; Assistant Physician, Vanderbilt Clinic; Instructor in medicine. [A.B., Columbia, 1899 and M.D., 1903. Assistant, 1908–'09; instructor, 1909–'12; associate, 1912–.]

EMILY C. SEAMAN: *Instructor.* [B.S., Adelphi College, 1899; A.M., Columbia, 1905 and Ph.D., 1912. Tutor, 1909–'10; instructor, 1910–.]

MAX KAHN: *Instructor.* Director of the chemical and physiological laboratories of Beth Israel Hospital. [M.D., Cornell University Medical College, 1910; A.M., Columbia, 1911 and Ph.D., 1912. Instructor, 1912–.]

LOUIS E. WISE: *Instructor.* [A.B., Columbia, 1907 and Ph.D., 1911. Instructor, 1912–.]

EDGAR G. MILLER, Jr.: *Assistant,* 1911–. [B.S., Gettysburg College, 1911.]

FREDERIC G. GOODRIDGE: *Assistant,* 1912–. [A.B., Harvard University, 1897; M.D., Columbia, 1901.]

ARTHUR KNUDSON: *Assistant,* 1912–. [A.B., University of Missouri, 1912.]

ETHEL WICKWIRE: *Assistant,* 1912–. [A.B., Tri-State College, 1909.]

TULA L. HARKEY: *Assistant,* 1912–. [A.B., Colorado College, 1909.]

BENJAMIN HOROWITZ: *Assistant,* 1913–. [B.S., Columbia, 1911 and A.M., 1912.]

CHRISTIAN SEIFERT: *Laboratory assistant,* 1898–.

STELLA WALDECK: *Recorder,* 1908–.

BLANCHE E. SHAFFER: *Laboratory assistant,* summer session, 1912.

JOSEPH S. HEPBURN: *University fellow,* 1912–'13. [A.B., Central High School, Philadelphia, 1903 and A.M., 1908; B. S., University of Pennsylvania, 1907 and M.S., 1907.]

* The work of the department was inaugurated in October, 1898, by Prof. R. H. Chittenden (lecturer and director), Dr. William J. Gies (instructor), Messrs. Alfred N. Richards and Allan C. Eustis (assistants), and Christian Seifert (laboratory assistant).

COURSES OFFERED BY THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY. 1911–1912

Courses 51, 105 and 215 are given during the first half-year only. Course 101 is given during the first half-year and is repeated (102) during the second half-year. Courses 104 and 110 (52) are given only during the second half year. All other courses are conducted throughout the entire academic year. All courses not otherwise specified are given at the College of Physicians and Surgeons.

(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. ELEMENTARY ORGANIC CHEMISTRY. Introductory to courses 101, 102 and 110 (52). (*Required of first year students of medicine.*) L, 1 hr. D. 1 hr. R, 2 hr., each section (2). Lw, 6 hr. each section (2). Profs. Gies and Howe, Drs. Wise and Goodridge, and Messrs. Miller and Knudson.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101–102. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (Teachers College, School of Practical Arts.) L, 2 hr. R. 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Dr. Seaman and Misses Wickwire and Harkey. (This course is designated "Chemistry 51" and "Household Arts Education 125" in the Teachers College Announcement.)

This course is designated "Chemistry s 51" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies, Dr. Seaman and Miss Shaffer.

104. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (Teachers College, School of Practical Arts.) L, 1 hr. Prof. Gies. (This course is designated "Chemistry 52" in the Teachers College Announcement.)

110 (52). GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R. 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Dr. Wise, and Messrs. Miller and Knudson.

This course is designated "*S*—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Dr. Smith.

209–210. CHEMISTRY OF NUTRITION. (School of Pharmacy. *Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

211–212. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* L, 1 hr. Lw, 4 hr. Dr. Eddy.

213–214. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (Teachers College, School of Practical Arts.) L, 1 hr. Lw, 5 hr. Prof. Howe, Dr. Seaman and Mr. Horowitz. (This course is designated "Household Arts Education 127" in the Teachers College Announcement.)

215. GENERAL BIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* L, 1 hr. Lw, 7 hr. Prof. Gies, Dr. Lothrop and Messrs. Miller and Knudson.

217–218. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS AND URINARY ANALYSIS IN GENERAL. L, 1 hr. Lw, 7 hr. Profs. Gies and Howe, Dr. Lothrop, and Messrs. Miller and Hepburn.

219–220. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* L, 2 hr. Lw, 14 hr. Profs. Gies and Howe, and Dr. Lothrop.

Courses in Nutrition (continued)

221–222. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies.

223–224. NUTRITION IN DISEASE. L, 1 hr. Profs. Gies and Howe, and Drs. Foster, Mosenthal, Kahn and Goodridge.

225–226. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Howe, and Dr. Lothrop.

TOXICOLOGY

231–232. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. Lw, 6 hr. Prof. Gies and Mr. Miller.

BOTANY

235–236. CHEMICAL PHYSIOLOGY OF PLANTS. (New York Botanical Garden.) L, 1 hr. Lw, 5 hr. Prof. Gies.

BACTERIOLOGY

241–242. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* L, 1 hr. Lw, 7 hr. Prof. Gies.

SANITATION

105. SANITARY CHEMISTRY. (Teachers College, School of Practical Arts). L, 1 hr. Lw, 3 hr. Dr. Seaman and Miss Harkey. (This course is designated "Chemistry 57" and "Household Arts Education 129" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301–302. BIOCHEMICAL SEMINAR. 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance, in any of the departmental laboratories.

LABORATORIES FOR ADVANCED WORK IN BIOCHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College, New York Botanical Garden and Bellevue Hospital. Each laboratory is well equipped for research in nutrition and all other phases of biological chemistry.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all past and present workers in the Department.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds scientific meetings regularly on the first Fridays in December, February and April, and on the first Monday in June. These meetings are open to all students in the University.

SUMMER SCHOOL COURSES

Summer session courses are mentioned in the foregoing references to Courses 101–102 and 110 (52). Prof. Gies will have charge of both courses next summer. He will also conduct a special lecture course in nutrition. The laboratories will be open for research throughout the summer.

ANNOUNCEMENTS

Professional Assistance Offered to Biological Chemists

The Columbia University Biochemical Association will be glad to cooperate confidentially with all who desire the services of biological chemists and with all who seek positions in biological chemistry.

Address inquiries to William J. Gies, 437 West 59th St., New York.

Journalistic

NEW JOURNAL. *Physiological Researches.* To appear at irregular intervals. Edited by *Burton E. Livingston,* Manager, Johns Hopkins University; *Daniel T. MacDougal,* Carnegie Institution of Washington; *Herbert M. Richards,* Columbia University. "The recent rapid advance of physiological science has been accompanied by a realization of the community of interest and uniformity of method which characterize the physiology of plants and of animals, and it has seemed highly desirable that the general physiological field thus indicated should possess an organ of publication in which its more comprehensive and technical papers might appear. This need is emphasized by the fact that present facilities for publication in physiology are generally taxed beyond their capacity and papers are consequently subject to long delays in appearance. It has therefore been decided to inaugurate a new series of scientific papers which will embrace contributions towards the advance of fundamental physiological knowledge."

"The plan of publication of the new series, for which the title of *Physiological Researches* has been adopted, is one in which practical ownership is vested in the contributors. It is hoped that the project will receive the interest and support of biologists of all classes. (Each volume will contain about 450 pages; each number will contain but a single contribution; and the numbers will be issued irregularly). Publication of the first contribution may be expected in a short time. Subscriptions will be received by the volume, the price being $5.00 per volume, payable in advance. Subscriptions to volume I, which are received prior to the date of publication of the first research, may be made at the reduced price of $4.00. At the date of the appearance of the first research the price will automatically become the regular one. Remittances should be made payable to *Physiological Researches,* and *all correspondence should be addressed to Physiological Researches, Station N, Baltimore, Maryland, U. S. A.*" (Editors' announcement.)

REDUCED SUBSCRIPTION PRICE OF THE JOURNAL OF BIOLOGICAL CHEMISTRY. The directors of the *Journal of Biological Chemistry* have announced that "beginning with the February issue of 1913 (Vol. 14, No. 1) the subscription price of the *Journal* to domestic subscribers will be reduced from $4.00 to $3.00 per volume; to foreign subscribers, $3.25. Any one engaged in biochemical work who subscribes for the *Journal* at this rate (beginning with Vol. 14) may secure Volumes 1–13 for $20.00, plus cost of transportation. The price at which a complete set has hitherto been sold is $50.00. Subscribers for the Journal who wish to complete their files may secure early volumes for $1.50 each, plus cost of transportation. Address: Alfred N. Richards, Secretary, University of Pennsylvania.

Meetings of Societies and Congresses

AMERICAN CHEMICAL SOCIETY: Annual meeting (47th) at Milwaukee, Wisconsin, *March 25–28*. Charles L. Parsons, Secretary, Box 505, Washington, D. C. At the last meeting of the Society the Council authorized the formation of a *Division of Biological Chemistry*. At that meeting the details of organization of the Division were entrusted to a committee. The committee will report in Milwaukee, the final organization of the Division will be perfected, and officers will be elected.

TENTH INTERNATIONAL CONGRESS OF AGRICULTURE: Ghent, Belgium, *June 8–13*. Secretary-general, Dr. P. de Vuyst, 22 Avenue des Germaines, Brussels. *American committee:* Dr. L. O. Howard, member of the International Commission on Agriculture and chief of the Bureau of Entomology; and Dr. A. C. True, director, Mr. John Hamilton, specialist in farmers' institutes, Dr. C. F. Langworthy, chief of nutrition investigations and Dr. J. I. Schulte, assistant agriculturist, of the Office of Experiment Stations.

AMERICAN MEDICAL ASSOCIATION, Annual meeting: Minneapolis, Minn., *June 17–20*. General secretary, Geo. H. Simmons, 535 Dearborn Ave., Chicago.

GENERAL MEETING OF THE INTERNATIONAL ASSOCIATION OF BOTANISTS: Copenhagen, *June 23*. Secretary-general, J. P. Lotsy, Haarlem, Holland.

SEVENTEENTH INTERNATIONAL CONGRESS OF MEDICINE: London, *Aug. 6–12*. General secretary, Dr. W. P. Herringham, 13 Hinde St., London, W.

FOURTH INTERNATIONAL CONGRESS ON SCHOOL HYGIENE: Buffalo, N. Y., *Aug. 25–30*. Secretary-general, Prof. Thomas A. Storey, College of the City of New York.

NINTH INTERNATIONAL PHYSIOLOGICAL CONGRESS: Groningen, Holland, *Sept. 2–6*. American Secretary, Prof. W. T. Porter, Harvard Medical School.

THIRD INTERNATIONAL CONGRESS OF REFRIGERATION: Washington, D. C., *Sept. 15* (opening meeting); Chicago, Sept. 17–23 (business and scientific meetings). Secretary-general, Mr. J. F. Nickerson, 431 So. Dearborn St., Chicago.

The Biochemical Bulletin

The BIOCHEMICAL BULLETIN is a quarterly biochemical review. It publishes results of original investigations in biological chemistry and presents miscellaneous items of personal and professional interest to chemical biologists. Original contributions to research, preliminary reports of investigations, abstracts of papers, addresses, reviews, descriptions of new methods and apparatus, practical suggestions to teachers, biographical notes, historical summaries, bibliographies, quotations, news items, proceedings of societies, personalia, views on current events in chemical biology, etc., are solicited.

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Remittances, manuscripts and correspondence should be addressed to the **Biochemical Bulletin**, 437 West 59th St., New York City.

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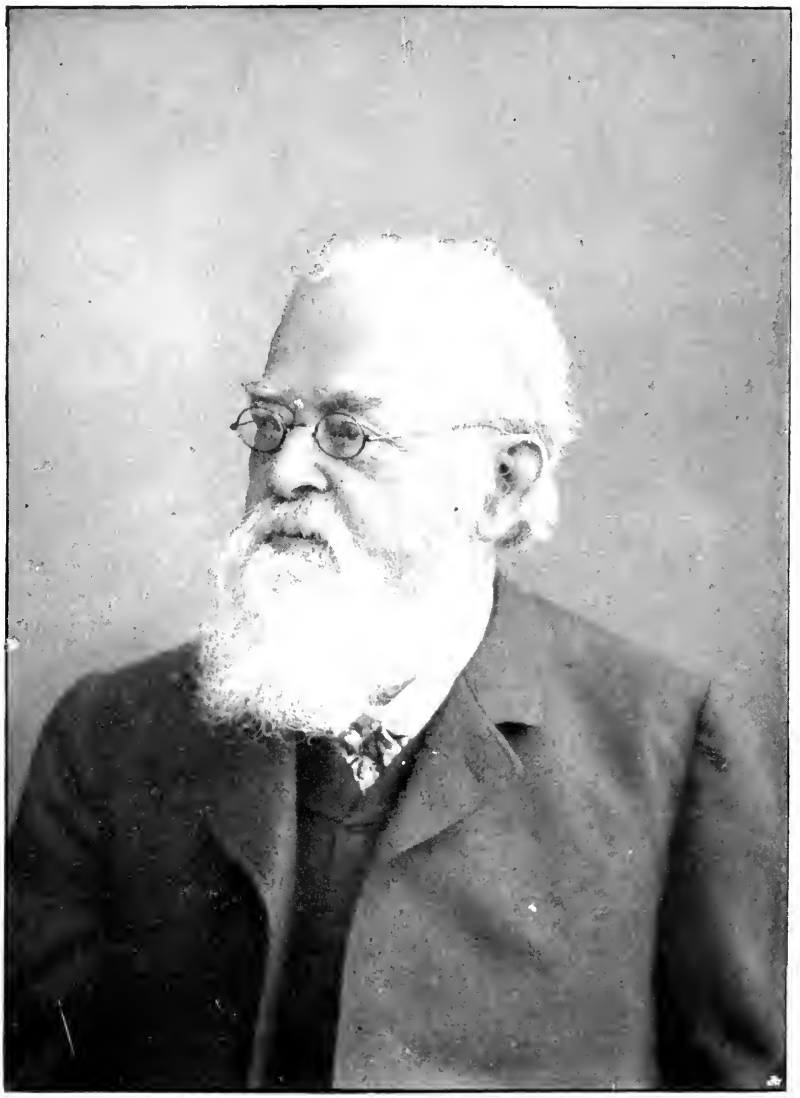

HEINRICH RITTHAUSEN.

BIOCHEMICAL BULLETIN

VOLUME II APRIL, 1913 No. 7

IN MEMORIAM

HEINRICH RITTHAUSEN

Born January 13, 1826 Died October 16, 1912

By the death of Heinrich Ritthausen in Berlin, on October 16, 1912, at the age of eighty-six, a long life spent in biochemical research was terminated. Beginning as a student under Liebig, and inspired by this great teacher, he made agricultural chemistry his life profession. His first work was as assistant to Professor Erdmann at Leipzig. From 1854 to 1856 he was director of the scientific department of the experiment station at Moeckern. He then became director of the station at Saarau in Schlesien. In 1858 he was appointed professor of chemistry and physics in the Royal Agricultural Academy at Waldau; in 1867, professor of chemistry and director of the experiment station at Poppelsdorf; and in 1873, professor of chemistry at Königsberg, where he remained until 1899, when his active career was concluded by advancing years. The latter part of his life was spent in Berlin.

At the present time, when the development of agricultural science has made plain the value of the agricultural experiment station not only to the farmer but to the entire community, the life of one who commenced his career in the first established institution of this kind is of special interest.

When Ritthausen began his work, the brilliant writings and lectures of Liebig had directed the attention of the whole world to the importance of applying the discoveries of science to the practice

of agriculture. As only the rudiments of a knowledge of the chemical and physical problems of the growth and maintenance of plants and animals had been acquired, it seemed a simple matter to instruct the farmer in proper methods to be employed in raising his crops and stock. No doubt remained in the minds of the early scientists who promoted this propaganda that the practical returns of their efforts would soon be realized.

Plants were to be fed with carbonic acid, nitrogen, and inorganic salts, and the proper quantity of each essential element of plant food was to be determined by chemical analysis of the tissues and ash of the plant, the fertilizer supplied and the soil in which it grew.

Animals were supposed to be composed of substances directly or indirectly assimilated from their foods. Their heat and mechanical energy was supplied by the carbohydrates and fats; the albumin, fibrin, casein and gelatin present in their blood, muscle, milk, etc., was furnished by identical proteins contained in the various vegetable products with which they were fed. Here again chemical analysis was to furnish the guide to proper practice, which should replace crude and wasteful methods founded in ignorance of the real factors involved.

Little did the enthusiasts who established the first agricultural experiment station realize what was before them nor what discoveries in every branch of biological science their efforts were to lead to. Little did they dream of enzymes and hydrolyses, of toxins and antitoxins and specificity of living tissues, of optical isomers and tautomeric compounds, of nucleic acids and pyrimidines, of amino-acids and polypeptides, of colloids and surface tension, nor of multitudes of other discoveries which have followed chiefly from the inspiration which Liebig imparted to those who worked with him.

All these discoveries had to be made before the practical problems of agriculture could be satisfactorily dealt with by the scientist; and it is evident that Ritthausen was one of the first to realize this, for we find him, after a short experience in attempting an immediate application of chemistry to the feeding of cattle, turning his attention to a careful study of the protein constituents of their vegetable foods.

His earliest paper on this subject, which appeared in the *Journal für praktische Chemie* in 1862, described the proteins of wheat; and for five successive years he published papers on this same subject. In the course of this work he isolated glutaminic acid from the products of hydrolysis of the gluten proteins, a discovery which ranks among the more important made by biochemists. He then extended his investigations to seeds of importance for nutrition, hardly a year passing when he did not contribute two or more papers on the results of his work.

In 1872 he published a review of his earlier work under the title *Die Eiweisskörper der Getreidearten, Hülsenfrüchten und Oelsamen.* This was the first attempt made to furnish an account of what had been learned respecting the properties of proteins of vegetable origin. Although this work contained much of value to animal physiologists, and was suggestive in many ways in connection with the problems then claiming their attention, few of them appear to have read it with the care that it deserved. Authors of text-books on physiological chemistry, for many years after, discovered in it nothing more than the fact that Ritthausen employed dilute alkaline solutions in isolating his preparations, and consequently dismissed his results with the statement that all his products were altered in their preparation and so deserved little consideration on the part of physiologists. Although such a criticism did not apply to the proteins soluble in strong alcohol which Ritthausen had described, and which after fifty years have become of much importance in the study of problems of nutrition, these remained for many years unknown to nearly every physiological chemist. After the publication of this review, Ritthausen continued his work until it included most of the seeds used for feeding men and animals.

When Hoppe-Seyler and Weyl introduced neutral saline solutions as solvents for many of the proteins of animal and vegetable origin, Ritthausen's results were regarded with increasing disfavor by physiological chemists. Undiscouraged by the unfair treatment accorded him, Ritthausen re-examined by the aid of salt solutions nearly all of the seeds which he had previously studied, and also showed that most of his earlier preparations were still soluble in

neutral salt solution and were unaltered in elementary composition. Little attention was, however, paid to this later work, although his papers were filled with information that has proved more helpful in developing our present knowledge of the chemistry of proteins in general than has most of that furnished by his critics.

Among the many proteins which he accurately described were several that could easily be obtained in well-formed crystals, a fact which, at that time, was of great importance in protein chemistry. Thus, in 1881, he described a crystalline protein of the hemp-seed and the method for its preparation, which is essentially that now in use. For more than twenty years this protein remained almost unknown although in recent years, under the name of edestin, it has been employed in hundreds of physiological experiments in connection with a great variety of problems in protein chemistry. As a result of his later work he proved that wide differences exist between different food proteins; and he was the first to direct attention to this fact, and to discuss its probable bearing on their relative value in nutrition.

Ritthausen's studies were not confined solely to the vegetable proteins, as is evident from his extensive bibliography which appears at page 339. He made many investigations of other constituents of seeds, obtaining vicin and convicin from vetch seeds, and discovered in the cotton-seed "melitose" now known as raffinose.

If we are to judge Ritthausen's work fairly we must remember that it was begun under the influence of Liebig's erroneous assumption that only a few forms of protein existed; that at that time organic chemistry was in its infancy; that few methods were known by which proteins could be isolated from the tissues containing them, or by which the different proteins could be separated from one another and be purified; that the only means for preventing the changes caused by bacteria and enzymes were low temperatures; and that the facilities for conducting such investigations were very limited. To the writer, who has had a long experience in this same field, under the vastly more favorable conditions prevailing a generation later, it is astonishing that Ritthausen accomplished so much, and that the data he secured were in the main so accurate. What-

ever may have been the shortcomings of Ritthausen's work, the fact remains that he made a most valuable contribution to biological chemistry; and that instead of criticism, he deserves our gratitude and admiration for his patience and perseverance in one of the most difficult fields of investigation.

THOMAS B. OSBORNE.

Connecticut Agricultural Experiment Station,
New Haven.

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Untersuchungen des Grünfutters von dem amerikanischen Zahnmais und dem österreichischen Mais (mit E. Wolff); 1854, 3, 1.

Vergleichende Untersuchung des schwedischen und des gewöhnlichen rothen Klees (mit E. Wolff); 1854, 3, 11.

Chemische Untersuchung von Gras, Heu und Grummet (mit E. Wolff); 1854, 3, 18.

Chemische Untersuchung der Runkelrübe: (*a*) Einfluss des Blattens auf die Zusammensetzung; (*b*) Einfluss der Grösse auf die Zusammensetzung; (*c*) Einfluss der Varietät auf die Zusammensetzung (mit E. Wolff); 1854, 3, 22.

Beobachtungen über die Milchproduktion bei dem Uebergang von der Winterfütterung zu der Grünfütterung (mit J. G. Bähr und E. Wolff); 1854, 3, 38.

Ueber den Einfluss des im Dampf gekochten Futters auf die Milchproduktion (mit J. G. Bähr); 1855, 4, 1.

Ueber den Einfluss der Zuckerrüben auf Milchproduktion (mit J. G. Bähr); 1855, 4, 13.

Düngungsversuche mit Knochenmehl, guanisirtem Knochenmehl, Blutdünger, und Guano (mit J. G. Bähr und W. Knop); 1855, 4, 15.

Versuche mit Ueberdüngung von Chilsalpeter, Kochsalz und Guano bei Weizen und Roggen (von J. G. Bähr, mitgetheilt von H. Ritthausen); 1855, 4, 22.

[1] Compiled from Nobbe's Quellenverzeichniss der hauptsächlichsten in den Jahren 1852 bis 1877 von den Versuchs-Stationen veröffentlichten wissenschaftlichen Arbeiten, in *Entwicklung u. Thätigkeit d. landwirtschaftlichen Versuchs-Stationen, etc.*, 1877, pp. 284–435 (Berlin).

Düngung des Roggens mit Peruanischem Guano, Chilsalpeter, gebranntem, reinen Knochenmehl und Polenz'schen Guano (von J. G. Bähr, mitgetheilt von H. Ritthausen); 1855, 4, 29.

Versuche mit verschiedenen Sorten Guano und Knochenmehl, Rapskuchenmehl und Stallmist zu Weizen und Kartoffeln (von J. G. Bähr, mitgetheilt von H. Ritthausen); 1855, 4, 31.

Einfluss der Düngung des Klee's mit Asche und Gyps; 1855, 4, 41.

Untersuchungen des schwedischen und rothen Klees; 1855, 4, 65.

Veränderungen des Heus von Rothklee durch Auswaschung von Regen; 1855, 4, 73.

Vergleichende Untersuchung der Wintergerste, Annat- und Probsteigerste; 1855, 4, 76.

Ueber den Einfluss der Lupinen auf die Milchproduktion, ein Fütterungsversuch (mit J. G. Bähr); 1856, 5, 1.

Ueber die Zusammensetzung und den Nahrungswerth einiger in der Landwirtschaft als Futtermittel angewendeter Fabrikationsrückstände (Kartoffelschlempe, Malz, Presshefe, Getreideschlempe); 1856, 5, 15.

Ueber einige Eigenschaften von Kulturpflanzen, die in gleicher Vegetationszeit einen verschiedenen Grad der Entwickelung zeigen (mit H. Scheven); 1856, 5, 67.

Entwicklung und Thätigkeit der land- und forstwirthschaftlichen Versuchs-Stationen in den ersten 25 Jahren ihres Bestehens; Festschrift zur Feier des 25 jährigen Jubiläums der Versuchs-Station Möckern; 1877, p. 56. (A statement, made by Professor Ritthausen at the request of Prof. G. Kühn, in regard to work conducted by or under him while Director of the Experiment Station at Möckern.)

3. Jahresberichte der Versuchs-Station Ida Marienhütte (bei Saarau) nach Breslau, gegrundet A. D. 1857

(Aus den Mittheilungen des landwirtschaftlichen Centralvereins für Schlesien)[2]

Zusammensetzung der Kuhmilch; 1, 59.

Ueber Dünger-Fabrikation; 1, 60.

Analysen des Bodens der Ida-Marienhütte (mit P. Bretschneider); 1, 82.

[2] Compiled from Nobbe's agricultural bibliography, 1877. See footnote, page 342. The numerals for the years of publication are not given in Nobbe's bibliography.

Versuche über Samendüngung; 1, 85.
Versuche mit Ueberdüngung des Roggens; 1, 95.
Düngungsversuche bei Rüben (*Beta*); 1, 104.
Untersuchung von in gleicher Vegetationszeit, ungleich entwickelten Kulturpflanzen; 1, 134.
Untersuchung eines Torfes und seiner Asche; 1, 145.
Bestimmung der Asche in Vegetabilien; 1, 147.
Bestimmung der Phosphorsäure; 1, 148.
Bestimmung der Kieselsäure in Pflanzenaschen; 1, 149.
Analysen des Bodens der Ida-Marienhütte (mit P. Bretschneider); 2, 36.
Untersuchung von Zuckerrüben; 2, 66.
Untersuchung von Zuckerrüben; 4, 59.
Analysen des Bodens der Ida-Marienhütte (mit P. Bretschneider); 6, 100.

4. Sächsiches Amts- und Amzeigeblatt

Versuche über den Nahrungswerth der Kartoffelschlempe in Vergleich zu Kartoffeln und Malz, und süsser Maische, bei gleichen Mengen Rohmaterial (Kartoffeln und Malz): Fütterungsversuche mit Kühen (mit J. G. Bähr); 1856, p. 87.
Fütterungsversuche mit Kühen ueber den Einfluss von geschrotenem und gekochtem Getreide auf Milchproduktion (mit J. G. Bähr); 1856, p. 96.
Ueber die Zusammensetzung einiger Wurzelgewächse (Rüben, Kohlrüben und Strunkkraut) und den Einfluss der Grösse und Schwere, sowie starker Düngung auf die Zusammensetzung derselben; 1857, p. 73.
Versuch über die Verdaulichkeit der Holzfaser des Futters beim Rind (mit H. Scheven); 1858, p. 58.

5. Landwirtschaftlichen Versuchs-Stationen

(Mittheilungen aus dem Agriculturchemischen Laboratorium der Universität Königsberg i. Pr.)
Untersuchungen ueber den Einfluss einer an Stickstoff und Phosphorsäure reichen Düngung auf die Zusammensetzung der Pflanze und der Samen von Sommerweizen (mit R. Pott); 1873, 16, 384.
Ueber die Einwirkung freier Phosphorsäure auf kohlensauren Kalk; 1877, 20, 401.
Ueber den Fettgehalt der käuflichen Kleberpräparate; 1877, 20, 408.

Analysen einiger Futtermittel; 1877, **20**, 409.
Ueber den angeblichen Gehalt des Roggensamens an Stearinsäure; 1877, **20**, 412.
Berichtigung zu der Mittheilung von M. von Sivera: Ueber den Stickstoffgehalt des Torfbodens; 1880, **25**, 169.
Ueber Zerstörung von Fett durch Schimmelpilze (mit H. Baumann); 1896, **47**, 389.
Ueber die Berechnung der Proteinstoffe in den Pflanzensamen aus dem gefundenen Gehalte an Stickstoff; 1896, **47**, 391.

6. Berichte der deutschen chemischen Gesellschaft

Ueber Vicin: Bestandtheil der Samen von *Vicia sativa*; 1876, **9**, 301.
Wassergehalt und Reaktion des Alloxantins; 1896, **29**, 892.
Ueber Alloxantin als Spaltungsprodukt des Convicins aus Saubohnen (*Vicia faba minor*) und Wicken (*Vicia sativa*); 1896, **29**, 894.
Ueber Galactit aus den Samen der gelben Lupine; 1896, **29**, 896.
Reaktionen des Alloxantins aus Convicin der Saubohnen und Wicken; 1896, **29**, 2106.
Vicin ein Glycosid; 1896, **29**, 2108.
Ueber Leucinimid, ein Spaltungsprodukt der Eiweisskörper beim Kochen mit Säuren; 1896, **29**, 2109.

7. Archiv für die gesammte Physiologie (Pflüger)

Die Eiweisskörper der Pflanzensamen; 1877, **15**, 260.
Ueber den Stickstoffgehalt der Pflanzen-Eiweisskörper nach den Methoden von Dumas und Will-Varrentrapp (mit H. Settegast); 1878, **16**, 293.
Ueber die Zusammensetzung der Proteinsubstanz der Bertholletia- (Para-) Nüsse; 1878, **16**, 301.
Ueber den Stickstoffgehalt der Pflanzen-Eiweisskörper nach den Methoden von Dumas und Will-Varrentrapp; 1878, **18**, 236.
Ueber die Eiweisskörper der Ricinussamen, der Proteinkörper, sowie der Krystalloide dieser Samen; 1879, **19**, 15.
Ueber die Eiweisskörper verschiedener Oelsamen; 1880, **21**, 81.

8. Chemiker-Zeitung

In Weingeist lösliches Gummi aus Roggen: Secalin; 1897, **21**, 717.
Zur Darstellung der Alkaloide der gelben Lupinen (*Lup. luteus*); 1897, **21**, 718.

9. Book

Die Eiweisskorper der Getreidearten, Hülsenfrüchte und Ölsamen; Beiträge zur Physiologie der Samen der Kulturgewächse, der Nahrungs- und Futtermittel; pages 252. Bonn (Max Cohen und Sohn), 1872.

10. Miscellaneous publications

Versuche über Düngung von Rüben; *Chemische Ackermann*, 1858, p. 130; abs. in *Jahresber. Agr. Chem.*, 1858, 1, 226.

Die Aschen einiger Futterpflanzen; *Mittheilungen aus Waldau*, 1859, p. 91; abs. in *Jahresber. Agr. Chem.*, 1859, 2, 84.

"Mug," ein Dungmittel; *Wochenblatt der Annalen der Landwirtschaft*, 1861, p. 8; abs. in *Jahresber. Agr. Chem.*, 1861, 4, 105.

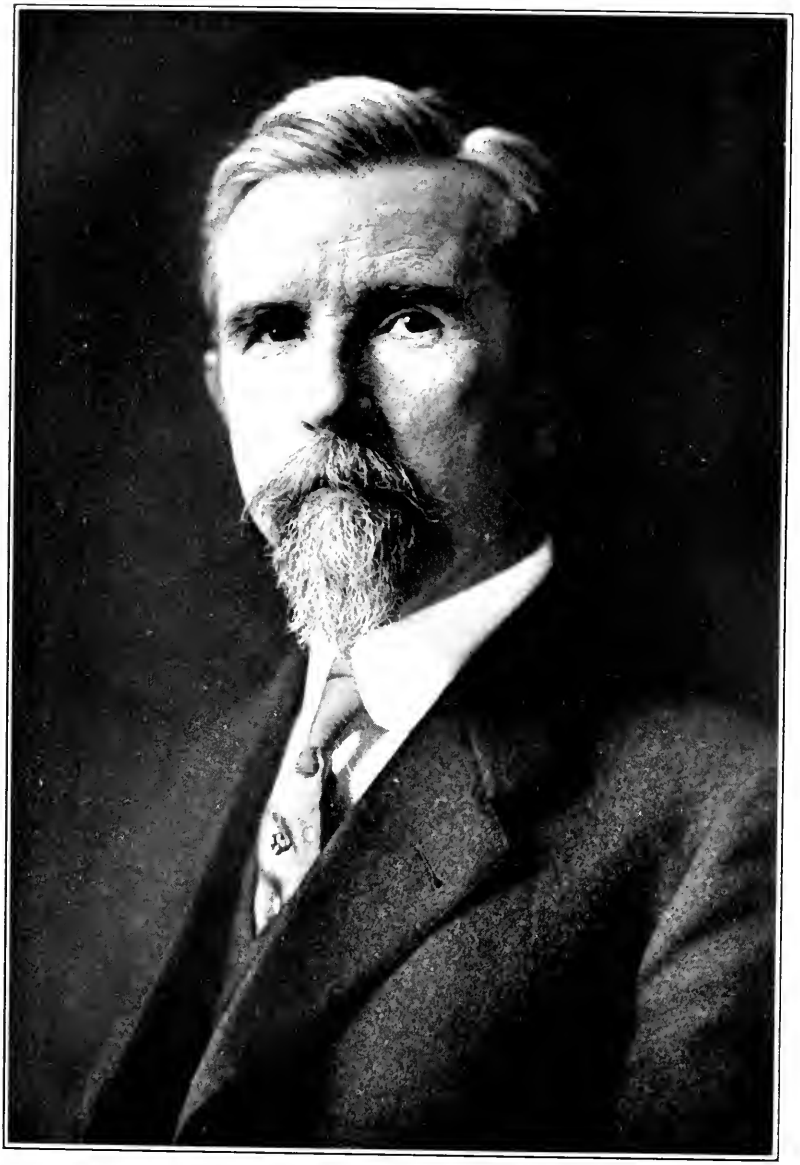
Das Verhalten der freien Phosphorsäure der Superphosphate; *Landwirtsch. Zeitung für das nordöstliche Deutschland*, 1875, 11; abs. in *Jahresber. Agr. Chem.*, 1875, 18, 51.

Verlust an Düngstoffen im Boden einer Düngerstätte (mit Ritschmann); *Agriculturch. Centralbl.*, 1876, p. 35; abs. in *Jahresber. Agr. Chem.*, 1876, 19, 40.

Ueber Proteinkörner, Krystalloide und krystallisirtes Eiweiss. *Schriften der Physikalisch-Ökonomischen Gesellschaft zu Königsberg;* 1881, 22, 15.

Lewis W. Fetzer.

Office of Experiment Stations,
U. S. Department of Agriculture,
and Georgetown University,
Washington, D. C.



Russell H. Chittenden

DINNER TO PROFESSOR CHITTENDEN

Testimonial by his pupils

In December, 1911, a number of the former pupils of Prof. Russell H. Chittenden, at a conference in Baltimore, concluded "that the time had arrived when it would be appropriate to provide some formal expression of the esteem in which Professor Chittenden is held by those who appreciate his contributions to physiological chemistry and education." Drs. S. W. Lambert, F. S. Meara, Holmes C. Jackson, S. P. Beebe, and William J. Gies were requested to serve as a provisional committee of five, to consider the matter further and to proceed with organization and execution of plans, "if some step in this direction seemed appropriate, after further consideration."

The provisional committee of five decided to organize Professor Chittenden's pupils for the purpose indicated, and invited twenty additional former pupils of Professor Chittenden's to serve with them as a Committee of Twenty Five, as follows: John A. Hartwell, *chairman*, T. S. Arbuthnot, S. P. Beebe, Joseph A. Blake, Harvey Cushing, H. H. Donaldson, Isadore Dyer, P. B. Hawk, Theodore C. Janeway, Elliott P. Joslin, J. H. M. Knox, Samuel W. Lambert, P. A. Levene, Frank S. Meara, Lafayette B. Mendel, Charles Norris, Thomas B. Osborne, Alfred N. Richards, E. W. Rockwood, W. T. Sedgwick, W. Gilman Thompson, H. Gideon Wells, E. B. Wilson, Holmes C. Jackson, *treasurer*, and William J. Gies, *secretary*. The Committee of Twenty-Five requested Drs. Hartwell, Beebe, Janeway, Jackson, and Gies to serve as a subcommittee for the execution of the committee's plans.

The general committee has invited Professor Chittenden's pupils to coöperate in raising a *Russell H. Chittenden Fund*, to be presented to the Yale corporation without any other condition than that it be used for the advancement of the work of the department of physiological chemistry in the Sheffield Scientific School.

The committee also authorized the secretary to invite Professor

Chittenden to be the guest of his pupils at a dinner in New York on March 1. In the formal invitation, the secretary wrote to Professor Chittenden, in part, as follows:

The above-named Committee of Twenty Five has instructed me to invite you to be the guest of your many pupils and friends at a dinner in your honor in New York City on March 1, 1913. It is our desire not only to have the pleasure of your company but also to extend to you our personal and professional greetings, and to evidence our friendship and respect.

The dinner in honor of Professor Chittenden was held at Delmonico's, on Saturday evening, March 1, and proved to be a delightful event in every particular. Although many who expected to be present were unable to attend, and sent letters of regret, about seventy-five pupils and a dozen invited friends comprised the enthusiastic company that made the dinner a cordial testimonial of affection and esteem for Professor Chittenden.

Among those who were unable to accept invitations to be present at the dinner, and to speak afterwards, were President Hadley, of Yale, and Prof. William H. Welch, of Johns Hopkins University. In a letter expressing regret for his unavoidable absence, President Hadley wrote, in part, as follows:

When you are having the Chittenden dinner, I shall be three thousand miles away. But I do not want to let the occasion go by without a word of greeting. Our universities are on the lookout for men who are either discoverers or teachers or organizers. In Chittenden Yale has a man who is all three.

Professor Welch sent a telegram in which he said:

Deeply regret unavoidable absence from banquet. Affectionate greetings and heartiest congratulations to Chittenden—the man and friend, the great teacher, investigator, administrator—who has rendered inestimable service to science, Yale, and country. May many years of health and vigorous work be his.

Seated at the speakers' table were Professors Harvey Cushing, Henry H. Donaldson, John A. Hartwell, Russell H. Chittenden,

THE MEMBERS OF THE GOVERNING BOARD

OF THE

SHEFFIELD SCIENTIFIC SCHOOL OF YALE UNIVERSITY

EXTEND CONGRATULATIONS TO THEIR COLLEAGUE

THE DIRECTOR OF THE GOVERNING BOARD

PROFESSOR

RUSSELL HENRY CHITTENDEN

PH. D., SC. D., LL. D.

ON THE OCCASION OF THE TESTIMONIAL DINNER

GIVEN IN HIS HONOR BY HIS PUPILS

AND FRIENDS ON

MARCH FIRST NINETEEN HUNDRED AND THIRTEEN

IN NEW YORK CITY

THE EVENT WHICH HAS CALLED FORTH THIS PERSONAL MANIFESTATION OF ESTEEM AFFORDS AN OPPORTUNITY TO THE MEMBERS OF THE GOVERNING BOARD TO GIVE FORMAL EXPRESSION TO THEIR HIGH ESTIMATE OF PROFESSOR CHITTENDEN'S CONTRIBUTIONS TO SCIENCE AND EDUCATION AND THEIR GRATIFICATION AT THE FAVORABLE RECOGNITION WHICH HAS BEEN ACCORDED TO HIS DISTINGUISHED SERVICES IN THE PROMOTION OF PHYSIOLOGICAL RESEARCH

THE SUCCESS WITH WHICH PROFESSOR CHITTENDEN HAS FURNISHED INSPIRATION FOR THE LIFE WORK OF OTHERS IS WORTHY OF COMMENDATION; HIS ENERGY RECALLS THE WORDS OF ANOTHER EMINENT STUDENT OF NUTRITION:

> "THE GREATEST JOY OF THOSE WHO ARE STEEPED IN WORK AND WHO HAVE SUCCEEDED IN FINDING NEW TRUTHS AND IN UNDERSTANDING THE RELATION OF THINGS TO EACH OTHER, LIES IN WORK ITSELF."

TO THE GRATITUDE AND REGARD OF PROFESSOR CHITTENDEN'S PUPILS HIS COLLEAGUES OF THE GOVERNING BOARD NOW DESIRE TO ADD THE CORDIAL ASSURANCE OF THEIR BEST WISHES AND THEIR PERSONAL GREETINGS.

William G. Hunter — Charles L. Hastings — Alexander Jay du Bois

[illegible] — Horace L. Wells — L. P. Brecknridge

Charles Schuchert — Charles E. Clarke — Ross T. Harrison

[illegible] — [illegible] — Guy L. Calkins

Lafayette B. Mendel — Charles Scott — J. E. Macallum

[illegible] — Wesley R. Coe — William C. Abbott

John Dan Irving — Harry M. Foote — Alexander W. Evans

Greetings to Professor Chittenden by his Colleagues of the Governing Board of the Sheffield Scientific School

Frank S. Meara, Graham Lusk, William T. Sedgwick, William T. Porter and Elliott P. Joslin.

At the conclusion of the dinner, the chairman of the committee, Dr. Hartwell, extended to Professor Chittenden the affectionate greetings of his pupils and friends, and informed him of the establishment of the *Russell H. Chittenden Fund for the Advancement of Physiological Chemistry in the Sheffield Scientific School.* Dr.

Faces of the gold medal presented to Professor Chittenden by the National Institute of Social Sciences

Hartwell stated that the amount of the fund and the time of its presentation to the Yale corporation will be announced at an early date. Dr. Hartwell concluded his remarks by introducing the toastmaster, Dr. Meara, who officiated in the graceful and inimitable manner in which he is accustomed to preside on such occasions. Informal after-dinner addresses were then made by Drs. Cushing, Donaldson, Joslin, Levene, Lusk and Sedgwick, to which Professor Chittenden responded.

The speakers who preceded Professor Chittenden paid eloquent tribute to the personality, influence, service, and achievements which have made Professor Chittenden the Dean of American biological chemists. Professor Chittenden replied earnestly and with deep feeling to the cordial tribute which had been conveyed in the sentiments of the speakers, in the abundant evidence of warm approval with which each address was received, and in the evident heartiness of his own reception.

Immediately after the conclusion of Professor Chittenden's address, the toastmaster announced that the National Institute of Social Sciences had voted a gold medal to Professor Chittenden in recognition of the distinction he has attained in original investigation in the field of physiological chemistry. Dr. H. Holbrook Curtis, secretary of the Institute, made the presentation. The faces of the medal are shown on page 353.

Prof. Lafayette B. Mendel followed Dr. Curtis with a presentation of engrossed congratulatory resolutions which had been adopted by Professor Chittenden's associates in the Governing Board of the Sheffield Scientific School. (See page 351.)

The accompanying group portrait was made just after seats had been taken at the tables. The names of all in attendance, arranged in table groups, are appended:[1]

Speakers' table

Harvey Cushing	Russell H. Chittenden	William T. Sedgwick
H. H. Donaldson	Frank S. Meara	William T. Porter
John A. Hartwell	Graham Lusk	Elliott P. Joslin

Table 1. Lafayette B. Mendel, S. J. Meltzer, Jacques Loeb, Yandell Henderson, Simon Flexner, P. A. Levene, Frederic S. Lee.

Table 2. Henry Hun, William Browning, H. H. Curtis, Henry Ling Taylor, W. M. Kenna, Harry Saltzstein, Frank C. Gephart, H. G. Barbour.

Table 3. John Rogers, Wm. L. Culbert, R. H. Wylie, William Armstrong, Joseph A. Blake, W. L. Griswold, G. Wyckoff Cummins.

Table 4. Theodore C. Janeway, J. H. M. Knox, S. P. Goodhart, Chas. H. Studin, George S. C. Badger, Joseph S. Wheelwright, N. R. Norton, Joseph H. Pratt.

Table 5. Charles Norris, A. N. Richards, E. K. Dunham, John A. Mandel, H. D. Dakin, George B. Wallace, William J. Gies, Holmes C. Jackson, William C. Lusk.

[1] The men at tables 11 and 12, when the photograph was taken, subsequently reassembled at tables 9, 10 and 2. One or two additional rearrangements account for the disagreement between the indications of the portrait and the table lists.


DINNER GIVEN IN HONOR OF
PROFESSOR RUSSEL H. CHITTENDEN
THEIR ADMIRATION AND AFFECTION
THEIR COLLEAGUE AND TEACHER

Table 6. S. P. Beebe, G. A. Hanford, A. L. Dean, Frank P. Underhill, Benjamin White, Oswald T. Avery, Leo F. Rettger, S. R. Benedict.

Table 7. Isadore Dyer, Wm. C. Wurtemberg, Robert Taylor Wheeler, Donald Guthrie, Henry H. Janeway, A. W. Elting, Charles L. Scudder.

Table 8. Lewis F. Frissell, Seth M. Milliken, Robert P. Wadhams, William P. Healy, Norman E. Ditman, W. W. Herrick, M. Heminway Merriman, Cyrus W. Field.

Table 9. Frank C. Yeomans, Alfred Jerome Brown, J. L. Bendell, Isaac F. Harris, Stanley D. Beard, Frank E. Hale, E. Monroe Bailey, Otto G. Hüpfel.

Table 10. Israel S. Kleiner, Lewis H. Weed, Orville H. Schell, Simon B. Kleiner, Morris S. Fine, Victor C. Myers, Warren W. Hilditch, Henry C. Courten.

Adjournment occurred at a very late hour, but many tarried to discuss informally the happy events of the evening and to talk over "old times" in Chittenden's laboratory.

'94 S.

New York City.

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

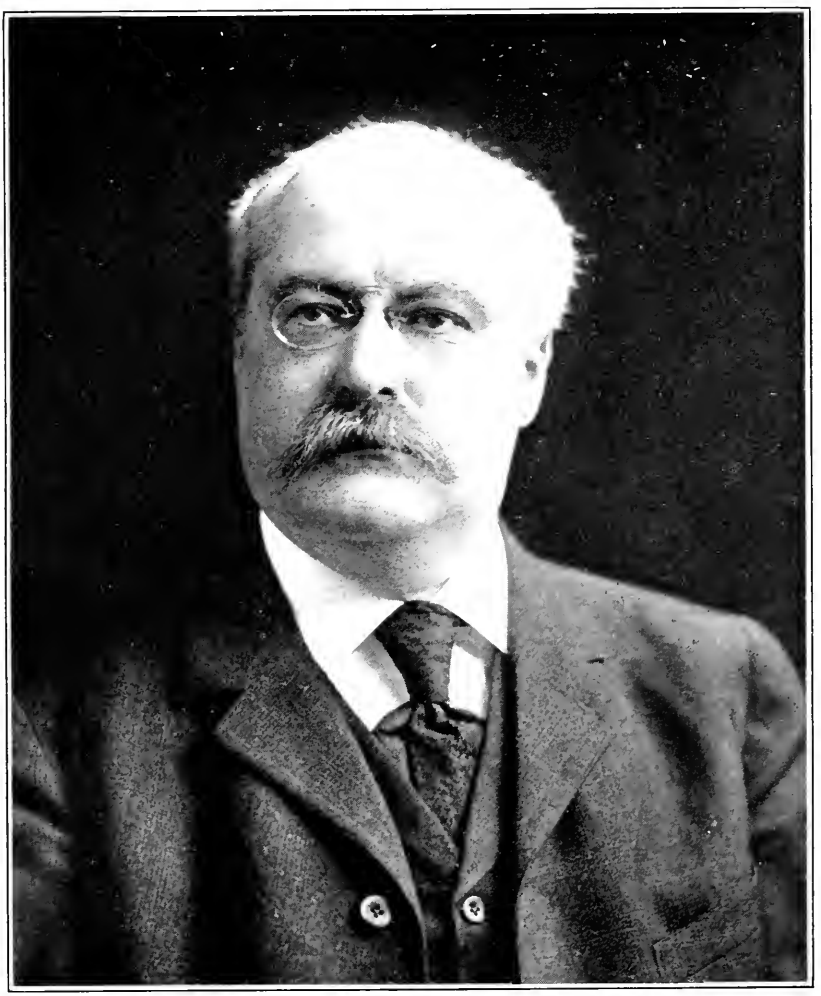
Tenth anniversary meeting and dinner

The tenth anniversary of the establishment of the Society for Experimental Biology and Medicine was celebrated in New York on the 19th of February. The *fifty second* regular scientific meeting was held, at 4 p. m., at the College of Physicians and Surgeons, in the lecture room adjoining the main biochemical laboratory. After the conclusion of the scientific meeting, at about 7 p. m., the members adjourned to the Hofbräu House (39th Street and Broadway), where, in a body, they feasted on a beefsteak dinner prepared under the auspices of a committee of which Prof. Graham Lusk was chairman.

The scientific session was the most interesting and important in the history of the society. The nature of the proceedings is shown by the appended copy of the official program:

G. N. Calkins: Further light on the conjugation of paramecium.—*W. H. Manwaring and J. Bronfenbrenner:* On lysis of tubercle bacilli (II); *On chemotherapeutics of tuberculosis.—*W. S. Halstead:* Hypertrophy of the thyroid; Partial occlusion of the aorta by bands of living tissue.—*G. H. A. Clowes:* Hay fever, with demonstration.—**A. I. Ringer:* Further studies on the fate of fatty acids in the diabetic organism.—*R. M. Pearce and P. F. Williams:* Experience with Abderhalden's test for pregnancy.—*L. L. Woodruff:* The kernplasma relation during the life of a pedigreed race of *Oxythricha fallax.—Richard Weil:* A new factor in anaphylaxis.—*E. E. Butterfield:* The reaction between oxygen and hemoglobin.—**F. S. Lee and S. Everingham:* The myoneural junction in fatigue.—*F. H. Pike:* A demonstration of the effects of electrical stimulation of the labyrinth of the ear.—*E. L. Scott:* The relation of pancreatic extract to the sugar of the blood.—*A. F. Hess:* The pancreatic lipase of infants in acute intestinal disturbances.—*W. H. Park, L. W. Famulener and E. J. Banzhaf:* Influence of protein concentration on absorption of antibodies in subcutaneous injections.—

*On the official program, but not abstracted in the *Proc. Soc. Exp. Biol. and Med.*, 1913, x, pp. 65–122.



S. J. Meltzer

Portrait of the Founder of the Society for Experimental Biology and Medicine.
Reproduced from Volume II (1904–'05) of the Society's Proceedings

G. C. Robinson: The influence of the vagus nerves on the faradized auricles in the dog heart.—*B. S. Oppenheimer and H. B. Williams:* Prolonged complete heart block with frequent changes in the idio-ventricular complexes.—*C. J. Wiggers and E. F. DuBois:* Methods for the production of temporary valvular lesions.—*A. J. Goldfarb:* The influence of the central nervous system on regeneration; The effect of salinity upon regeneration.—*B. T. Terry:* Variations in the amount of transformed atoxyl (trypanotoxyl) produced by varying the strength of atoxyl incubated with blood.—*W. J. MacNeal and A. F. Chace:* Some observations on bacteria of the duodenum.—*A. E. Cohn:* The effects of morphin on the mechanism of the dog heart after removal of one vagus nerve.—*T. S. Githens:* The influence of temperature on the minimal dose of strychnin and the onset of tetanus in the frog.—**J. E. McWhorter and F. Prime* (by invitation): Cinematographic demonstration of the growth of tissues.—**F. S. Lee:* Cinematographic demonstration of the beating heart.—**R. Burton-Opitz:* Demonstration of the vasomotor nerves of the liver.—**H. B. Williams:* Demonstration of the electrovagogram.—*Wm. de B. MacNider:* The difference in the effect of Gréhant's anesthetic and of morphin-ether on the total output and composition of the urine in normal dogs.—*Sutherland Simpson:* The rate of growth in the dog.—*Andrew Hunter:* The influence of experimental cretinism upon nitrogenous metabolism in the sheep.—*DeWitt Stetten and Jacob Rosenbloom:* Metabolism studies in a case of hypopituitarism, with infantilism of the Lorain type.—*J. P. Atkinson and C. B. Fitzpatrick:* On the presence of pressor substances in experimental immunity.—*G. H. A. Clowes, Francis C. Goldsborough and F. West:* On a complement-deviation reaction exhibited in pregnancy.—*G. H. A. Clowes and Francis C. Goldsborough:* On the antitryptic reaction exhibited in pregnancy.

Prior to adjournment an election of officers for 1913–'14 occurred, with the following results: President, *James Ewing* (reelected); vice president, *Cyrus W. Field;* secretary, *Holmes C. Jackson;* treasurer, *Charles Norris* (reëlected). The new members elected were Russell L. Cecil, Cary Eggleston, K. George Falk, Davenport Hooker, Paul E. Howe and Charles J. West.

The dinner was a very enjoyable event. The accompanying group portrait shows the condition of the party at midnight. All

* On the official program, but not abstracted in the *Proc. Soc. Exp. Biol. and Med.*, 1913, x, pp. 65–122.

those at the reader's extreme left and right who did not get into the picture were under the table when the photograph was taken.[1]

The president, Prof. James Ewing, ably and entertainingly conducted the after-dinner proceedings. Informal speeches were made, at the call of the President, by the distinguished founder, Dr. S. J. Meltzer, also by Drs. Graham Lusk, Frederic S. Lee, G. H. A. Clowes, Jacques Loeb, William H. Park and William J. Gies.

The speakers felicitated Dr. Meltzer on the happiness of the idea that led him to found the society; they also complimented him on the society's past service, and on its vigor and effectiveness at the tenth anniversary of its birth. There was a strong note of congratulation of the society itself on the prospect of steady growth in efficiency and usefulness.

The names of the members present at the meeting or at the dinner, or both, are appended:

J. P. Atkinson
John Auer
J. H. Austin
F. W. Bancroft
S. P. Beebe
Jacob Bronfenbrenner
E. E. Butterfield
G. N. Calkins
G. H. A. Clowes
A. E. Cohn
Rufus I. Cole
J. W. Draper
E. F. Du Bois
E. K. Dunham
A. B. Eisenbrey
C. A. Elsberg
Haven Emerson
James Ewing
L. W. Famulener
Cyrus W. Field
C. B. Fitzpatrick
Simon Flexner
N. B. Foster
William J. Gies
T. S. Githens
A. J. Goldfarb
W. S. Halstead
Isaac F. Harris
Alfred F. Hess
Paul E. Howe
H. C. Jackson
Walter A. Jacobs
H. H. Janeway
Don R. Joseph
Ludwig Kast
I. S. Kleiner
R. A. Lambert
Frederic S. Lee
P. A. Levene
Isaac Levin
Charles C. Lieb
Jacques Loeb
W. F. Longcope
Graham Lusk
W. G. MacCallum
W. J. MacNeal
F. H. McCrudden
A. R. Mandel
John A. Mandel
W. H. Manwaring
S. J. Meltzer
G. M. Meyer
H. O. Mosenthal
John R. Murlin
J. B. Murphy
V. C. Myers
Hideyo Noguchi
Charles Norris
B. S. Oppenheimer
A. M. Pappenheimer
William H. Park
R. M. Pearce
F. H. Pike
A. I. Ringer
G. C. Robinson
Peyton Rous
E. L. Scott
G. G. Scott
H. D. Senior
M. Sittenfeld
Edna Steinhardt
H. A. Stewart

[1] Drs. Auer, Bancroft, Dunham, Eisenbrey, Field, Hess, Jackson, Mandel brothers, Norris, Oppenheimer, Park, Pearce, Senior, Swift, Wadsworth, Wallace, Wood. Anticipating the fate of the Michigan editor in the Roosevelt water-wagon case, we wish to add that we do not believe this situation implies anything more than the facts themselves indicate.

Members of the Society for Experimental Biology and Medicine at the Dinner, on February 19, 1913.

H. F. Swift	George B. Wallace	Anna W. Williams
B. T. Terry	Richard Weil	H. B. Williams
D. D. Van Slyke	C. J. West	Francis C. Wood
A. B. Wadsworth	C. J. Wiggers	

The Society for Experimental Biology and Medicine has been an important influence in the development of biological and medical science, particularly in New York. It has stimulated aspiration, quickened activity, increased productivity, afforded a congenial and ready means of expression, and opened a suitable channel for communication, during a period of awakening in the biological and medical sciences in New York. It continues in this rôle as an influential factor in the advancement of science in this country.

The growth of the society is indicated by the appended tabulation of its total membership at the end of each successive academic year since its foundation in 1903:

Year	Total	Increase	Year	Total	Increase	Year	Total	Increase
1903	19	—	1907	140	21	1911	222	17
1904	55	36	1908	162	22	1912	239	17
1905	87	32	1909	185	23	1913:		
1906	119	32	1910	205	20	(Feb. 19)	255	16

Biological chemists may be interested in the following statistical summary relating to the Society for Experimental Biology and Medicine:

Of the seven men at the conference in Prof. Graham Lusk's home preliminary to organization, on January 19, 1903, four were biological chemists.

The society was formally organized at a meeting in the biochemical laboratory of Columbia University, at the College of Physicians and Surgeons, N. Y., on Feb. 25, 1903.

Two of the three authors of the constitution, and two of the first five officers, were biological chemists.

The following members of the American Society of Biological Chemists are members of the Society for Experimental Biology and Medicine:

J. J. Abel, J. G. Adami, H. M. Adler, C. L. Alsberg, J. P. Atkinson, E. J. Banzhaf, S. P. Beebe, F. G. Benedict, S. R. Benedict, W. N. Berg,

F. J. Birchard, Russell Burton-Opitz, R. H. Chittenden, A. C. Crawford, H. D. Dakin, E. K. Dunham, C. W. Field, Otto Folin, N. B. Foster, C. Stuart Gager, R. B. Gibson, William J. Gies, Shinkishi Hatai, R. A. Hatcher, P. B. Hawk, Paul E. Howe, W. H. Howell, Reid Hunt, Andrew Hunter, H. C. Jackson, W. A. Jacobs, Walter Jones, J. H. Kastle, I. S. Kleiner, Oskar Klotz, J. B. Leathes, P. A. Levene, Jacques Loeb, A. S. Loevenhart, Graham Lusk, A. B. Macallum, J. J. R. Macleod, W. deB. MacNider, J. A. Mandel, F. H. McCrudden, L. B. Mendel, G. M. Meyer, J. R. Murlin, V. C. Myers, F. G. Novy, T. B. Osborne, Franz Pfaff, A. N. Richards, A. I. Ringer, T. B. Robertson, Jacob Rosenbloom, William Salant, P. A. Shaffer, H. C. Sherman, Torald Sollmann, L. B. Stookey, A. E. Taylor, F. P. Underhill, D. D. Van Slyke, G. B. Wallace, H. G. Wells, C. G. L. Wolf.

Of the 775 communications to the Society for Experimental Biology and Medicine at its first fifty-two meetings, 430—more than half—were largely or entirely biochemical in character.

NINETEEN O. THREE

New York City

METHODS FOR THE ELECTROMETRIC DETERMINATION OF THE CONCENTRATION OF HYDROGEN IONS IN BIOLOGICAL FLUIDS

K. A. HASSELBALCH

(*Finsen Institute, Copenhagen, Denmark*)

(WITH PLATE 3)

The great importance of the reaction of the medium in many biological processes has long been appreciated and has led to a series of more or less successful endeavors to measure its degree. It is only within the most recent years, however, that the methods of measurement have been so far perfected as to enable us to say that the "true reaction" of biological fluids can now be measured with sufficient accuracy for most purposes.

This is due, in the first instance, to the insight into the nature of the question which has been derived from the electrolytic dissociation theory: the "true reaction" of a liquid is not determined by its concentration of free acid or alkali, but by its concentration of hydrogen and hydroxyl ions—or, practically speaking, by the concentration of hydrogen ions alone, for the product of the two is a constant. Since the dissociation of acid or base in a liquid, and thereby also its hydrogen-ion concentration, is in many respects dependent on the nature of the dissolved substances, the true reaction of the liquid cannot be determined by merely measuring the quantity of alkali or acid that must be added to a certain quantity of the liquid in order to effect a particular change of color in the indicator used. As is now known, such a titration shows only that, at the moment, a certain hydrogen-ion concentration, to which the indicator reacts, has been reached, the original hydrogen-ion concentration of the liquid remaining unknown.

Thus, the determination of the true reaction of a liquid requires some procedure by which the concentration of the hydrogen ion is

not altered. Only two methods of the latter kind are in practical employment, namely, the colorimetric and the electrometric.

The colorimetric method is based on the above-mentioned fact, that a series of indicators shows certain color nuances with known hydrogen-ion concentrations, which must be determined electrometrically, so that the electrometric determination of the hydrogen-ion concentration must at any rate be considered as the fundamental method. The colorimetric method has been indicated by Friedenthal and Salm;[1] its field has been considerably widened and its trustworthiness assured by the thorough investigations and improvements of S. P. L. Sörensen and his collaborators. I shall not discuss the technical details of the method but merely refer to Sörensen's latest summary of his work.[2]

We owe the electrometric method orginally to Nernst.[3] It was first applied to biological fluids by Bugarsky and Liebermann,[4] and by Höber.[5] It is based on the fact that a hydrogen-saturated metal electrode in a hydrogen-saturated liquid gives rise to a difference of potential between the electrode and the liquid, which is dependent on the hydrogen-ion concentration according to known laws. The determination of this difference of potential thus makes it possible to determine the hydrogen-ion concentration of the liquid.

The experimental method generally employed to measure the difference of potential between the hydrogen-saturated electrode and the hydrogen-saturated liquid has been so often described in its main features, most recently by Sörensen[6] in the above-cited work, that it needs no attention here. The present paper deals with the difficulty of obtaining the condition presupposed by the method, *viz., saturation of the electrode and liquid with hydrogen, without any alteration in the hydrogen-ion concentration of the liquid.*

Biological fluids, as is well known, usually contain volatile acids (or bases) which determine, in great part, their hydrogen-ion concentration, so that the *normal* electrometric method, by which liquid and electrode are saturated with a current of hydrogen bubbled

[1] Friedenthal and Salm: *Zeitschr. f. Elektroch.*, **10**, 1904; **12**, 1906; *13*, 1907.
[2] Sörensen: *Ergebnisse der Physiologie*, **12**, 1912.
[3] Nernst: *Zeitschr. f. physikal. Chemie*, **4**, 1889.
[4] Bugarsky and Liebermann: *Pflüger's Arch.*, **72**, 1898.
[5] Höber: *Pflüger's Arch.*, **81**, 1900.
[6] Sörensen: *Loc. cit.*

through the fluid, cannot be used. Let us take an extreme case and see what even a slight carbonic-acid tension—according to ordinary ideas—may mean for the hydrogen-ion concentration of a liquid. Sea-water and the surrounding atmosphere have the same carbonic acid tension—*ca.* $0.04/100 \times 760 = 0.3$ mm. On driving all the carbonic acid from the sea-water we should cause the hydrogen-ion concentration to sink from *ca.* 10^{-8} to *ca.* 10^{-9} or, using Sörensen's terminology,[7] the hydrogen-ion exponent, p_H, would rise from 8 to 9. If, therefore, in this case we saturated the liquid and electrode with a current of hydrogen, quite an erroneous result would be obtained.

A similar error, though less in amount, would also arise if we had recourse to the auxiliary method applied in such cases at the beginning of this century, namely, if the hydrogen-saturated electrode remained in contact with the liquid and we waited until the potential became constant, *i. e.,* until equilibrium had been attained in the diffusion between the liquid and the hydrogen atmosphere. For instance, a sample of sea-water (kept in a bottle for nearly a year), whose p_H was in reality 7.55, showed $p_H = 7.74$ on using this method. The error may be reduced if, with Michaëlis,[8] we take a small quantity of hydrogen and let the electrode only just touch the surface of the liquid. But the most satisfactory method of proceeding seems to me the following:[9]

A current of pure hydrogen, saturated with moisture, is led through the vessel containing the electrode until the latter has become saturated with hydrogen. The experimental liquid, which is stored in such a way that it retains its natural tension of volatile acid (or base), is now led into the vessel in such a quantity that the electrode reaches more or less deeply into the fluid (see below) and the vessel is then closed. By shaking the vessel, the establishment of diffusion equilibrium between liquid and hydrogen, and attainment of constancy in the measured potential, are accelerated. This constancy, however, has been obtained by the loss of part of the volatile acid (or base) from the liquid to the hydrogen and

[7] Sörensen: *Bioch. Zeitschr.,* **21**, 1909.
[8] Michaëlis: *Ibid.,* **18**, 1909; **46**, 1912.
[9] Hasselbalch: *Ibid.,* **30**, p. 317, 1910; **38**, p. 77, 1913; **49**, p. 450, 1913.

would, therefore, indicate a too alkaline (or too acid) reaction of the fluid. The liquid is now renewed without changing the gas-mixture around the electrode, and shaking is repeated. It is easily seen that electromotive constancy may now be obtained without any, or at least without any appreciable, alteration of the tension of the volatile acid (or base), *i. e.*, without alteration of the original hydrogen-ion concentration of the liquid.

This procedure may be repeated, if necessary, until the renewal of liquid no longer causes any alteration in the measured potential. For blood, urine, and probably the majority of biological fluids, a single renewal is sufficient. Sea-water and similar solutions, which are poor in "reaction regulators"[10] (*Henderson*[11]), are *eo ipso* far more susceptible to the change in carbonic-acid tension resulting from the method of measurement and would require three to four or, according to circumstances, even a larger number of renewals of the liquid before constancy is reached. In such cases it is easier, and more correct, to extrapolate graphically from the first three measurements (*i. e.*, after two renewals of the liquid) in order to get the final value.

The procedure described here permits one inconsiderable error, for which a correction may be made, if necessary. When diffusion equilibrium between the hydrogen and the liquid has been obtained, none of the components are, strictly speaking, any longer saturated with moist hydrogen at the existing barometric pressure but at a somewhat lower pressure. The potential changes, however, in accord with the logarithm of the hydrogen pressure, so that, *e. g.*, a fall in the hydrogen pressure from 760 to 700 (and a greater fall is practically inconceivable) would drop the measured potential to a value *ca.* 1 milli-volt too low. An error like this lies very near the limit of error of the whole method but may be eliminated, as already mentioned, by analysis of the hydrogen mixture and by calculation.

Solutions which are poor in "reaction regulators," but whose hydrogen-ion concentration (owing to the volatile acid or base they contain) must necessarily be measured in the above-mentioned way (if it cannot be measured colorimetrically), have been found to

[10] Compounds which, by their presence, diminish the effect on the hydrogen-ion concentration of changes in the proportion of acid or base.

[11] Henderson: *Ergebnisse der Physiologie,* **8**, 1909.

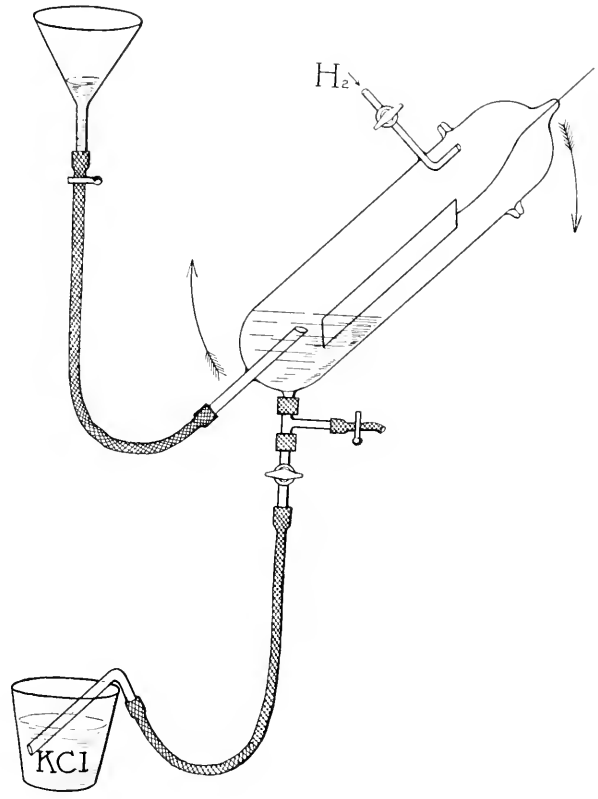


FIG. 1.

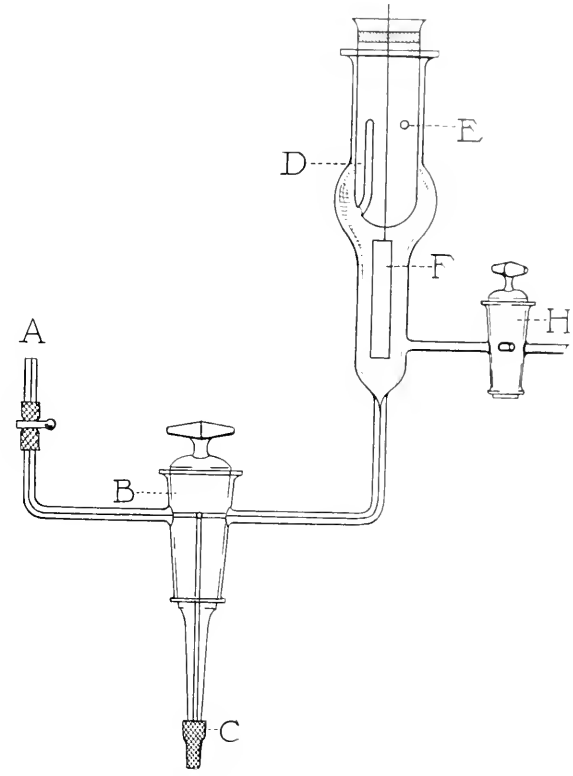


FIG. 2.

HASSELBALCH: METHODS FOR THE ELECTROMETRIC DETERMINATION OF THE CONCENTRATION OF HYDROGEN IONS IN BIOLOGICAL FLUIDS.

present the difficulty that the potential changes in an unaccountable manner when the liquid in the electrode vessel is at rest. For this reason I have proposed, as a normal method in measuring biological fluids, that the shaking should be mechanical and permanent, even during the reading of the electrometer. The electrode vessel is seen in Plate 3, Fig. 1. The arrows indicate the direction and extent of the movement. While the shaking takes place, the electrode is constantly immersed in the liquid.

It is easily seen that this electrode vessel, by another arrangement of the T-tube, may also be used in measurements which permit hydrogen to be led through the liquid. This property of the vessel may be of use, *e. g.*, in efforts to control the correctness of the electrode by measurement of "standard solutions" of a known hydrogen-ion concentration.

Fig. 2 (Plate 3) shows an electrode vessel used by me for small quantities of fluid, especially in determinations of the hydrogen-ion concentration in 2–3 c.c. of human blood to which some hirudin is added. The blood may be taken from the lobe of the ear; it is saturated in a glass syringe by rotation with about 20 c.c. of the alveolar air of the individual. The electrode, F, is saturated with a current of hydrogen flowing in the direction A → B → D → E. D is a groove in the inner part of a ground glass stopper lubricated with vaseline and, during the flow of the hydrogen, it is turned so as to be opposite the hole E in the outer wall of the apparatus. The saturation with hydrogen being completed, D is turned, as shown in Fig. 2 (Plate 3), and the cock B, which must be quite free from vaseline, is turned around so that the first portion of the liquid from the syringe passes through A and down into the rubber tube C. The electrical connection between the liquid in the electrode vessel and the solution of potassium chlorid (Fig. 1, Plate 3) takes place along this route. When cock B is then turned as shown in the figure (Fig. 2, Plate 3), and cock H (which is carefully lubricated) is opened, the liquid rises in the electrode vessel as high as the side-tube; H is now closed, B turned around, and the syringe disconnected. The shaking of the apparatus and the reading of the electrometer may now be started.

When we are dealing with blood or other fluids containing dis-

sociable oxygen compounds, electromotive constancy is not attained until the moment when the layer of liquid into which the electrode projects is completely reduced. In such cases it may be useful, by saving time, to apply the suggestion of Michaëlis,[12] namely, to let the electrode just touch the surface of the liquid.

I am of the opinion that by following the lines indicated above, we shall be able to measure the hydrogen-ion concentration of biological fluids in many cases where it has hitherto been considered impossible, or where we have had to be satisfied with rough approximations. There are undoubtedly numerous questions in biology and pathology which these improvements in method may help to solve.

[12] Michaëlis: *Loc. cit.*

A METHOD FOR THE DETERMINATION OF TRYPTOPHAN DERIVED FROM PROTEIN

JESSE A. SANDERS AND CLARENCE E. MAY

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Introduction. Tryptophan is a protein cleavage product that is never obtained abundantly. So far as we know the tryptophan yield has been determined quantitatively in the case of but two proteins, namely casein[1] and wheat gliadin.[2] Only traces of tryptophan can be obtained from other proteins; and some proteins, especially gelatin, fail to yield it, if the indications of the usual test with glyoxylic acid and sulfuric acid are reliable.

Although tryptophan cannot be abundantly obtained from proteins, considerable importance is attached to it because it is produced in the tryptic digestion of protein and, in putrefaction, yields indol. The quantity of indican in urine indicates, in a general way, the extent of intestinal putrefaction. One usually accepts that conclusion without considering the details of the tryptophan transformation, which involves the necessary presence of tryptophan precursors in the original protein molecules; the degree of digestion of the particular proteins that yield tryptophan; the conversion of tryptophan into indol rather than skatol; followed by the absorption of indol, its oxidation to indoxyl, its esterification and its excretion in the urine in the form of the potassium ethereal sulfate.

Owing to the evanescent nature of tryptophan, its isolation from tryptic digestion mixtures has been the subject of many investigations. Although Hopkins and Cole, Abderhalden, and others, have

[1] Abderhalden: *Zeit. f. physiol Chem.*, 1905, xliv, p. 23; Abderhalden and Samuely: *Ibid.*, p. 276. (100 gm. of gliadin yield about 1.0 gm. of tryptophan; 100 gm. of casein yield 1.5 gm. of tryptophan.)

[2] Osborne and Clapp: *Amer. Jour. Physiol.*, 1906, xvii, p. 231; Osborne and Guest: *Jour. of Biol. Chem.*, 1911, ix, p. 426. (Hydrolysis of gliadin; revised gliadin-tryptophan figures.)

used the mercury sulfate-sulfuric acid method[3] in work on casein, this treatment has always been reported as giving figures somewhat lower than actual values. Tryptophan diminishes in quantity after a time, and may disappear, during the progress of tryptic digestion. In this laboratory we have found that the mercury sulfate-sulfuric acid method of Hopkins and Cole does not completely precipitate the tryptophan present in the digestion mixture. After precipitating the tryptophan-mercury-sulfate product from a casein digestion mixture, filtering, neutralizing with calcium hydroxide and removing the calcium sulfate and insoluble calcium salts, we obtained a filtrate that gave a characteristic tryptophan test with glyoxylic and sulfuric acids. Obviously the Hopkins-Cole method did not completely precipitate tryptophan. Because of the smallness of the amounts of tryptophan usually derived from proteins, this method is necessarily dependent on the use of relatively large quantities of protein. The tedious nature of the methods for the purification of large amounts of proteins led us to endeavor to devise an accurate process involving the use of small amounts of protein.

The solution of the problem seemed to depend on perfecting a method for the quantitative determination of small amounts of indol. We desired to use β-napthoquinone mono-sodium sulfonate, such as Herter[4] employed in his work on indol, but could not find it on the market. We prepared a substance that reacted with indol, giving a deep violet colored solution such as Herter obtained, but the substance formed by the combination of indol with our supposed β-napthoquinone mono-sodium sulfonate was not soluble in chloroform. Herter used chloroform to extract the indol-containing compound. Further use of our reagent was abandoned. It is probable that we had an isomer of Herter's reagent differing mainly from his in its reaction with chloroform. We prepared our reagent by cautiously oxidizing "Eikonogen," the photographic developer, by means of concentrated nitric acid. The oxidation was quite satisfactory but the substance obtained was evidently an isomer, bearing the sulfonic acid radical on a benzene nucleus other than the one holding the quinone linkages.

[3] Hopkins and Cole: *Jour. of Physiol.*, 1901–2, xxvii, p. 418; *Ibid.*, 1903, xxix, p. 451. (Mercury sulfate-sulfuric acid method; isolation of tryptophan.)

[4] Herter and Foster: *Jour. of Biol. Chem.*, 1905–6, i, p. 257; *Ibid.*, 1906–7, ii, p. 267. (β-napthoquinone reaction with indol.)

General method. We studied the production of indol in the tryptic digestion of casein, the tryptophan yield from which is known approximately. We used small amounts (1.0–1.75 gm.) of casein, digesting them with strong pancreatin solutions free from tryptophan, as determined by negative response to the glyoxylic-sulfuric acid test. The digestive periods differed in length. At the end of each, the mixture was neutralized, reinforced with neutral salts, and then made alkaline to one of several degrees of alkalinity. After sterilization in an autoclave, the mixtures were inoculated with mixed fecal bacteria from the stools of an individual on a mixed diet. No bacteria were isolated for purposes of identification. The organisms were allowed to develop in the digestion mixtures at 37° C. for periods of different length. The reaction mixtures, after neutralizing and making them alkaline with a known amount of alkali, were distilled with steam until about 700 c.c. of distillate had been obtained. The distillate was diluted to 1,000 c.c. and an aliquot portion was treated with 0.2 per cent. sodium nitrite and conc. sulfuric acid solutions. A control solution containing 0.25 per cent. of indol (Kahlbaum) was treated in the same manner. Each nitroso-indol solution was then allowed to stand until the maximum color developed.[5] We used the Wolf colorimeter for the tinctorial comparisons, and found that even with the small amounts of indol obtained (see figures later) an error of 3 per cent. was very easily detected by difference in the intensity of the resulting colorations.

We experienced some difficulty, at first, in mixing definite amounts of the indol solution with the nitrite and sulfuric acid solutions, and water, which would give uniform shade and intensity of color. Later we obtained very constant results by taking an aliquot portion of the indol solution, adding the nitrite solution and enough water to fill the cylinder of the apparatus almost to the 100 mark, then adding the conc. sulfuric acid solution and sufficient water to fill to the mark. On mixing uniformly, a very faint though distinct

[5] Moraczewski: *Zeit. f. physiol. Chem.*, 1908, lv, pp. 42–47; *Chem. Abstr.*, 1908, ii, p. 2578. (Colorimetric determination of indol in feces. The abstract of the original article contains an error that should be corrected: the sodium nitrite solution has a concentration of *0.2 per cent.* instead of 2.0 per cent. See also, Levene and Rouiller: *Jour. of Biol. Chem.*, 1906–7, ii, p. 481. A bromine-tryptophan colorimetric method for the determination of tryptophan.)

color developed which reached its maximum intensity in about half an hour.

Details of the experiments. About 500 c.c. of skimmed milk were diluted in a precipitation jar with five volumes of water. The casein was precipitated by the addition of 12.5 c.c. of 10 per cent. acetic acid solution. The casein was repeatedly washed with water by decantation and then dissolved in standard sodium hydroxid solution (enough to dissolve the casein without leaving a large excess of alkali). The liquid required about 150 c.c. of *n*/2 sodium hydroxid solution to produce a permanent alkalinity, using azolitmin paper as indicator. After dilution to a definite volume and filtration, two nitrogen determinations were made by the Kjeldahl method. It was found that each 100 c.c. of the solution contained 0.8755 gm. of casein. Of the remaining solution 500 c.c. were neutralized with phenolthalein as the indicator and treated with 0.4 gm. of sodium carbonate for each 100 c.c. volume of the neutral liquid. Then 25 c.c. of a saturated pancreatin (commercial) solution and xylene, as a preservative, were added. Incubation was continued at 37° C. for 24 hours, when an equal portion of the pancreatin solution was added; a third portion was added at the end of 48 hours. The incubation was then continued for forty-four days. Steam was passed through the alkaline solution to remove the xylene. The digestion was apparently complete; common tests for tryptophan indicated its presence. The mixture was neutralized and reinforced with neutral salts, such as Hopkins and Cole used in their work—5 gm. Rochelle salt, 0.2 gm. ammonium phosphate and 0.1 gm. magnesium sulfate, per liter. No gelatin was added. The total volume was now made up to one liter and divided into four equal portions. Each portion contained cleavage products corresponding to 1.0944 gm. of casein.

Portion *A* was sterilized in an autoclave and inoculated with a 24 hour slant agar growth of intestinal bacteria.[6] Portion *B* was

[6] The method of inoculation was as follows: The bacteria were grown first in ordinary broth inoculated from feces. After 24 hours, agar slants were made in the usual way. When these were 24 hours old, the organisms were detached by means of sterile water and a sterile wire, and the liquid containing them was poured directly into the flask to be inoculated. In order to establish a definite degree of alkalinity, the digestion mixtures, after steam distillation, were

treated with sodium carbonate (0.4 gm. per 100 c.c.), sterilized and inoculated with some of the same 24 hour growth of bacteria. Portion *C* was made alkaline with sodium carbonate to 0.8 per cent.; portion *D* to 1.0 per cent. Both were inoculated as in *A* and *B*. After four days of incubation, the four flasks were reinoculated with fresh 24 hour cultures of the bacteria and again incubated. After eight days' incubation, *flask A* was removed from the oven. Active indol-producing bacteria were present in the mixture. The original putrefaction mixture, neutral to litmus and giving a faint odor of indol, was made alkaline with sodium carbonate (to 0.4 per cent.). With steam distillation, all the indol passed into the first 300 c.c. of distillate.

Determination of indol. A standard indol solution was made by dissolving 0.25 gm. of the pure substance in a liter of water. Twenty-five c.c. of the original distillate from *flask A,* diluted to about 90 c.c., were treated with 10 drops of a 0.2 per cent. sodium nitrite solution, and six drops of conc. sulfuric acid solution, diluted to 100 c.c., and mixed uniformly. The liquid was allowed to stand until the maximum rose-red color of the nitroso-indol developed, when it showed the same intensity of color as that produced by 1.3 c.c. of the standard indol solution diluted in the same manner to 100 c.c. The total distillate contained 3.9 mg. of indol. In *flask B,* incubation was continued for nine days after the second inoculation. At the end of that time, indol-producing bacteria were still active. The reaction-mixture being distinctly alkaline, no alkali was added prior to steam distillation. All the indol appeared in the first 650 c.c. of distillate, which was diluted to 700 c.c. and thoroughly mixed. Of this solution 50 c.c. contained as much indol as 1.2 c.c. of the standard solution. The total indol content of *flask B* was 4.2 mg. *Flask C* was incubated twenty-six days. The mixture smelled strongly of indol and was alkaline in reaction. No additional alkali was added. A distillate of 1000 c.c. was obtained, each 50 c.c. of which contained as much indol as 2.05 c.c. of the standard indol solution. The total content of indol in this putrefaction mixture was 10.25 mg. *Flask D* was incubated twenty-five

titrated with *n*/10 hydrochloric acid solution and then neutralized quantitatively. The required weight of sodium carbonate was then added to the neutral solution to give the desired alkalinity.

days. It contained indol and was alkaline in reaction. Of 1 liter of distillate obtained by steam distillation, each 50 c.c. contained the quantity of indol present in 2.0 c.c. of the standard solution, indicating that the indol in the putrefactive mixture amounted to 10.0 mg. A summary of the analytic data is appended.

Flask	Casein, gm.	Digestion, days	Bacterial action, days	Indol, mg.
A	1.0944	44	8	3.9
B	1.0944	44	13	4.2
C	1.0944	44	26	10.25
D	1.0944	44	25	10.00

The yield of indol could be derived from 1.7872 gm. of tryptophan in the case of sample *C* and from 1.7436 gm. of tryptophan in the case of sample *D*. The weight of casein corresponding to the amount of indol found must have yielded the calculated weight of tryptophan. This being the case, 100 gm. of casein yield either 1.593 gm. of tryptophan (*C*) or 1.633 gm. of tryptophan (*D*), as the minimum amounts.

Hopkins and Cole claim that intestinal bacteria form small amounts of indol-acetic acid and other indol-containing substances, but we have not found these in our putrefactive mixtures, although they may have been formed in very small amounts, for which reason we give the two results as indicating the *minimum* amounts of indol-yielding radicals in casein. Our results are as high as those obtained by other investigators. It is likely, of course, that the method will be improved by the further study we hope to give it, especially in its application to other common proteins. The present paper presents only preliminary results.

The method, as outlined, is slow but it promises to be a satisfactory process for the determination of one of the cleavage products of protein material that hitherto has been difficult to determine quantitatively.

PHYSICAL CHEMISTRY OF MUSCLE PLASMA[1]

FILIPPO BOTTAZZI

(*Physiological Institute, University of Naples, Italy*)

My experiments have been made on striated muscles of oxen, dogs, *Scyllium stellare* and *Dentex vulgaris,* and on plain muscles (*M. retractor penis*) of oxen. In the case of the dogs, the muscles were removed after flushing the blood vessels with 0.9 per cent. solution of sodium chlorid (sometimes cooled to 4–5° C.). In nearly all cases the muscles were preserved in dry vessels at low temperatures. They were freed from fatty and connective tissues, then minced, thoroughly pounded with quartz sand and infusorial earth, and plasma obtained in a Buchner press, generally at a maximum pressure of about 350 atmospheres. In some experiments the irritability of the animal (*Scyllium*) was abolished by gradually cooling it to about —2° C., so that on cutting off the body musculature no contraction ensued. The muscle plasma (about 600–800 c.c.) was collected in dry vessels, centrifuged for an hour and preserved in a refrigerator.

The plasma of striated mammalian muscle was always deep red in color and rather turbid; that of fish muscle was less colored. The plasma of plain muscle was always opalescent and almost colorless. The microscopic examination, made with powerful apochromatic objectives, revealed no trace of morphologic elements or granules in the centrifuged plasma, which always appeared to be perfectly homogeneous. But ultramicroscopic examination revealed the presence of innumerable very small and highly brilliant *granules,* mixed with a relatively small number of coarse *particles,* which have nothing to do with the granules, being composed of fat, glycogen and nuclear or sarcoplasmic fragments. The existence of the ultra-

[1] Presented at the eighty-first meeting of the British Association for the Advancement of Science, in Dundee, September, 1912. In these researches I was aided by my assistant Dr. G. Quagliariello.

microscopic granules was never observed before; this is the most important result of my investigations.

The number or concentration of the granules in the original plasma is so great that the ultramicroscopic field appears almost uniformly luminous—the individual granules cannot be distinctly seen. But when the plasma is diluted with Ringer solution, the granules are separated, and then appear as distinct brilliant corpuscles endowed with lively Brownian movements on a darkish homogeneous background. They are not precipitation-particles of a dissolved muscle protein, because they do not disappear under the action of dilute alkali. Precipitation of such protein might be caused by lactic acid produced in the muscles, but in that case the particles would be dissolved by alkali—we do not know of any acid-precipitated proteins that are not resoluble in alkalies. No ordinary reagent causes the granules to disappear at a low temperature. Moreover, acid *increases* the number of particles, by precipitating a special dissolved muscle protein.

The granules are present in almost equal number in plasmas expressed from muscles which have been cooled to a low degree and which, therefore, are non-irritant, *i. e.*, from muscles in which acid production is greatly diminished. The concentration of the granules is greater in the plasma of striated muscle than in that of plain muscle.

Normal muscle plasma is, then, a suspension of ultramicroscopic granules in a liquid which, besides containing mineral salts and extractives, certainly holds protein in a state of true solution. Accordingly, the plasma, freed from the granules, is an optically homogeneous fluid, but on adding to it a weak acid solution, or on heating it at 55° C., additional particles appear—true *precipitation-particles* of a dissolved muscle protein, which may be termed *myo-protein,* while we may give the name *myosin* to the protein of which the plasma granules are made. The existence of other muscle proteins in the muscle plasma has not been proved.

The observed plasma granules are apparently a degradation or cleavage product of material in the myofibrils, plain or striated, and preexist in all muscle plasma. Their preexistence is not only easily conceivable, but we are also obliged to assume it, when we reflect

that both materials—fibrillar and sarcoplasmic—exist in muscle fibres in two distinct phases, which of course must also remain distinct in the plasma. The fibrillar phase being represented in the plasma in the form of granules, these are probably constituent elements of the fibrils, in harmony with the views of Heidenhain.

The granular material tends to flocculate spontaneously; but spontaneous agglutination and sedimentation of the granules occurs very slowly, because of their smallness and the high viscosity of the suspension fluid. Dilution with water, or with neutral, faintly acid or alkaline solutions, dialysis, or heating to about 30° C., accelerates the process; but it also occurs, in from about 12 to 24 hours, as I have said, when all accelerating action of physical or chemical agents is excluded. This aggregation, followed by precipitation, of the granular material is essentially the so-called *"spontaneous coagulation"* of muscle plasma or extract; it is therefore neither an enzymic-coagulation nor a heat-coagulation of dissolved protein. I have never observed phenomena like those described by Kühne—of nearly instantaneous clotting of cold muscle plasma when raised to room temperature.

Precipitation of the granules is greatly accelerated by heating muscle plasma to between 38° and 54° C., when, after a few minutes, a heavy precipitate is produced, from which there separates a clear yellowish-red fluid, *muscle serum*. This phenomenon, which many authors interpret as one of *heat coagulation* of a dissolved protein, is, on the contrary, the effect of rapid aggregation and precipitation of the suspended granules. When their concentration is very great, massive clotting of the plasma occurs.

The precipitate which appears during the first 24–48 hours of dialysis of plasma is composed of the granular material, and is not formed from a dissolved protein. Sometimes the plasma transforms itself into something like a blood coagulum.

Heat-coagulation of dissolved myoprotein is a continuous process, which does not appear to be complete even at 80° C. As we cannot deny that it begins at a temperature as low as 50° C., we are bound to admit that the precipitate of granules, formed at 54°–55° C., probably also contains a little myoprotein. In opposition to von Fürth and others, I have observed that the (dissolved)

myoprotein is totally precipitated by strong and prolonged dialysis. As this precipitation process is also a continuous one, I do not deny that it begins during the first 24–48 hours; therefore the precipitate of granules which forms early during dialysis may also contain a little myoprotein.

A trace of dissolved protein can always be found in plasma which has been dialyzed continuously for several months. It is probably serumalbumin, which cannot be wholly eliminated.

Muscle pigments (hemoglobin, MacMunn's myohematin) are partly removed from the plasma by the agglutinated granules—adsorbed by them, and are also precipitated in some degree with myoprotein by prolonged dialysis.

The granules and myoprotein, freed from electrolytes by sufficiently long dialysis, move toward the anode, when put under the influence of a strong electric current; they carry electronegative charges.

For muscle plasma I have determined the quantity of plasma as per cent. of fresh muscle, the total solid and total protein contents and ash yield, the specific gravity, lowering of the freezing point, electric conductivity, viscosity, surface tension and chemical reaction. Tables 1–3 contain the results of these determinations.

TABLE I

General data pertaining to muscle and muscle plasma

Muscle	Plasma No.	Muscle		Plasma	
		Weight, kg.	Pressure, atm.	Weight, gm.	Per cent. of muscle
Scyllium: Striated	7	1.067	350	666	62
Ox: Striated	8	0.938	350	592	63
Dentex: Striated	9	0.894	50	217	24
Ox: Plain	5	1.184	350	580	50
Dog: Striated	11	0.829	350	339	40
Ox: Plain	12	1.190	50–350	562	47

The plasma varied in quantity from 40 to 63 per cent. (volume), for pressures which never exceeded 350 atmospheres. The dry residue from plain muscle plasma was less than that from striated muscle plasma. The total protein content was relatively low—less than that of blood serum. Muscle plasmas are very rich in their

TABLE 2

Percentage data for water, total solid and total protein contents, and ash yield, of muscle plasma

A. Plain muscle

Muscle plasma	Water	Total solids	Total protein	Ash	Organic solids minus protein
3: Ox	93.14	6.86	3.63	1.32	1.91
4: Ox	97.57	6.43	3.37	1.30	1.76
5: Ox	93.808	6.192	3.15	1.148	1.894
6: Ox	94.126	5.874	2.75	1.37	1.754
12: Mixed—	93.27	6.73	3.37	1.30	2.06
Fraction a	92.76	7.24	4.13	——	——
Fraction b	93.60	6.40	2.90	——	——
Fraction c	93.99	6.01	2.67	——	——
B. Striated muscle					
2: Ox	91.086	8.914	4.53	1.739	2.645
8: Ox	92.57	7.43	3.65	0.85	2.93
9: *Dentex*	83.90	16.10	4.10	1.82	3.18
7: *Scyllium*	90.36	9.64	3.04	1.80	4.80
10: *Scyllium*	——	——	3.36	——	——
10a: *Scyllium*	——	——	2.38	——	——
11: Dog	87.37	12.63	3.85	1.50	7.28

TABLE 3

Data pertaining to physico-chemical properties of muscle plasma

A. Plain muscle

Plasma No.	Muscle	Specific gravity	Lowering of the freezing point (Δ)	Electric conductivity ($K_{18°}$)	Viscosity (ρ 25°)	Surface tension at 25° $\left(100\frac{Z_W}{Z}\right)$	Chemical reaction ($C_H \cdot 10^7$)	Remarks regarding the plasma
3	Ox	1.026	—	—	—	—	—	Normal, fresh
4	Ox	1.024	0.806°	—	—	—	—	Normal, fresh
5	Ox	1.023	0.761°	—	4.13	73.15	7.44	Normal, fresh
6	Ox	1.021	0.730°	0.0144	3.04	76.76	6.18	Normal, fresh
12	Mixed	1.024	0.804°	—	4.48	73.34	7.30	
	Fraction a	1.026	0.812°	—	5.98	69.34	—	Expressed at 50 atm.
	Fraction b	1.025	0.812°	—	3.68	74.67	—	Expressed at 200 atm.
	Fraction c	1.023	0.810°	—	2.96	76.27	—	Expressed at 350 atm.
B. Striated muscle								
7	*Scyllium*	1.027	2.455°	0.0147	1.59	—	11.1	Muscles were not cooled
8	Ox	1.027	0.868°	0.0107	1.75	78.19	31.4	Normal conditions
9	*Dentex*	1.049	1.196°	0.0120	2.82	76.22	12.5	From muscles of cooled animal
10	*Scyllium*	1.024	2.337°	0.0149	3.71	72.9	5.65	From three *Scyllia* (cooled to from −2° to −3° C.)
	Scyllium: Frac. 10a	1.024	2.494°	0.0150	1.71	73.1	6.50	
11	Dog	1.039	1.088°	—	2.24	68.90	10.6	Three hours after expression
	Dog (1)	1.037	1.016°	—	—	70.00	35.0	Data obtained the following day

yield of ash and content of organic non-protein substances. Generally, the dry residue and the protein contents are inversely proportional to the pressure at which the plasma is expressed. Since the specific gravity (1,021–1,027, as a rule) is quite near that of blood serum, I believe the salts and extractives, lipoids, glycogen, etc., help to account for it.

The osmotic pressure is always very high ($\Delta = 0.730$–$1.088°$ C. for mammals; $\Delta = 2.337$–$2.494°$ C. for *Scyllium;* $\Delta = 1.196°$ C. for *Dentex*), higher than that of the blood.

The reaction is always acid ($C_H \cdot 10^7 = 5.65$–12.5; but there are also higher values: $C_H \cdot 10^7 = 31.4$ f. e.). Acidity is lower in plasma of plain muscle and of cooled striated muscle, higher in the plasma of striated mammalian muscle. As a rule, the hydrogen-ion concentration increases with time; but as this augmentation is rather feeble, I believe, with Fletcher, that the maximum production of acid substances occurs in muscles soon after their separation from the body.

The high osmotic pressure of muscle plasma is probably due mainly to the substances that determine the acid reaction.

The low electric conductivity ($K_{18°} = 0.0107$–0.0144 for mammals; 0.0147–0.0150 for *Scyllium;* 0.0120 for *Dentex*) and high viscosity ($\rho_{25°} = 1.59$–2.82; 3.04–5.98 for plain mammalian muscle) of the plasmas are explained by their corpuscular composition. But the very high viscosity of the plasma of plain muscle is probably caused by some particular protein derived from the connective tissue.

The surface tension ($100 \frac{Z_W}{Z} = 68.90$–$78.19$) is generally higher than that of the blood serum.

My results are, for the most part, in opposition to those obtained by previous authors. But the new interpretation of phenomena like those of spontaneous coagulation and heat coagulation, etc., was suggested to me mainly by the granular constitution of muscle plasma. As I stated above, this is the most important result of my investigations, a result which hereafter must be recognized by all who study problems pertaining to the chemistry and physical chemistry of the contents of the muscle fiber.

I have endeavored to corroborate my hypothesis, that the granules are disintegrated fibrillar material, by trying to stain the granules with some pigment which would selectively color the muscle fibrils; and also by attempting to show that the granules possessed double refractive power. My attempts have been unsuccessful, however, although this was not unexpected, because of the ultra-microscopic dimensions of the granules.

FASTING STUDIES

11. A note on the composition of muscle from fasting dogs[1]

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The variations in the composition of the different forms of muscle in the normal individual have received considerable attention. A comparison of the nitrogen and moisture contents of heart and striated muscle reveals a lower percentage of nitrogen and a higher percentage of moisture in the heart muscle than in the skeletal muscle. The proportions of these and other constituents in the fasting muscle have not been studied extensively. The changes which occur in the composition of muscle during fasting are significant for the solution of general problems relating to the effects of fasting.

In a study of the influence of fasting upon the creatine content of dog muscle, determinations were made of the proportions of nitrogen, moisture, fat and creatine in normal and fasting muscle; and also of the nitrogen and creatine in heart muscle. In these preliminary experiments particular attention was paid to the percentages of nitrogen and creatine: an attempt was made to show the relation between the nitrogen and creatine contents of muscle, so that the ratio of creatine-nitrogen to total nitrogen might be used as an index of the changes due to pathological conditions. This factor should be more significant than the percentage of creatine in fresh muscle, and as accurate as that for the creatine content in muscle on a fat- and moisture-free basis. These results, as well as the variations in the creatine content of muscle, will be discussed in a later paper.[2]

[1] Presented before the Columbia University Biochemical Association, December 6, 1912; BIOCHEMICAL BULLETIN, 1913, ii, p. 288.

[2] Howe and Hawk: Presented before the recent annual meeting of the American Physiological Society, but not abstracted in the proceedings.

The procedure employed in determining some variations due to fasting was as follows: muscles (*M. semitendinosus* and *M. biceps femoris*) of *normal* animals were analyzed for moisture, fat, phosphorus and creatine; and, in one case, the heart was analyzed for nitrogen and creatine.

Muscles of the same kind were removed aseptically from normal dogs under ether anesthesia[3] and analyzed for nitrogen and creatine. After a prolonged fast the corresponding muscles of the opposite legs of the "operated" dogs, as well as the remaining muscles on the same side, were analyzed for total nitrogen and creatine, and in two cases for moisture and fat. With such a procedure the changes which resulted from fasting were studied on the *same* individual, also in fasted animals as compared with *different* normal controls. The "operated" dogs recovered readily and the wounds healed rapidly even when the fast was begun immediately after the operation.

The analytical methods employed were as follows: Total nitrogen was determined by the Kjeldahl process; moisture by drying in a vacuum over sulfuric acid at room temperature; fat by the ether-extraction process, and creatine by the Folin procedure as modified for meat by Emmett and Grindley.[4] The creatine was extracted according to the methods of Grindley and Woods[5] and of Mellanby.[6]

The table on page 388 contains the more significant data.

A consideration of this data shows an increase in the percentage of moisture, and a decrease in the percentages of nitrogen and creatine, in the striated muscle as a result of fasting. From the data on a single normal heart and a single fasting heart there appears to have been a decrease in the nitrogen and an increase in the creatine content of the fasting heart. We also note that the changes which take place in the same fasting individual, as contrasted with the fasting changes when compared with different control animals, are approximately the same.

[3] We wish to thank Dr. O. O. Stanley, of the University of Illinois, and Dr. C. T. Moss, of the Michael Reese Hospital, of Chicago, Illinois, for their aid in the removal of the muscles.

[4] Emmett and Grindley: *Jour. of Biol. Chem.*, 1907, iii, p. 491.

[5] Grindley and Woods: *Ibid.*, 1906–'07, ii, p. 309.

[6] Mellanby: *Jour. of Physiol.*, 1908, xxxvi, p. 453.

Data pertaining to the composition of muscle from fasting dogs

Dog	Kind of muscle	Moisture, per cent.	Nitrogen, per cent.	Fat, per cent.	Creatine, per cent.
E.	Normal leg	73.4	3.42	2.4[7]	0.31
D.	Normal leg	73.4	3.51	2.2[8]	0.33
	Normal heart	——	2.95	——	0.23
B.	Normal leg	——	3.34	——	0.34
	Fasting leg	81.2	2.82	0.56	0.31
	Fasting heart[9]	——	2.65	——	0.30
C.	Fasting leg[10]	81.8	2.95	0.49	0.19
A.	Normal leg	——	3.99	——	0.42
	Fasting leg[11]	——	3.61	——	0.39

The increase in the moisture content of fasting muscle may be associated, in part, with the decrease in the fat content; in normal animals there is a decrease in the percentage of moisture associated with an increase in the fat content of muscle.[12] The increase in the moisture content of fasting muscle may also be due to changes in the colloidal state or the molecular condition of the cellular constituents. This increase in moisture is more significant when we consider that there is apparently a greater decrease in the cytoplasm than in the nuclei of the cells as a result of a fast;[13] the nucleus and the connective tissue, the substances which would then predominate, normally contain less water than the cytoplasm. The increase in the moisture content of fasting muscle has been noted by other investigators.

The *lower* absolute nitrogen content of fasting muscle, when considered on the basis of fresh muscle, becomes an *increased relative* nitrogen content, when the values for nitrogen are calculated

[7] Content of phosphorus = 0.16 per cent.

[8] Content of phosphorus = 0.21 per cent.

[9] A sixty-four day fast which resulted in a loss of 62 per cent. of the original weight. The animal received 320 c.c. of water daily.

[10] A twenty-one day fast.

[11] A fifteen day fast, which resulted in a loss of 38 per cent. of the original weight. The animal did not receive water.

[12] This fact, together with certain other deductions, has been corroborated by data in a personal communication from Professor P. F. Trowbridge of the University of Missouri.

[13] Morgulis: *Archiv für Entwicklungsmechanik der Organismen*, 1911, xxxii, p. 169.

on a moisture- and fat-free basis. An alteration in the direction of increased nitrogen content is more in harmony with the changes that actually take place in the muscle. The relatively greater decrease in the volume of the cytoplasm than of the nucleus, and the apparent relative increase in the connective tissue, are modifications in the direction of a higher nitrogen content of the muscle. The proteins which predominate in the connective tissue and in the nuclei of muscle contain higher percentages of nitrogen than do the proteins which make up the major portion of the cytoplasm of the cells.

In addition to changes in the chemical nature of fasting muscle, certain physical modifications arise: normal muscle is firm to the touch and, when hashed, may be readily handled without sticking to the fingers; fasting muscle, on the other hand, is soft to the touch and, when hashed, adheres tenaciously to the fingers.

While the lowered percentage of nitrogen in the fresh skeletal muscle, as a result of fasting, may be due to diminutions in the contents of moisture and fat, the difference in the composition of heart and skeletal muscle cannot be explained in this way. If the nitrogen content of normal striated and heart muscle be calculated to the moisture- and fat-free basis, there still remains a greater percentage of nitrogen in the striated muscle. That there are differences in the relative proportions of the soluble protein and the stroma in heart, and in striated and smooth muscle, has been shown by Saxl,[14] who finds that seven-eighths of the skeletal muscle consists of soluble proteins while but one-third of the heart muscle is of this nature.

A careful differential study of the proteins of fasting muscle should throw some light upon the nature of the disintegrative processes which take place in the tissues as a result of fasting. Such a study is contemplated.

[14] Saxl: *Beiträge z. chem. Physiol. u. Pathol.*, 1907, ix, p. 1.

SOME NOTES ON THE FORM OF THE CURVE OF CARBON-DIOXIDE EXCRETION RESULTING FROM MUSCULAR WORK FOLLOWING FORCED BREATHING[1]

G. O. HIGLEY

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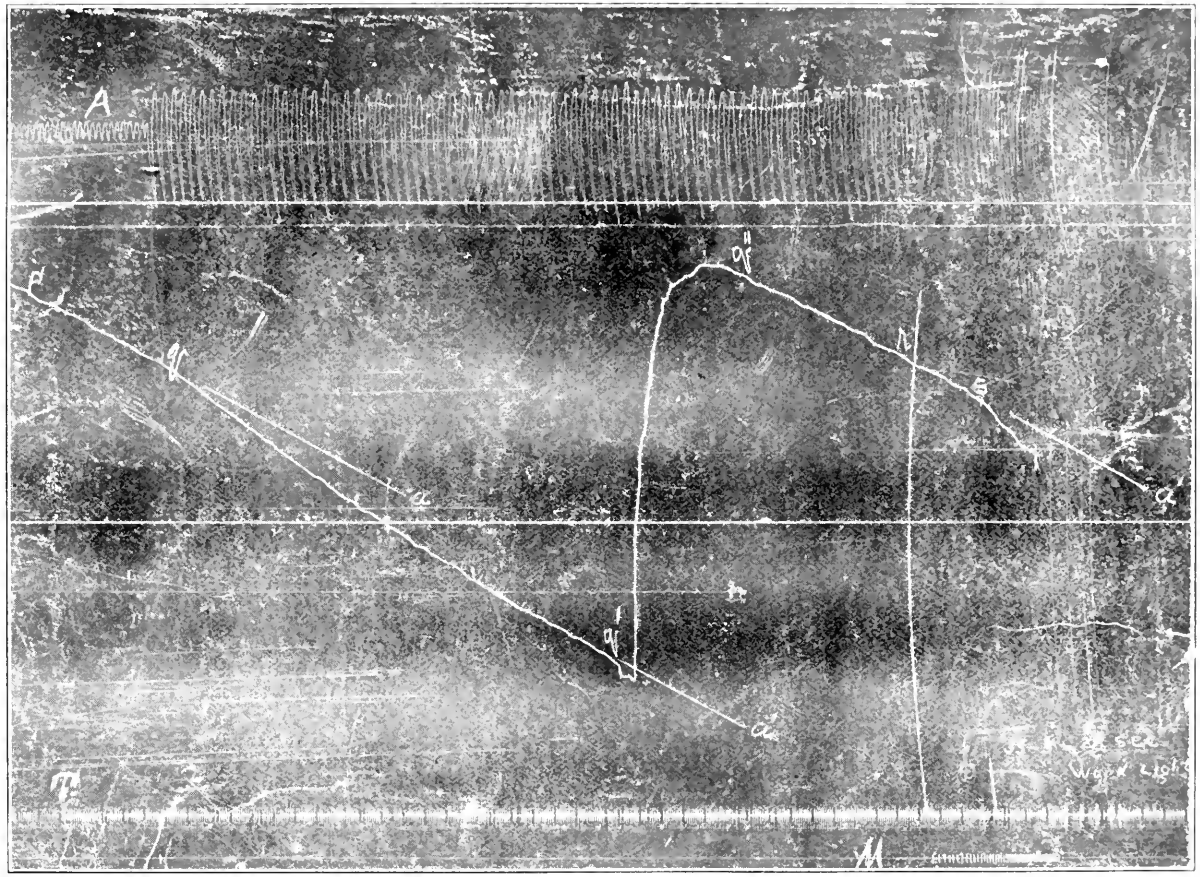
(WITH PLATE 4)

In an earlier research[2] an attempt was made to determine how soon after the beginning of work an increase in the production of carbon dioxide begins to show itself in the expired air. This time (the latent period) was at first found to vary from three to fourteen seconds. Now, clearly, such periods are not long enough to correspond with the time required for the carbon dioxide formed in the muscles at the first muscular contraction to reach the outside air. It must first diffuse into the blood from the tissues where it is formed, then traverse the venous half of the systemic circulation, the right side of the heart, and the arterial half of the pulmonary circulation, and finally diffuse into the air of the alveoli before any of it can appear in the breath. From the conclusions of Stewart and others it appeared that from fifteen to twenty seconds is the least possible time required for the blood to traverse this distance, to say nothing of the diffusion time. It was finally found that the sudden increase in the rate of excretion of carbon dioxide, after the beginning of work, was due primarily to a better ventilation of the lungs, while the continuation of the increase was due to the ventilation of the blood and tissues as well. A recognition of this fact led to the following modification of the method for the determination of the latent period of carbon dioxide excretion:

[1] This paper was accepted for publication by the officers of Section VIII, *d*, Eighth International Congress of Applied Chemistry, and was read before the Section at a stated meeting on September 11, 1912; BIOCHEMICAL BULLETIN, 1912, ii, p. 153.

[2] Higley and Bowen: *American Journal of Physiology*, 1904, xii, p. 311.



HIGLEY: SOME NOTES ON THE FORM OF THE CURVE OF CARBON-DIOXIDE EXCRETION RESULTING FROM MUSCULAR WORK FOLLOWING FORCED BREATHING.

Curve of CO_2 excretion: Pqq'q''rss'; respiration: A; time: T; work (bicycle): M. [Reproduction of author's original faded tracing.]

After the "normal" rate of excretion at rest had been determined, the subject began forced breathing at a predetermined rate, continuing this for a minute or so until the curve of carbon dioxide excretion had apparently assumed its permanent direction. At this point, at a signal from the experimenter, the subject began to drive the bicycle as in the preceding experiments. The effect was marked, the new rate of excretion being sharply defined from the normal rate preceding it. The further increase in the rate of excretion, after the beginning of work, was now not so prompt in its appearance, and came on more gradually, reaching its maximum after a minute or so, depending on the work.

In these experiments the latent period of increase due to work was from seventeen to twenty-two seconds. It is evident that as the latent period will vary with the rapidity of the circulation, the rapidity of diffusion, and the rate of work, a more definite figure was not to be expected.

Shortly after the publication of these results by Bowen and the writer, a communication was received from Prof. N. Zuntz, calling attention to the gradual character of the change in rate of excretion of carbon dioxide after the beginning of work (as already mentioned) and kindly suggesting a modification of the method of carrying out the latent-period experiments. According to Prof. Zuntz, if the forced breathing were continued for *five* minutes instead of *one* minute, as already stated, the blood and tissues would become thoroughly ventilated; the direction of the curve of carbon dioxide would become parallel to that before forced breathing began; and, furthermore, with the beginning of work, the carbon dioxide curve, after the latent period of twenty seconds, would change much more sharply than it did in the published record. The writer accordingly made a series of experiments in which the forced breathing was continued for from five to seven minutes before bicycle work has begun.

The results of one of these experiments are seen in Plate 4 in which **A** is the pneumograph record, **Pqrs** the carbon dioxide curve, **T** the chronograph record, and **M** the bicycle record. The line **Pq'q"r**, as in the previous paper,[3] represents the rate of excretion

[3] Higley and Bowen: *Loc. cit.*

before the beginning of forced breathing; the line **Pqq'q''r** (broken by the arresting of the beam and the addition of four gram weights) represents the curve of carbon dioxide during forced breathing; **r** is the position on the curve of the carbon-dioxide-writing lever at the instant when work was begun; and **s** *is the point where the curve changes as a result of the work.*

This research was conducted on two subjects. It was found very difficult to maintain respiration of uniform depth for five minutes, since there is a decided tendency to make the respiration shallower. Indeed, notwithstanding the great care on the part of the subject, the pneumograph record indicated, in some cases, a lessened depth of respiration toward the end of the forced respiration period.

In the case of one subject the curve for rate of excretion of carbon dioxide returned, during the period of forced respiration, practically to the original value. With the other subject the return was less perfect. It would seem that as a result of the additional work of the respiratory organs a return of the rate of excretion to the value during normal respiration could not be expected.

While, therefore, the writer is able to confirm Prof. Zuntz's prediction regarding the sharpness of the change, as a result of work, in the curve of carbon dioxide after continued forced respiration, he can confirm only in part Prof. Zuntz's prediction on the return of the curve, during forced respiration, to the direction which it had before forced respiration was begun.

This work was done in the physiological laboratory of the University of Michigan, Ann Arbor, Michigan.

THE INFLUENCE OF BAROMETRIC PRESSURE ON CARBON-DIOXIDE EXCRETION IN MAN[1]

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(WITH PLATE 5)

Introduction. This work was suggested by that of Lombard[2] on "Some of the influences which affect the power of muscular contraction." In that research, which was made with the ergograph, Lombard found that, in general, there was a fall of muscular power during the day, this result being noted on eighteen out of a series of twenty-three days. However, on certain days, the fall in power due to fatigue was slight and on five days the power was greater at the last experiment than at the first. These exceptions led to the suspicion that barometric changes had an influence on muscular endurance. When, later, a comparison was made between Lombard's endurance curve and the curve of barometric height, it was found that, while no constant relationship existed between the two variables, they varied in the same sense on twenty out of twenty-three days; *i. e.*, in general "when the barometer rose during the day, or fell less than on the preceding day, the muscular endurance either rose, or fell less than on the preceding day."

It has been shown, furthermore, that while a diminution of barometric pressure increases both the respiration rate and the volume of air respired, after allowance is made for the increase of volume due to the lower pressure the volume respired is less (Speck).

Now, the effect of increasing barometric pressure upon the power of the muscular system might possibly be due to some influence

[1] This paper was accepted for publication by the officers of Section VIII, *d*, Eighth International Congress of Applied Chemistry, and was read before the Section at a stated meeting on September 11, 1912; BIOCHEMICAL BULLETIN, 1912, ii, p. 153.

[2] Lombard: *Journal of Physiology*, 1892, xiii, p. 1.

exerted through the nervous and circulatory systems tending to increase the readiness of metabolism; if such were the case then a variation in barometric height should be accompanied by a variation, in the same sense, in the rate of excretion of carbon dioxide.

Plan of the experiments. It seemed that a series of experiments carried out for a month on three healthy subjects might throw light on this question, and also give interesting results as regards the effect of other conditions on the rate of carbon dioxide excretion. A series of respiration experiments was planned, accordingly, for three subjects, *A* and *B*, students in the University of Michigan, and the writer, *C*. *A* and *B* were 24 and 22 years of age respectively, and weighed, without clothing, 158 and 159½ pounds. *C* was 46 years of age and weighed, exclusive of clothing, 148 pounds. Each subject was to live his regular daily life except that no vigorous muscular exercise was to be engaged in immediately preceding any experiment and that nothing whatever was to be eaten between meals. The plan of work is indicated in the appended summary, where the data for the third part of each experiment are placed below those for the first and second:

Subject	Hour of rising	Reclined	First experiment	Breakfast	Time until next experiment	Reclined	Second experiment begun	Dinner
A	6	6:45	7:00	7:40–8:00	4 hr.	11:45	12:00	12:40
B	6	7:05	7:20	8:00–8:20	4 hr.	12:05	12:20	1:00
C	6	7:25	7:40	8:00–8:20	4⅓ hr.	12:25	12:40	1:00

Subject	Time until next experiment	Reclined	Third experiment begun	Lunch	Experimenter
A	4 hrs.	4:45	5:00	5:40	*C*
B	4 hrs.	5:00	5:20	6:00	*A*
C	4⅓ hrs.	5:25	5:40	6:00	*B*

The routine of work was as follows: The subjects rose at 6 o'clock, reaching the laboratory at about 6:35. *A* reclined upon a couch at 6:45 in preparation for the first experiment. *B* and *C* prepared all the apparatus, making the initial calibration of the balance, weighing the guard tubes, reading and recording the barometric height, the outdoor and room temperature, etc. In order to enable the experimenter to judge the better as to the physical

condition of each subject, mouth temperature and pulse were also taken and recorded. This routine at the laboratory was followed at 12 M. and 5 P. M.

TABLE I

Data showing the excretion of carbon dioxide by subjects A, B and C, in milligrams per minute.

Date	Subject *A*.			Subject *B*.			Subject *C*.		
Dec.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.
23	406	422	—	381	498	567	406	390	447
24	438	460	447	489	422	541	419	409	409
26	442	448	466	514	429	(743)	422	403	428
27	422	453	466	535	434	548	390	403	390
28	403	448	(635)	488	507	498	397	375	419
29	407	422	381	520	553	546	382	387	456
30	438	483	405	529	495	518	419	381	374
31	425	470	444	(647)	489	476	390	362	438
Jan.									
2	433	487	480	438	(611)	570	393	422	473
3	470	436	422	416	462	508	394	377	422
4	507	435	480	537	442	553	—	386	448
5	469	473	442	528	466	515	410	—	396
6	458	466	442	—	525	531	449	403	476
7	465	442	432	439	560	466	406	386	411
9	416	436	436	410	442	462	390	380	448
10	416	459	448	455	506	—	383	425	473
11	456	439	426	453	422	526	363	402	337
12	446	—	—	543	—	—	388	—	—
13	—	—	—	—	—	—	—	—	—
14	405	462	445	—	476	504	398	427	—
16	436	496	449	469	531	440	402	396	437
17	412	453	—	418	460	—	—	459	—
18	377	407	462	—	459	469	364	442	435
19	472	487	422	445	474	409	403	438	429
20	469	476	436	402	455	432	402	474	419
21	462	436	493	399	442	493	406	448	434
23	428	481	429	509	442	436	396	449	429
24	422	517	495	500	459	537	409	460	429
25	422	475	402	—	528	486	422	468	428
26	495	561	—	—	—	—	422	422	—
Averages.....	438	462	443	472	476	501	401	414	427

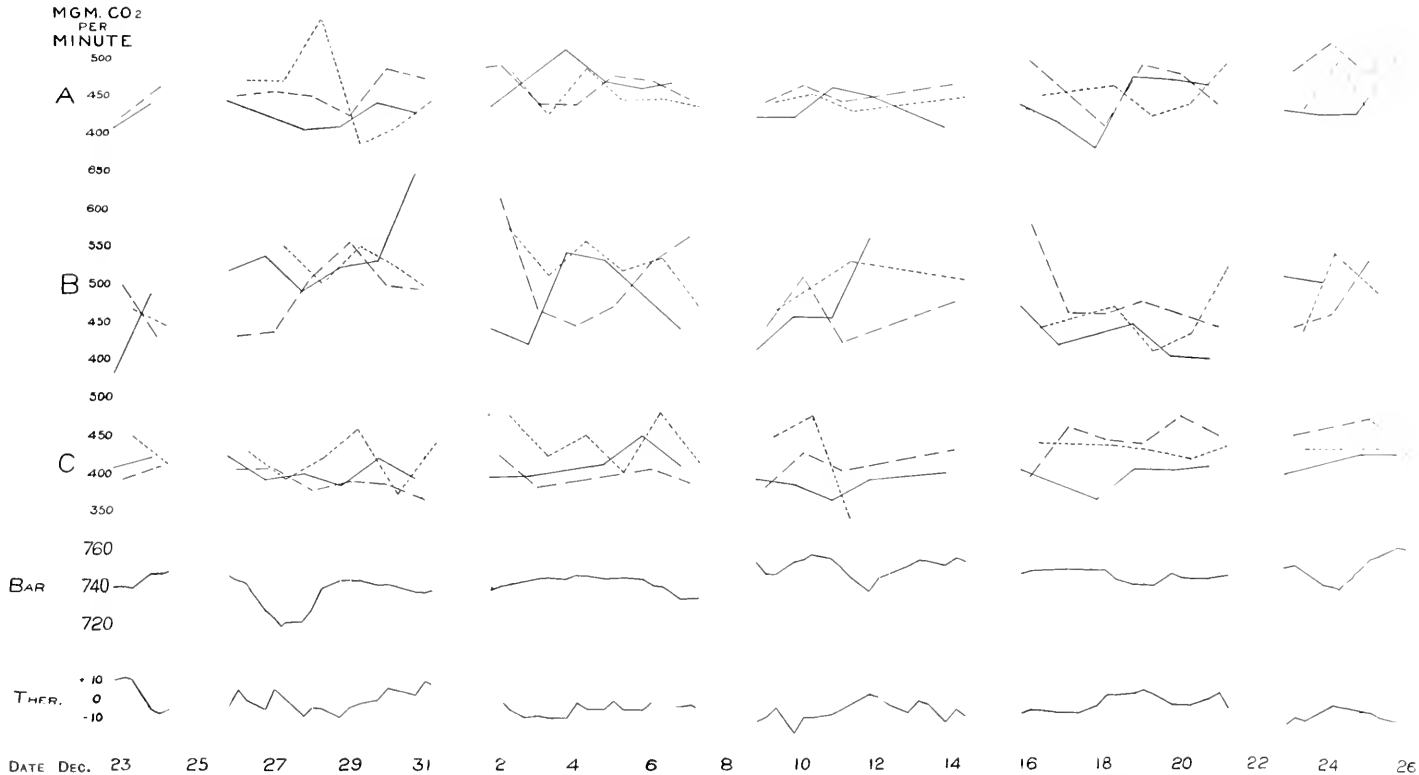
Results. The results of this series of experiments are shown in Table I, in milligrams of carbon-dioxide excretion per minute. It will be noted that *A*'s average for the midday experiments is considerbly higher than that for the morning and evening experiments.

This is due, in part at least, to the fact that this subject took his heartiest meal in the morning. The excretion of carbon dioxide for *B* and *C*, on the other hand, was greatest in the evening, since these subjects took their dinner at 1 P. M.

The remarkably high excretion shown for *A* at the evening experiment of December 28 (635 mg., while the average for that hour for this subject is only 443 mg.) is explained as follows: This subject went skating in the afternoon of that day and at about 2:30 o'clock had the misfortune to break through the ice, becoming wet to the neck. On being rescued, he walked about two miles in his frozen clothing, exposed meanwhile to a strong wind at a temperature of about —6° C. On reaching his room he took a thorough rubdown, made a change of clothing, rested for one and one half hours, and appeared at the laboratory at the usual hour for the experiments, with the result stated above. It will be noted that all of this subject's values for the following day, especially that of the evening, were much below the average, indicating a reaction from the exposure and excitement of the preceding day. The high excretion of the morning of January 4 is supposed to be due to lunch eaten late on the preceding evening; that of 12 o'clock, January 26, to an exceptionally heavy morning meal; and the low result of the evening of January 19 to an especially light midday meal.

The irregularity of results obtained from *B* are somewhat difficult to explain. Those of the morning of December 27, 30 and 31, were due to lunch eaten late the preceding evening and in the case of the two latter results, also in part to excessive haste to reach the laboratory in time for the regular experiment. Other high results, especially those of 5 P. M., December 26, and of 12 M., January 2, were undoubtedly due to indigestion.

Passing now to a study of the relation of carbon dioxide excretion to barometric changes, Plate 5 will be found to embody, in the form of curves, the results already given in Table 1, with time as abscissae, and milligrams of carbon dioxide per minute as ordinates; it presents curves for *A*, *B* and *C*, together with that for the barometer in millimeters of mercury and of the outdoor temperature in degrees centigrade. The temperature of the room was practically constant throughout the series of experiments. Three curves are



HIGLEY: THE INFLUENCE OF BAROMETRIC PRESSURE ON CARBON-DIOXIDE EXCRETION IN MAN

Curves showing CO_2 excretion by subjects A, B and C, in mg. per minute. *Morning:* solid lines; *midday:* long-dash lines; *evening:* short-dash lines. (See Table 1.) Note corresponding curves for barometric pressure and out-door temperature.

given for each subject where the necessary data were at hand. *In each case the morning, midday and evening curves are represented, respectively, by solid, long-dash and short-dash lines.*

Analysis of the results. *Comparison of the data for barometric pressure and carbon dioxide excretion.* Before proceeding to a rigorous mathematical investigation of the relationship between barometric change and carbon dioxide excretion, it seemed desirable to make a comparison of these two variables at a number of the dates on which especially marked barometric fluctuations took place, since in such cases the effect would be more pronounced and less likely to be masked by other varying conditions, such as amount and character of the preceding meal, character of muscular exercise, etc. To facilitate such a comparison Table 2 was prepared; it indicates experiment number; dates between which the comparison is made; barometric height, rise or fall; subject; carbon dioxide for the two days between which comparison is made; rise or fall of excretion; and relation between barometric change and carbon dioxide excretion, whether *direct* or *inverse*. Taking first the morning values, it was found that the barometer rose between 7 A. M., December 23, and 7 A. M., December 24, from 739 to 746, or 7 mm. During the same period the excretion of carbon dioxide of the three subjects changed as follows: That of *A* from 406 to 438 mg. per minute, an increase of 32 mg.; that of *B* from 381 to 489, an increase of 108 mg., and that of *C* from 406 to 419, an increase of 13 mg. per minute. Thus with *rising* barometer there was an *increase* in the rate of excretion of carbon dioxide in the case of each subject. A similar result is obtained in four other morning experiments (two subjects). In three morning experiments there are two direct results each. One experiment shows two *indirect* results, *i. e.*, there is a change in carbon dioxide excretion which is opposite in sign to that in the barometer.

Summing up the results of the morning experiments we have the following: Eleven experiments were carried out on *A*, seven on *B*, and eleven on *C*. The degree of correspondence of barometric change with carbon dioxide excretion was:

A, 7 cases out of 11, or 63.6 per cent.
B, 6 cases out of 7, or 85.7 per cent.
C, 6 cases out of 11, or 55.5 per cent.

TABLE 2

Data obtained at 7 a.m.

No.	Date, Dec.	Barometer			Subject	Excretion of carbon dioxide			Relation between barometric change and carbon dioxide excretion.	
		Heights, mm.	Rise, mm.	Fall, mm.		Per minute, mg.	Rise, mg.	Fall, mg.	Direct	Inverse
1	23–24	739 –746	7.	—	*A*	406–438	32	—	32	
					B	381–489	108	—	108	
					C	406–419	13	—	13	
2	26–28	745.1–721	—	24.1	*A*	442–403	—	39	39	
					B	514–488	—	26	26	
					C	422–397	—	25	25	
3	28–29	721 –742.1	21.1	—	*A*	403–407	4	—	4	
					B	488–520	32	—	32	
					C	397–382	—	15	—	15
4	29–31	742.5–736	—	6.1	*A*	407–425	18	—	—	18
					B	——	—	—	—	
					C	382–390	8	—	—	8
5	Jan. 2–4	737.1–743.5	6.4	—	*A*	433–507	74	—	74	
					B	438–537	99	—	99	
					C	393–394	1	—	1	
6	5–7	743.5–732.5	—	11.	*A*	469–465	—	4	4	
					B	528–439	—	89	89	
					C	410–406	—	4	4	
7	11–12	753.9–737.1	—	16.8	*A*	456–446	—	10	10	
					B	453–543	90	—	—	90
					C	363–388	25	—	—	25
8	12–14	737.1–751.2	14.1	—	*A*	446–405	—	41	—	41
					B	——	—	—	—	
					C	388–398	10	—	10	
9	18–19	748.2–739.3	—	8.9	*A*	377–472	95	—	—	95
					B	——	—	—	—	
					C	364–403	39	—	—	39
10	23–24	749 –739.8	—	9.2	*A*	428–422	—	6	6	
					B	509–500	—	9	9	
					C	396–409	13	—	—	13
11	24–26	739.8–758.8	19.	—	*A*	422–495	73	—	73	
					B	——	—	—	—	
					C	409–422	13	—	13	

The direct results from the midday experiments were as follows:

A, 3 cases out of 7, or 42.8 per cent.
B, 3 cases out of 9, or 33.3 per cent.
C, 6 cases out of 9, or 66.6 per cent.

From the evening experiments the direct results were:

A, 3 cases out of 6, or 50 per cent.
B, 4 cases out of 9, or 44.4 per cent.
C, 4 cases out of 8, or 50 per cent.

TABLE 3

Data showing the relation of carbon dioxide excretion to barometric change, at noon for subject A.

Barometer reading, mm.	Carbon dioxide excretion, mg.	X	Y	X^2	Y^2	Products (XY)	
						Negative	Positive
739.1	422	− 4.2	−40	17.64	1,600	——	168
746.2	460	2.9	− 2	8.41	4	5.8	——
742.5	448	− 0.8	−14	0.64	196	——	11.2
722.1	453	−21.2	− 9	449.44	81	——	190.8
726.9	448	−16.4	−14	268.96	196	——	229.6
742.5	422	− 0.8	−40	0.64	1,600	——	32
740.1	483	− 3.2	21	10.24	441	67.2	——
736.1	470	− 7.2	8	51.84	64	57.6	——
738.9	487	− 4.4	25	19.36	625	110.	——
742.9	436	− 0.4	−26	0.16	676	——	10.4
745.1	435	1.8	−27	3.24	729	48.6	——
743.5	473	0.2	11	0.04	121	——	2.2
739.9	466	− 3.4	4	11.56	16	13.6	——
732.9	442	−10.4	−20	108.16	400	——	208
745.8	436	2.5	−26	6.25	676	65.	——
753.2	459	9.9	− 3	98.01	9	29.7	——
749.3	439	6.6	−23	36.00	529	151.8	——
753.6	462	10.3	0	106.09	0	——	——
747.5	496	4.2	34	17.64	1,156	——	142.8
748.1	453	4.8	− 9	23.04	81	43.2	——
747.9	407	4.6	−55	21.16	3,025	253.0	——
740.1	487	− 3.2	25	10.24	625	80.	——
744.1	476	0.8	14	0.64	196	——	11.2
743.9	436	0.6	−26	0.36	676	15.6	——
749.8	481	6.5	19	42.25	361	——	123.5
738.8	517	− 4.5	55	20.25	3,025	247.5	——
752.9	475	9.6	13	92.16	169	——	124.8
758.4	561	15.1	99	228.01	9,801	——	1,494.9
				1,642.43	27,078	1,174.6	2,749.4
							1,174.8
							1,574.6

$$\sigma_1 = \sqrt{\frac{1,642.43}{28}} = 7.65, \qquad \sigma_2 = \sqrt{\frac{27,078}{28}} = 31.1$$

$$\Sigma(xy) = 1,574.6$$

Coefficient of correlation :

$$\frac{\Sigma(xy)}{N\sigma_1\sigma_2} = \frac{1,574.6}{28 \times 7.65 \times 31.1} = +0.236$$

$$\text{Regression} = \frac{0.236\partial_2}{\partial_1} = 0.95$$

Or, out of a total of seventy-seven experiments, there was direct relationship between barometric change and carbon dioxide excretion in forty-two experiments, or 54.5 per cent.

It will be seen from these results that the apparent degree of correspondence, so far as it is revealed by this method of analysis, is greater in the morning experiments than in those carried out at midday or in the evening. This is probably due to the fact that in the morning not merely the digestive organs, but the whole system, is in a more uniform condition than at any other time during the twenty-four hours.

Application of the method of least squares. It now seemed desirable to subject the results obtained in this series of experiments to a more rigorous analysis than that just described, with a view of discovering what is the degree of correlation between the two variables, the barometric height and the rate of excretion of carbon dioxide, during muscular rest. The data obtained in the experiments were, therefore, examined by the method of least squares, which was applied separately to the three sets of data from each subject in order that the effect of different times of day might be determined separately.

In Table 3 are given the barometric height and the corresponding carbon dioxide excretion;[3] the problem is to find the correlation between these two quantities, and also the regression of carbon dioxide on barometric height, *i. e.*, the amount of change in excretion of carbon dioxide for a millimeter change in barometric height. The means of columns 1 and 2 are obtained in the usual manner, by dividing the total in each column by the number of experiments (N). Having obtained these means, two additional columns are formed, giving the deviation of each observation from the mean of its column. In columns 5 and 6 are entered the squares of the deviations (X^2 and Y^2). The standard deviation (σ_1) is now obtained by dividing the sum of the squares in the fifth column by the number of experiments, N, and extracting the square root of the quotient; the standard deviation for y is, of course, found in the same manner.

$$\sigma_1 = \sqrt{\frac{\Sigma x^2}{N}} = \sqrt{\frac{1642.4}{28}} = 7.65 \quad \text{and} \quad \sigma_2 = \sqrt{\frac{\Sigma y^2}{N}} = \sqrt{\frac{27078}{28}} = 31.1$$

[3] The data are those obtained from experiments on subject *A* at noon.

The products XY are now collected, the negative in column 7 and the positive in column 8, and the totals determined. We have, then, $\Sigma(XY) = 2{,}749.4 - 1{,}174.8 = 1{,}574.6$.

From this the coefficient of correlation (r) is obtained:

$$r = \frac{\Sigma(xy)}{N\sigma_1\sigma_2} = \frac{1{,}574.6}{28 \times 7.65 \times 31.1} = +0.236$$

The positive sign of this coefficient indicates, of course, that the relationship between barometric change and carbon dioxide excretion in this case is *direct* or that the two variables change in the same sense. Since a coefficient of correlation of 1 indicates *perfect correlation,* the result obtained in the series of experiments represented in Table 1 indicates a slight degree of correlation. The probable error of a correlation coefficient of this value for a series of 25 observations is at least 0.13 so that the value of r is 0.16 ± 0.13.

The results of the whole series of experiments are summed up in Table 4.

TABLE 4

General Summary

Subject	Hour	x^2	y^2	N	σ_1	σ_2	$S(xy)$	Coefficient of correlation, r	Probable error	Regression
A	7 A.M.	1,769.7	24,951	29	7.8	29.3	828.	+0.12	±0.13	0.45
A	12 M.	1,642.4	27,078	28	7.65	31.1	1,574.6	+0.236	±0.126	0.95
A	5 P.M.	1,315.71	17,620	24	7.4	27.1	589.9	−0.12	±0.126	0.44
B	7 A.M.	1,324.02	59,721	24	7.4	49.8	1,787.7	−0.2	±0.129	0.13
B	12 M.	1,313.02	40,919	26	6.9	44.2	818.3	−0.04	±0.124	0.09
B	5 P.M.	1,158.7	102,631	25	6.5	64.0	1,926.1	−0.23	±0.13	0.17
C	7 A.M.	1,228.2	8,693	27	8.0	17.9	1,228.2	+0.316	±0.12	0.7
C	12 M.	1,652.0	26,484	27	7.8	31.2	2,627.6	+0.39	±0.11	1.5
C	5 P.M.	1,232.6	23,076	25	7.02	30.38	1,325.1	+0.248	±0.125	1.07

Conclusions. There were indications in this work of an influence of barometric change on carbon dioxide excretion in the case of one subject, *C,* since there were three positive coefficients of correlation having the value of 0.316, 0.39, and 0.248, for morning, noon, and evening experiments (perfect correlation would be indicated by a coefficient of 1); a slight direct influence is also indicated in the case of *A,* whose coefficients were 0.12, 0.236 and −0.12. In the case of *B,* whose values of carbon dioxide excre-

tion throughout the work were quite irregular, there were three *negative* coefficients with values —0.2, —0.04, and —0.18.

These results are, perhaps, what might have been expected. The barometric change is evidently a minor influence and its effect is therefore liable to be masked by other influences, such as exercise, amount and character of meals, etc. Moreover, the effect upon the muscular endurance noted by Prof. Lombard in his own case has not been verified in the case of other subjects. The writer is of the opinion that if a series of parallel ergographic and respiration experiments were made on a number of subjects, it would be found that positive effects of barometric changes on muscular endurance are accompanied in general by positive coefficients of correlation of barometric change with rate of excretion of carbon dioxide.

This work was done in the Physiological Laboratory of the University of Michigan, with the apparatus described by Higley and Bowen (*American Journal of Physiology*, 1904–'05, xii, p. 311).

THE RELATION OF ACAPNIA TO SHOCK, AND A CONSIDERATION OF THE MECHANICAL EFFECTS OF ARTIFICIAL HYPER-RESPIRATION UPON THE CIRCULATION

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It has been claimed that the most important factor in the causation of shock is diminution of the carbon-dioxide content in the blood and that this diminution is a regular consequence of all influences resulting in shock. That carbon dioxide exercises significant physiological functions cannot be denied; determination, therefore, of the true significance of the diminution of its normal proportion in the blood is important and bears a special relation to the various methods of artificial respiration utilized in thoracic surgery.

This study was undertaken for the purpose of investigating the relation of acapnia to shock. All experiments were performed on dogs.

The *first series* of experiments was conducted for the purpose of studying the effect of variation in intrapulmonic air-pressure upon the blood-pressure. The thorax was opened laterally, a T-tube connected with a water-manometer was tied in a small bronchus, and the heart enclosed in a Henderson cardimeter connected with a recording tambour. The blood-pressure was recorded from the carotid artery. The thorax was then closed and intratracheal insufflation was given from an apparatus provided with an exhaust valve, which reduced the pressure to approximately zero from four to twelve times per minute. When the machine was running at a pressure of 6 mm. of Hg there was an average rise of blood pressure of 15 mm., each time the exhaust valve operated.

[1] The work presented in this paper was begun in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons; BIOCHEMICAL BULLETIN, 1912, ii, p. 175.

In one experiment with an increase in intrabronchial pressure of from 8 to 30 mm. Hg, the blood pressure fell from 122 to 55 mm. Hg, and the volumetric tracing indicated that the output from the heart had diminished about 44 per cent. These variations in blood-pressure were completed within a few seconds after the change in intrabronchial pressure, and could be duplicated at will. A rise of intrabronchial pressure above 8 to 10 mm. Hg always caused a fall in blood-pressure and it was concluded that the variation in pressure was the result of a diminution of the venous return to the heart, resulting from compression of the veins in the thorax. In view of the marked changes in the blood-pressure and output of the heart resulting from small variations in intrapulmonic pressure, it is evident that, in any experiments planned for the purpose of estimating the part played in the production of shock by a diminution of carbon-dioxide content, induced by artificial hyper-respiration, the effects of the increase of intrapulmonic pressure upon the return flow of blood to the heart must be considered.

With the *second series* of animals, Henderson's experiments were duplicated, the dogs being artificially respired by means of a force-and-suction pump, working about seventy times per minute. The animals were given morphin, and ether was administered only when necessary. In these experiments, blood-pressure fell about 40 per cent. within one minute after artificial respiration was begun, and then decreased more slowly throughout the experiment to between 40 and 50 mm. Hg. At the end of the experiment, when the artificial respiration was stopped, the blood pressure increased 60 to 90 per cent. within a few seconds. In all experiments the blood analysis showed that the carbon-dioxide content, at the end, was only 40 to 50 per cent. of the original amount. These animals, at the end of two to three hours of artificial respiration, were all in a condition of deep shock. This degree of shock was indicated by a rapid pulse, a low blood-pressure, and a marked degree of insensibility to sensory stimulation. Three of the animals so treated lived three days (dying of secondary effects of the experiment), and one lived twenty-four hours. None of them died from the immediate effects of the experiment. During these experiments, when the artificial respiration was interrupted or permanently

stopped at the end of the experiment, the period of apnea lasted only one or two minutes, so that no death resulted directly from asphyxia dependent in turn upon acapnia. The absence of a prolonged period of apnea is explained by the fact that the effect of ether was not added to that of morphin.

With a *third series* of animals the experiments just described were duplicated, with the exception that the carbon-dioxide content of the blood was maintained at its normal level, or slightly above it. The conservation of the carbon dioxide was accomplished by inserting a large rubber bag, to act as a reservoir, between the suction pump and the force pump, thus creating an almost perfectly closed circuit; the dog thus rebreathed expired air. To replace the small amount of air and carbon dioxide lost from the animal's trachea, carbon dioxide was administered from a tank into the rubber bag, where it mixed with air drawn in from the trachea. In these experiments the animals went into the same degree of shock in two or three hours as those of the second series, in which the carbon-dioxide content of the blood was diminished to 40 per cent. of the original volume. One animal died on the table just before the completion of the experiment, the others lived for from one to three days. Blood-pressure changes in the two series were similar but a characteristic of the experiments, in which the carbon-dioxide content was kept at or a little above the normal, was a less rapid and weaker heart-beat than that observed when the carbon-dioxide content was diminished.

No other conclusions can be drawn from the experiments of Series 1 and 2 than that the reduction in the carbon-dioxide content of the blood was not an important factor in the causation of shock produced by hyper-respiration, and that in shock so produced, the essential factor was an interference with the venous return to the heart.

In the *fourth series* of experiments the effects of aerating and handling the intestines were studied. A celluloid window was placed in the abdominal wall, and a stream of warm moistened air was passed over the intestines for a period of three hours. During this procedure the animals breathed normally, the blood-pressure was 163 mm. Hg, the content of carbon dioxide was slightly

diminished, and there was no evidence of shock. Beneath the celluloid the absence of peristalsis could be observed as well as the efficiency of the aeration and failure of the intestines to become dry. The celluloid was then removed, the intestines spread out, and the aeration continued. After 45 minutes the carbon-dioxide determination indicated a content of 38.8 vol. per cent., and blood-pressure was 153 mm. Hg. The intestines were then handled; in ten minutes blood-pressure had fallen to 98 mm., in twenty minutes to 56 mm. Hg, and in forty minutes there was still 31.6 vol. per cent. of carbon dioxide in the arterial blood.

In another experiment the intestines were exposed and aerated (not handled). The carbon-dioxide content of the blood was maintained by connecting a long tube with the trachea. After one hour and a half, blood-pressure had changed but 1 mm. Hg, and the animal was in good condition. The intestines were then handled and in ten minutes the blood-pressure fell from 122 to 60 mm. Hg. The carbon-dioxide content was 45.1 vol. per cent. In twenty-five minutes the blood-pressure was 46 mm. Hg, the carbon-dioxide content normal, and the dog in a severe degree of shock.

In these abdominal experiments the primary factor concerned is unquestionably the manipulation of the intestines and not any diminution of carbon-dioxide content caused thereby. It will be remembered that in the similar experiments with aeration of the intestines reported by Henderson, the intestines were handled gently. We have been unable to find any mention in his paper of aeration of the abdominal cavity with air alone beneath a celluloid membrane as a control.

Henderson's control experiment, in which he did not secure shock, included aeration (with a stream of air plus carbon dioxide) of the abdominal cavity beneath a celluloid window in the abdominal wall. Our own experiments show that aeration of the intestines without the addition of carbon dioxide does not produce shock.

CLEAVAGE OF PYROMUCURIC ACID BY MOLD ENZYMES

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In a recent paper[1] we have presented data showing that the formol-titrimetric method of Sörensen can be applied to the determination of the cleavage of hippuric acid by enzymes. The six saprophytic fungi studied were found to produce an enzyme capable of hydrolyzing as much as 90 to 100 per cent. of a solution of sodium hippurate in the presence of toluene as an antiseptic. The production of enzyme was independent of the presence of the corresponding substrate in the nutrient medium upon which the fungus was cultivated, and the age of the culture, within the limits studied, had little influence upon this enzymic activity.

If we assume that the synthesis in the animal organism of hippuric acid from benzoic acid is an enzymic process, the synthesis of the corresponding derivatives from substituted benzoic acids might well be attributed to the same cause. It is known, for example, that *o*-brombenzoic, salicylic, toluic and other substituted benzoic acids, when administered orally, are conjugated with glycocoll and excreted through the kidneys as *o*-brombenzoylglycocoll, salicyluric, toluric, etc., acids. Likewise, a homologue of benzoic acid, *e. g.*, phenylacetic, may be conjugated in the same way and eliminated as phenylaceturic acid. It is quite unlikely that these substituted benzoic acids all require separate enzymes for their conjugation with glycocoll, and it is equally improbable that the hydrolysis of the substituted hippuric acids would require specific enzymes.

This reasoning may be extended, also, to analogous compounds where the benzene nucleus is replaced by a heterocycle. For example, α-pyridine carboxylic acid is united in exactly the same manner with glycocoll and excreted as α-pyridinuric acid. The

[1] Dox and Neidig: *Zeitschr. f. physiol. Chem.*, 1913, lxxxv, p. 68.

striking analogy between benzene and the two heterocycles, furfurane and thiophene, led Jaffé[2] to a study of the behavior of the corresponding derivatives of these substances in the animal organism. As was anticipated, furfurol behaved exactly as did benzaldehyde, undergoing oxidation to the acid and then conjugation with glycocoll, and was eliminated principally as pyromucuric acid. Similarly, thiophenic acid was excreted as thiophenuric acid.

None of these heterocyclic analogues of hippuric acid have, to our knowledge, been studied with reference to their cleavage. Knowing from previous work that the lower fungi produce an enzyme capable of hydrolyzing hippuric acid, we thought it would be of interest to test their activity toward one of these heterocyclic compounds.

With this object in view the following experiments were undertaken. Cultures of seven molds were grown for two weeks on the nutrient medium previously described.[3] The extraction of enzyme was effected by the following method: The mycelium was washed with distilled water, ground in a mortar with fragments of glass and the juice obtained at a pressure of 350 kg. per sq. cm. In each case about 20 c.c. of extract were obtained, 10 c.c. of which were used in the enzyme experiment and 10 c.c. in the control. In the enzyme experiment, 25 c.c. of a 1 per cent. solution of pyromucuric acid,[4] previously neutralized with sodium hydroxide, were added

TABLE I

Data pertaining to the cleavage of pyromucuric acid

Source of enzyme	Titration $n/10$ $Ba(OH)_2$ c.c.	Control $n/10$ $Ba(OH)_2$ c.c.	Difference	Cleavage %
Aspergillus fumigatus	10.9	5.6	5.3	35.8
Aspergillus niger	19.1	12.0	7.1	48.0
Aspergillus clavatus	20.4	13.3	7.1	48.0
Penicillium roqueforti	7.0	2.8	4.2	28.4
Penicillium camemberti	13.1	6.3	6.8	45.9
Penicillium expansum	11.0	5.0	6.0	40.5
Fusarium oxysporium	13.8	10.5	3.3	22.3
Emulsin (Kahlbaum)	4.9	0.2	4.7	31.8
Taka-diastase (Parke-Davis)	0.6	0.4	0.2	1.4

[2] Jaffé and Cohn: *Berichte,* 1887, xx, p. 2311; Jaffé and Levy: *Berichte,* 1888, xxi, p. 3458.

[3] Dox and Neidig: *Loc. cit.*

[4] The pyromucuric acid was prepared by the method of Jaffé and Cohn. It

and to the control, 25 c.c. of water. Toluene was used throughout as an antiseptic. After two weeks, during which time the flasks were shaken at frequent intervals, the formol-titration was made, with the results given in Table 1.

Comparing the foregoing results with those obtained with hippuric acid,[5] it will be noted that the extent of the cleavage of the acid into its components is in this case considerably less. This can hardly be taken as evidence in support of any assumption that we are dealing with two separate enzymes. Quantitative differences observed in work of this nature have little significance, unless the same enzyme preparation has been employed and a uniformity of all other conditions maintained.

TABLE 2

Data pertaining to the formation of ammonia.

Organism	Titration $n/10$ H_2SO_4 c.c.	Control $n/10$ H_2SO_4 c.c.	Ammonia $n/10$ H_2SO_4 c.c.
Aspergillus fumigatus	0.90	0.53	0.37
Aspergillus niger	1.00	0.68	0.32
Aspergillus clavatus	1.00	0.78	0.22
Penicillium roqueforti	0.35	0.33	0.02
Penicillium camemberti	0.90	0.63	0.27
Penicillium expansum	1.65	0.88	0.77
Fusarium oxysporium	2.00	1.68	0.32

As in our previous work with hippuric acid, the contents of the flasks after this titration were distilled with magnesium oxide for the determination of ammonia.

Ammonia can hardly be regarded as a direct product of the cleavage of pyromucuric acid. The small amount found probably results from further decomposition of the glycocoll by another enzyme. This cleavage of glycocoll is, however, so slight as to be practically negligible.

Thiophenuric acid was not available at the time these experiments were carried out. We propose to test the activity of mold enzymes toward this substance at a future date.

melted at 163° C.; 0.25 gm. required 14.8 c.c. of $n/10$ $Ba(OH)_2$ solution for neutralization; theory, 14.8 c.c.

[5] Dox and Neidig: *Loc. cit.*

ANALYSIS OF THE ASH OF THE CASTOR BEAN

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In the discussion, following the presentation at the February meeting of the Columbia University Biochemical Association, of the results of investigations conducted in this laboratory on the effect of manganous sulfate on the action of lipase in the castor bean, *Ricinus communis*,[1] Professor Gies suggested that the effect produced *in vitro* by comparatively large amounts of manganese was very possibly induced in the plant by much smaller amounts, and that it would be of particular interest to test the ash of the seed for its presence.[2] The ash of the seed was therefore tested for manganese; and, at the same time, silica, magnesia, lime and phosphoric acid were determined.

A sample of the kernels of the seed, cold-pressed, ground, exhaustively extracted with ether, as for use in lipolytic experiments,[3] and dried in vacuo over sulfuric acid, was slowly ignited in a platinum dish. The black residue was treated several times with nitric acid and re-ignited till free from carbon. The residue was weighed, taken up with water and nitric acid, and the solution filtered. Calcium sulfate was precipitated in the filtrate by sulfuric acid and alcohol; and, in the filtrate from this precipitate, magnesium and phosphorus were determined in separate aliquot parts, each as magnesium pyrophosphate.[4]

Of the powdered kernels, 4.7698 grams gave 0.3483 gram of ash, or 7.3 per cent. Of this, 0.0018 gram was insoluble and

[1] Falk and Hamlin: *Jour. Amer. Chem. Soc.*, 1913, xxxv, p. 210. An abstract of this paper appears in this issue of the BIOCHEMICAL BULLETIN (p. 455).

[2] The importance of infinitesimal amounts of manganese in plant growth has been repeatedly pointed out by G. Bertrand. For a recent presentation of his views, see his general lecture delivered before the Eighth International Congress of Applied Chemistry, New York, September, 1912.

[3] Falk and Hamlin: *Loc. cit.*

[4] Abderhalden: *Handbuch der bioch. Arbeitsmeth.*, 1912, vi, p. 381.

taken as silica; this amounted to 0.5 per cent. of the ash. The calcium sulfate weighed 0.0326 gram, which represented 0.0134 gram of calcium oxide, or 3.8 per cent. of the ash. Of the filtrate diluted to 500 c.c., two portions of 200 c.c. each were taken for the determination of magnesium and phosphorus. In one, 0.1052 gram of magnesium pyrophosphate represented 0.0671 gram of phosphorus pentoxide, or 48.2 per cent. of the total ash. In the other 0.0798 gram of magnesium pyrophosphate represented 0.0289 gram of magnesium oxide or 20.7 per cent. of the total ash.

To test for manganese[5] 5.000 grams of the oil-free powdered kernels were ignited as above and the ash taken up with 4 c.c. of nitric acid solution (sp. gr. 1.20) and water, and this liquid filtered. It was diluted to 20 c.c. and a 10 c.c. portion was boiled with 0.5 gram of lead peroxide for several minutes, the precipitate allowed to settle, and the liquid decanted into a test tube. Next a solution containing 20 milligrams of manganous sulfate per 5 c.c. was diluted with nitric acid solution and water to fifty times its original volume, and treated in the same way. It was found that the solution of the ash was matched in color by a solution 1/700 as concentrated as the original manganous sulfate solution; therefore 5 grams of the kernel powder contain $4 \times 20 \times 1/700$ milligrams of manganous sulfate, or 0.000028 gram of manganese, which is 0.00056 per cent. The results are summarized below:

	SiO_2	CaO	MgO	P_2O_5	Mn	Total ash
Per cent. in dry, oil-free kernel.....	0.04	0.28	1.51	3.52	0.00056	7.3
Per cent. in ash.....	0.5	3.9	20.7	48.2	0.0076	——

Schulze and Godet, who analyzed the ash of this seed,[6] obtained the following data for *dry* but *not* oil-free kernels:

	CaO	MgO	P_2O_5	Total ash
Per cent. in dry substance.....	0.15	0.72	1.16	3.64
Per cent. in ash.....	4.0	19.8	31.9	——

These results indicate that the cold-pressing and ether extraction in my own work removed substance amounting to about half the weight of the kernel.

[5] Noyes, Bray and Spear: *Tech. Quart.*, 1908, xxi, p. 116.

[6] Schulze and Godet: *Z. f. physiol. Chem.*, 1908, lviii, pp. 156–61.

NOTES ON THE CHEMICAL NATURE OF THE "TANNIN MASSES" IN THE FRUIT OF THE PERSIMMON

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Introduction. The researches of Lloyd upon the nature of the ripening process in persimmons and dates led him to believe that the loss of astringency at maturity is due to the combination of tannin with a colloidal substance of carbohydrate nature in the "tannin mass." This combination takes place ordinarily at the time of ripening, probably under the influence of enzymes; but it may be hastened by artificial means, such as exposure to the vapors of acetic acid and ethyl nitrite, and to carbon dioxid under normal and supranormal pressures. Lloyd[1] defines his tannin mass as "the tannin idioblast containing tannin associated with a second colloid." After the union of tannin and this colloidal substance has taken place no more tannin can be extracted nor do alkaloids indicate its presence. It is evident, then, that a knowledge of the chemical substances in the tannin mass would facilitate further investigation of the mechanism of the ripening process.

Preparation of tannin masses. The tannin masses used in our experiments were prepared by Prof. F. E. Lloyd[2] as follows: Fully ripe persimmons (*Diospyros*) were shaken and macerated with water until they formed a thick paste, from which the heavy tannin masses settled out. This process was repeated until the separated masses were thoroughly washed by decantation and also purified from all debris. The resulting thick suspension of tannin

[1] Lloyd: BIOCHEMICAL BULLETIN, 1911, i, p. 7. See also *Plant World,* 1911, xiv, p. 1.

[2] Lloyd: *Zeitsch. f. Chem. und Ind. d. Kolloide,* 1911, ix, pp. 65–73. The material was shipped to this laboratory from Prof. Lloyd's laboratory at Auburn, Ala.

masses was kept under a layer of ether. This material had the appearance of a multitude of minute cylindrical particles which were colorless and transparent. Upon exposure to the air these particles soon became brown, probably from oxidation.

General properties of the tannin masses before hydrolysis. In both the Millon and xanthoproteic tests the tannin masses gradually assumed a dark brown color; a change apparently similar to that resulting from slow oxidation by the air. With Fehling or Fehling-Benedict solution the masses turned black at once, but no reduction was observed. Tests for pentoses with conc. hydrochloric acid solution and phloroglucinol caused the particles to become bright red, but this acid *alone* caused the same change. Iodin in potassium iodid solution produced no coloration. Repeated fusions with metallic sodium failed to indicate the presence of nitrogen. This observation was confirmed by subjecting 2 gm. of the material to the Kjeldahl process for nitrogen, with negative results.

The presence of phloroglucinol in the masses was shown by adding to them a little vanillin in hydrochloric acid solution when the particles were stained a beautiful magenta shade, a result similar to that obtained when pure phloroglucinol and vanillin react in the presence of traces of hydrochloric acid. Various other phenolic substances, however, form brightly colored condensation products with vanillin under such conditions.[3]

All tests for pentose by boiling with hydrochloric acid solution and allowing the vapors to act on anilin acetate paper, and also by boiling the particles with conc. hydrochloric acid solution plus orcin, were negative. No pentose is present, apparently, in spite of the red color given by the phloroglucinol-hydrochloric acid test, a result that may have been caused by the interaction of these substances with a phenolic substance (like vanillin) rather than by pentoses. With the Molisch reagent a very strong positive result was obtained and, furthermore, we found that exactly the same purplish ring was formed by the tannin masses with pure sulfuric acid *alone*.

[3] Hartwick and Winckel (*Archiv d. Pharm.*, 1904, ccxlii, p. 471), showed the presence of phloroglucinol in the tannin masses of *Ceratonia siliqua* and *Phoenix dactylifera*. Tichomirow (*Bull. Soc. Imp. d. Nat. Sci.*, Moskau, 1905) obtained the same reactions in the tannin masses of the persimmon, indicating the presence of a phenol.

Finally, the tannin masses stained deep blue with ferric chlorid solution and, as Lloyd found, this color was quickly destroyed by nitric acid. These properties of the tannin masses show that the latter contain neither reducing sugars nor protein; they also suggest that phloroglucinol occurs with tannin and cellulose material.

Hydrolysis of tannin masses with 0.2 per cent. and 2 per cent. hydrochloric acid solutions. The addition of 0.2 per cent. hydrochloric acid solution to tannin masses, with subsequent heating, caused them to disintegrate, giving the whole liquid a bright cherry red color. The tannin masses as such disappeared and a white flocculent residue remained suspended in the red liquid. The mixture was filtered and to the filtrate we added 0.5 per cent. sodium hydroxid solution until the acid was neutralized. Beyond this neutral point the red color disappeared, but on standing or after further treatment with alkali, a gradually increasing brownish coloration took its place.

The neutral filtrate was evaporated to dryness on the steam bath and an aqueous solution of this reddish residue was subjected to the following tests, with the results indicated: With ferric chlorid solution, a purplish black coloration was given; with Fehling-Benedict solution, a dark brownish precipitate was formed at once but it soon changed to a characteristic reduction when heated; the Molisch test was a typical positive one; with vanillin-hydrochloric acid solution a red color appeared; and a peculiar non-typical precipitate was produced when we attempted to form an osazone with phenylhydrazine hydrochlorid-sodium acetate mixture.

We filtered off the white amorphous residue and washed it free from chlorid. Upon testing it we found there was little if any reduction of Fehling-Benedict solution; with the vanillin-hydrochloric acid reagent there was no red coloration except in a few deeply stained particles (stone-cells?);[4] with ferric chlorid solution a brownish color appeared but no bluish shade. Finally, the residue,

[4] "Undoubtedly they were stone cells, as I observed the same thing. To get this reaction all one needs to do is to add hydrochloric acid to the mucilaginous pulp which includes stone cells, and these become colored. When I observed this I did not refer the reaction to the presence *in the tannin masses* of the phloroglucinol. This I later satisfied myself to be the case." (Lloyd: Personal communication.)

when suspended in the "biuret reagent," was slowly colored blue as the cellulose material absorbed copper from the alkaline solution.[5]

Next, the tannin masses were hydrolyzed with a 2 per cent. hydrochloric acid solution. The hydrolytic products, when treated in exactly the same manner, gave results identical with those just described. The more concentrated acid seemed to favor the formation of dark-colored products.

From the foregoing results it appears probable that tannin masses contain tannin and phloroglucinol combined with a third substance, from which union they are released when hydrolysis takes place. The acid gelatinizes that part (colloidal) of the masses which appears to be cellulose or some related substance.

Hydrolysis of tannin masses with 0.5 per cent. and 5 per cent. sodium hydroxid solutions. Treated with 0.5 per cent. or 5 per cent. solution of sodium hydroxid, "tannin masses" gave purplish brown mixtures containing a bulky gelatinous residue, which we removed by filtration. The filtrate was carefully neutralized with dilute hydrochloric acid solution and the reddish brown precipitate that formed was filtered off, washed, and dissolved in water. This liquid was tested for reducing power with Fehling-Benedict and ammoniacal silver solutions, both reagents showing weakly positive results. The Molisch reagent, and also ferric chlorid solution, gave dark non-typical colorations. With vanillin-sulfuric acid solution a typical red color was produced, when the liquid was evaporated.

The solution of the material *not precipitated* from alkaline solution by acid was now tested in the usual way for reducing power, presence of phloroglucinol, etc., with uncertain results, due to the dark color of the solution. When alkaline solutions of the hydrolytic products are exposed to the air, oxidation seems to occur and dark complex substances are formed. The gelatinous material which resisted hydrolysis was filtered and washed. It appeared to consist of the collapsed cell-walls of tannin masses. When dried it formed a light-colored scaly mass composed of small particles. On the whole, the results of alkaline treatment of the tannin masses

[5] Kantor and Gies: Biochemical Bulletin, 1911, i, p. 269.

are similar to those obtained with acids, except that alkali accelerates oxidation by atmospheric oxygen.

Properties of mixed solutions of pure tannin and phloroglucinol. Mixtures in varying proportions of solutions of pure tannic acid and phloroglucinol were tested and found to show many similarities to the hydrolytic products of the tannin masses. The Molisch test was strongly positive. With ferric chlorid such mixed solutions gave a purplish black color which was accompanied by the gradual formation of a precipitate of the same shade. The typical influence on Fehling-Benedict solution was observed; namely, heavy greenish precipitation at first but, upon heating, this changed to a characteristic red reduction. When some of the mixed solution was evaporated with the vanillin-hydrochloric acid reagent, a definitely positive result was obtained but the tannin seemed to cause a brownish tint not given by phloroglucinol alone.

Pure phloroglucinol solutions were negative to the Molisch test and also to the Fehling-Benedict test. Ferric chlorid solution produced a clear blue color with pure phloroglucinol but *did not give a precipitate,* thus differing from tannin, which forms a blackish precipitate at once under these conditions. Solutions of pure tannin yield a typical Molisch test and reduce Fehling-Benedict solution in the characteristic manner just described. It is evident that, in the presence of phloroglucinol, one cannot conclude that a purple color with ferric chlorid indicates tannin. The addition of nitric acid to the mixture already turned purple by ferric chlorid caused an instantaneous change to a brownish tint.

It is obvious, then, that, in the tests indicated, mixtures of tannin and phloroglucinol differ in no essential way from the hydrolytic products of the tannin masses.

Sources of the tannin and phloroglucinol in the tannin masses. The material in the tannin masses from which tannin and phloroglucinol are derived by hydrolysis is probably one of the so-called phloroglucin-tannoids, which were found by Graebe[6] to yield these hydrolytic products by treatment with acids, etc. Weinzierl[7] showed that phloroglucinol is widely distributed among plants but

[6] Graebe: *Ber. d. d. chem. Ges.,* 1903, xxxvi, p. 212.

[7] Weinzierl: *Oesterr. bot. Ztsch.,* 1874, xxvi, p. 285. See also Nickel: *Bot. Centralblatt.,* 1891, xlv, p. 394.

he was unable to say whether it occurs as a waste product or one useful in the synthetic processes.

Waage[8] made some interesting observations in regard to phloroglucinol in plants. He confirmed the statement that the substance is widely distributed in plants and suggested that it might arise during photosynthetic processes, just as glucose probably does. He felt that, because of the dark blue color given by phloroglucinol and ferric chlorid, and because of the bleaching of methylene blue by the former substance, one cannot rely on the previous deductions in regard to the presence of tannin in cell vacuoles based on these reactions for tannin. In fact, he criticized, on the same ground, the work of af Klercker[9] on tannin in vacuoles. Schiff[10] found that under suitable conditions, phloroglucinol and carbon dioxid combine to form phloro-tannic acid which, upon heating, yields a red substance like phlobaphene. Evidently, tannin is present along with phloroglucinol in many plants, but the physiological rôle of these substances is as little known as is the nature of the combination between them in cases like that of the tannin masses of the persimmon. Much more work on persimmons will be necessary to explain the part played by phloroglucinol in the loss of astringency in the mature fruit.

Summary. Tannin masses from the fruit of the persimmon, by hydrolysis with weak acid or alkali, yield tannin, phloroglucinol, and considerable insoluble colloidal residue. Hydrolysis of such tannin masses does not produce hexose or pentose.

The nature of the union between the tannin and phloroglucinol is unknown but it is probably similar to that of the phloroglucin-tannoids in various plants.

The colloidal residue that resists hydrolysis seems to be a cellulose-like substance, which readily forms gelatinous masses with water or alkaline solutions. Quantitative studies on large amounts of this third substance are desirable.

In the presence of phloroglucinol, the ferric chlorid test for tannin is unreliable.

[8] Waage: *Ber. d. d. bot. Ges.*, 1890, viii, p. 250.

[9] af Klercker: *Bihang till K. Svenska Vet.-Akad.*, 1888, xiii, p. 18 (repaged?).

[10] Schiff: *Ann. d. Chem.*, 1888, ccxlv, p. 36; 1889, cclii, p. 87.

A study of the conditions necessary for the formation, and also the hydrolysis, of the phloroglucinol-tannin combination might help to explain the nature of the ripening process in persimmons.

This work resulted from a suggestion by Prof. F. E. Lloyd to Prof. Wm. J. Gies, that material would be supplied in abundance for a study of the "tannin mass." I am indebted to Professor Lloyd not only for the material but also for many suggestions. To Professor Gies I am likewise indebted for helpful advice.

HISTON AND ITS PREPARATION

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I. INTRODUCTION

Several years ago I began a study of some artificial protein compounds, as an introduction to an inquiry into the nature of protein conditions in cells (7).[1] In preparing histon from calf thymus glands by the Huiskamp (10), Lilienfeld (19) and Bang (2) processes, I found that the ammonia-precipitated product from neutral histon-hydrochlorid solution was insoluble in water and hence not available for the intended study of histon compounds. This result led me to reject the ammonia-precipitated histon and to use, instead, a solution of histon hydrochlorid (made neutral by dialysis). Lately, in furtherance of this study, I have endeavored to prepare water-soluble basic histon, free from admixture with histon salt of any kind. Various methods of preparation have been proposed, but I find that thymus histon is almost invariably precipitated by the addition of an excess of ammonia to an acid solution of the substance, in spite of the fact that solubility in water is cited again and again as a property of histon. The only exceptions to this statement were found in Kossel's original paper (12) on goose-blood histon and in the description of Lota histon by Ehrström (8). I have spent much time in determining by various methods of preparation that the product precipitated by ammonia (thymus histon) is invariably insoluble in water. Recently, in reviewing Fleroff's paper (9) on para-histon, I found a definite statement that ammonia-precipitated thymus histon is insoluble in both hot and cold water, and may be washed with water for purification.

[1] Figures in the text enclosed in parenthesis refer to the numbered items in the bibliography at the end of this paper.

II. HISTORICAL REVIEW OF HISTON PREPARATIONS

Before proceeding to the description of my experiments the reader may find it interesting to examine a brief summary of the main points in this confusing situation as brought out in the literature of the subject.

1. **Histons already prepared.** The types of histons already prepared, with indications of sources and names of the original investigators, are shown in the appended summary:

Name	*Source*	*Prepared by*
Goose-blood	Corpuscles	Kossel (12)
Thymus	Calf thymus	Lilienfeld (19)
Salmin albumose	Unripe sperm of the salmon	Miescher and Schmiedeberg (22)
Arbacin	Testes of sea-urchin	Matthews (21)
Globin	Hemoglobin	Schulz (23)
Scombron	Mackerel sperm	Bang (2)
Para-histon	Thymus	Fleroff (9)
Gadus	Codfish sperm	Kossel and Kutscher (16)
Lota	Burbot sperm	Ehrström (8)
Hen blood	Blood corpuscles	Ackerman (1)
Centrophorus	Centrophorus sperm	Kossel (15)

2. **Methods of preparation.** The methods of preparation are indicated in the following summaries:

A. GOOSE-BLOOD HISTON: KOSSEL, 1884 (12). *Preparation I.* Kossel centrifuged blood corpuscles free from plasma and then dissolved them in water and ether. The residue was extracted with water and ether until free from color and the final colorless mass extracted with hydrochloric acid solution. The acid liquid was then saturated with sodium chlorid, the precipitated histon was filtered off, and purified by washing with acidified sodium chlorid solution and then dialyzing against distilled water until free from chlorid. The resulting solution was then treated with an excess of alcohol-ether and the precipitate dried to constant weight at 105° C. *Preparation II.* In the second preparation the hydrochloric acid solution was precipitated with ammonia instead of sodium chlorid.

B. THYMUS HISTON: LILIENFELD, 1893 (19). Thymus glands were freed from fat with a knife, minced and the hash pressed in a

hempen bag to remove the juice, which contained lymphocytes that were separated in a centrifuge. The lymphocytes were then extracted with water to extract nucleohiston. (In a modification of this process, the minced glands were extracted directly with water.) The nucleohiston was precipitated from the extract with acetic acid, and purified by re-solution in water to which a little sodium carbonate had been added and reprecipitating with acetic acid. The precipitate was then treated with 0.8 per cent. hydrochloric acid solution. From this solution of histon-hydrochloride, histon was precipitated with ammonia (added either before or after dialysing free from free acid). The ammonia-precipitated product was finally purified by washing with alcohol and ether, and dried to constant weight.

C. SALMIN HISTON: MIESCHER, 1896 (22). The nuclei of unripe salmon sperm were extracted with 0.25 per cent. hydrochloric acid solution, the extract filtered and (after dialysing to neutrality) the filtrate precipitated by saturation with ammonium sulfate or sodium chlorid.

D. ARBACIN HISTON: MATHEWS, 1897 (21). *Preparation I.* Dried sperm heads were extracted with 1–2 per cent. sulfuric acid solution and the acid extract poured into a large volume of alcohol to precipitate the histon-sulfuric acid complex. The precipitate was purified by washing with alcohol-ether and dried to constant weight. *Preparation II.* The alcohol-precipitated product was dissolved in water, the solution made ammoniacal and filtered (no precipitate at this point), the filtrate poured into alcohol, and the resulting precipitate washed free from ammonia and redissolved in a small volume of water. Ammonia added to this concentrated solution failed to completely precipitate the histon, which showed a strong tendency to remain dissolved in ammoniacal solutions. Matthews' studies were based on the use of alcohol-precipitate from acid extract, alcohol-precipitate from an ammoniacal water-solution, and ammoniacal water-solution neutralized with sulfuric acid.

E. GLOBIN HISTON: SCHULZ, 1898 (23). A solution of hemoglobin was treated with dilute hydrochloric acid solution and a brown precipitate obtained, soluble in the presence of a very slight excess of acid. When this precipitate was dissolved in acid and

one-fifth its volume of alcohol and ether added, the color passed into the ether leaving a clear water-alcohol solution, from which ammonia precipitated a yellowish flocculent mass of globin, that, when washed free from ammonia, began to dissolve, a few drops of acetic acid solution completing the process. Dialysed free from acid, a clear, neutral, slightly colored, odorless and tasteless solution of globin resulted. Schulz' tests were based on this solution and on the ammonia-precipitated product. The analyses were made on the latter, washed with alcohol and ether, and dried *in vacuo* to constant weight at a temperature of 100° C.

F. SCOMBRON HISTON: BANG, 1899 (2). Unripe mackerel sperm was heated with alcohol and the residue dried. This dried residue was then extracted with dilute hydrochloric acid solution and the histon precipitated from the filtrate with caustic soda, ammonia, or by saturation with sodium chlorid. The product was purified by re-solution in water containing a trace of acid, reprecipitation with the desired reagent, washing with alcohol and ether, and drying to constant weight. Bang's statement of "characteristic properties" of histon was based upon the water-solution of the products precipitated by sodium hydroxid or sodium chlorid. His analyses were based on the ammonia-precipitated product. (See pages 424 and 426.)

G. PARA-HISTON: FLEROFF, 1899 (9). *Preparation I.* Minced thymus glands were treated with alcohol and ether, and the residue extracted with 2 per cent. sulfuric acid solution (100 gm. of thymus to each 1,000 c.c. of acid solution). The filtered acid extract was then precipitated with three volumes of alcohol, the precipitate dissolved in hot water, and the solution heated with sodium picrate. The histon picrate was then reconverted into the sulfate by treatment with 2 per cent. sulfuric acid solution and ether, and reprecipitation with alcohol. This process was repeated twice. The final precipitate was then dissolved in water, freed from sulfate with barium hydroxid, and excess of barium precipitated with carbon dioxid. To this turbid, viscid liquid was then added an equal volume of alcohol, and ammonia, and the liquid filtered. Excess of alcohol added to the filtrate precipitated para-histon. This was still further purified by dissolving in water and repre-

cipitating with alcohol-ether. *Preparation II.* (*Levene method*). After transformation to picrate, and removal of picric acid with sulfuric acid as detailed above, the histon was precipitated from the sulfuric acid solution with ammonia. The filtrate containing the para-histon was then precipitated with alcohol, the precipitate purified by solution in hot water, and reprecipitation with alcohol-ether. The product was then dried to constant weight. Fleroff determined the properties of his material in studies of the alcohol precipitate and its water-solution.

H. Gadus histon: Kossel and Kutscher, 1900 (16). Dry codfish sperm was extracted many times with hydrochloric acid (20 c.c. conc. hydrochloric acid solution to each liter of water), the combined acid solutions then filtered, histon precipitated by saturation with sodium chlorid, and purified by dialyzing against running water until free from sodium chlorid and a clear water-solution obtained, which was then precipitated with ammonia, and the histon washed with ammonia-water, alcohol, and ether, and dried to constant weight.

I. Lota histon: Ehrström, 1901 (8). Dry sperm of the burbot was extracted with conc. hydrochloric acid solution at room temperature for an hour, the acid extract treated with 3–4 volumes of water, the filtrate neutralized with sodium hydroxid and diluted with five volumes of water to precipitate the histon. This product was purified by digesting the precipitate on a water bath with 0.5 per cent. hydrochloric acid solution, precipitating the histon with ammonia, washing the material with water, alcohol, ether, and drying to constant weight. Ehrström used a hydrochloric acid solution neutralized with sodium hydroxid for the determination of properties. His analyses were based on the ammonia-precipitated product.

J. Hen blood histon: Ackerman, 1904 (1). (*Plenge's method*). The hen blood was centrifuged with 0.9 per cent. sodium chlorid solution for the isolation of the corpuscles, which were extracted with alcohol for the removal of pigment and again centrifuged free from alcohol, and dried with alcohol and ether. Histon was obtained by extracting the dry material with 1 per cent. hydrochloric acid solution, precipitating with ammonia, and purifying by

washing with alcohol and ether, and drying to constant weight. Ackerman used the ammonia-precipitated product for his analyses.

K. CENTROPHORUS HISTON: KOSSEL AND KUTSCHER, 1906–7 (15). Report was given of the preparation but no details as to method or properties.

L. REVIEWS. The available reviews merely summarize the methods of preparation already in the literature. Oppenheimer (1909) recommends a method of preparing histon which is essentially that of Lilienfeld (19).[1] Abderhalden (1909–10) recommends the method of Kossel and Kutscher (16).[2] In our experience both of these methods result invariably in a product that is practically insoluble in water. The only other method reported in

TABLE I

Data pertaining to percentage elementary composition of histons

Kind	Precipitated by	C	H	O	N	S	P	Ash
1. Goose-blood (12).....	NaCl	50.88	7.05	—	—	—	—	—
		50.90	7.16	—	17.77	—	—	0.52
2. Goose-blood (12).....	NH_4OH	52.31	7.06	—	—	—	—	0.65
		52.14	7.20	—	18.46	—	—	0.66
3. Thymus (19, 2).....	NH_4OH	52.34	—	—	17.42	—	—	—
		52.31	7.31	—	18.35[3]	—	—	—
4. Salmin-albumose (22) .	NaCl or $(NH_4)_2SO_4$	51.21	7.60	23.44	17.64	—	—	—
5. Globin (23, 2)[4].....	NH_4OH	59.47	7.20	—	16.80	—	—	0.52
		—	—	—	16.89	0.42	0.00	0.84
6. Arbacin (21).....	Alcohol	—	—	—	15.91	—	—	—
7. Scombron (2).....	NH_4OH	49.86	7.23	—	19.79	0.79	—	—
8. Para-histon (9).....	Alcohol	51.91	7.31	20.32	17.97	—	—	—
9. Gadus (16).....	NH_4OH	—	—	—	18.65	—	—	—
		—	—	—	18.64	—	—	—
10. Lota (8).....	NH_4OH	—	—	—	16.46	—	—	—
		—	—	—	16.49	—	—	—
11. Hen-blood (1).....	NH_4OH	—	—	—	18.31	—	—	—
12. *Centrophorus and Sphærechinus* (15)	—	No data given by Kossel.						

[1] Extracted minced, fat-freed, glands with water and precipitated with acetic acid. Histon was extracted with 0.8 per cent. hydrochloric acid solution and the histon precipitated with ammonia.

[2] The fat-free glands were extracted with water and sufficient hydrochloric acid was added to make the strength of the acid 0.8 per cent. The filtered extract contained the histon, which was precipitated with ammonia.

[3] Second figure for N is that of Bang and Fleroff.

[4] All determinations by Bang except second N.

the literature is that of Lawrow (18). Ammonia is used to precipitate the histon and purification is secured by redissolving in hydrochloric acid solution and reprecipitating with ammonia. It introduces no new features.

III. PROPERTIES OF HISTONS

1. **Results of elementary analysis.** A summary of the available analytic data is presented in Table 1.

2. **Solubilities.** The solubilities of the histon products are indicated by the data in Table 2.

TABLE 2

Data pertaining to the solubilities of histons

Kind	Precipitated by	Reagents											
		Water	Alcohol	NH_4OH, in absence of salts	NH_4OH, in presence of salts	Alkalies	Salts of alkali earths	Cold HNO_3	Hot HNO_3	0.8% HCl	H_2SO_4, dilute	Neutral alkali salts
1. Goose-blood (12)	NaCl	S*	I*	I	I	S[1]	S[1]	I	S	S	S	I
2. Goose-blood (12)	NH_4OH	I	I	I	I	S[1]	S[1]	I	S	S	S	I
3. Goose-blood (2)	NaOH	S	I	S	I	S[1]	S[1]	I	S	S	S	I
4. Thymus	NH_4OH	I	I	S[1]	I	S[1]	S[1]	I	S	S[2]	S	I
5. Thymus	NaCl	S	I	S[1]	I	S[1]	S[1]	I	S	S	S	I
6. Salmin (22)	NaCl	S	I	—	I	S[1]	S[1]	I	S	S	S	I
7. Arbacin (21)	Alcohol	S	I	S[3]	S[3]	S[1]	S[1]	I	—	S	S	—
8. Globin (23)	NH_4OH	S	I[1]	S[1]	I	S[4]	S	I	S	S	S	I
9. Scombron (2)	NaOH	S	I	I	I	S[1]	S[1]	I	S	S	S	I
10. Gadus (16)	NaCl	S[5]	I	I	I	S[1]	S[1]	I	S	S	S	I
11. Lota (8)	NH_4OH	I	I	S[1]	I	S[1]	S[1]	S	S	S	S	I
12. Para-histon (9)	Alcohol	S	I	S	S	S	S	S	S	S	S	S
13. Hen-blood (1)	NH_4OH	—	I	—	I	—	—	—	—	S	—	—
14. *Centrophorus and Sphærechinus* (15)	—	(No data given by Kossel).										

* S = soluble; I = insoluble.

[1] Soluble in excess of the reagent.

[2] Becomes insoluble if allowed to stand.

[3] Precipitate dissolves in slight excess of ammonia.

[4] Alkali precipitates (30 per cent.) water solution.

[5] Kossel states properties are same as those of ordinary histon. Hence the properties are assumed to be identical with those of 1.

3. **Characteristic precipitation reactions** (Bang). Table 3 presents a summary of the characteristic precipitation tendencies of histons, as specified by Bang.

TABLE 3

Data pertaining to the precipitation of histons (Bang)

Kind	Nature of product in aqueous solution	Reagents										
		NH_4OH, salts absent	NH_4OH, salts present	Cold HNO_3	Hot HNO_3	Heat	Sodium phosphotungstate	Sodium phosphomolybdate	Sodium picrate	$K_4Fe(CN)_6$	Potassio-mercuric iodid	Protein solutions
1. Goose-blood (12) ..	NaCl ppt.	P[1]	P[2]	P*	N*	N	—	—	—	—	—	P
2. Goose-blood (2) ...	NaOH ppt.	P[1]	P[2]	P	N	N	P[3]	P[3]	—	P[3]	P[3]	P
3. Thymus.....	Histon-HCl	P[4]	P[2]	P	N	P	P	P	P	P	—	P
4. Thymus.....	NaCl ppt.	P[4]	P[2]	P	N	P	P	P	P	P	P	P
5. Salmin (22).....	Histon-HCl.	P[2]	P[2]	P	N	—	—	—	—	P	—	—
6. Arbacin (21).....	Histon-H_2SO_4	N[5]	N[5]	—	—	—	P[6]	—	P	P	—	P
7. Arbacin (21).....	Alc. ppt. (NH_4OH sol.)	N[5]	N[5]	—	—	—	P[6]	—	—	—	—	P
8. Globin (23).....	NH_4OH ppt.	P[1]	P[2]	P	N	N	N[6]	P[3]	—	P	—	P
9. Scombron (2).....	NaOH ppt.	P[2]	P[2]	P	N	P	P[6]	P[6]	P[6]	P[6]	—	P
10. Para-histon (9)....	Alc. ppt.	N	N	N	N	N	—	—	P	P	P[6]	P
11. Gadus (16).....	NaCl ppt.	P	P[2]	P	N	—	—	—	—	—	—	P
12. Lota (8).....	Histon-HCl	P[1]	P[2]	N	N	N	—	—	P[3]	P[3]	—	P
13. Hen-blood (1).....	Histon-HCl	—	P	—	—	—	—	—	—	—	—	—
14. *Centrophorus and Sphœrechinus* (15)	—	No data given.										

4. **General precipitation reactions.** Additional general data on the precipitation of histons are given in Table 4.

5. **Color reactions.** Available data pertaining to the responses of histons to protein color tests are indicated in Table 5.

* P = precipitated; N = not precipitated.

[1] Soluble in excess of the reagent.

[2] Insoluble in excess of the reagent.

[3] From neutral but not from alkaline solutions.

[4] Thymus is apparently insoluble in ammonia in the absence of salts provided only a small amount of ammonia is used to precipitate it. Once formed a large excess of ammonia can be added without dissolving the precipitate.

[5] No precipitate with ammonia unless the solution is very concentrated or alcohol is present.

[6] In neutral or weak alkaline solution.

TABLE 4

Additional general data pertaining to the precipitation of histons

Kind	Nature of product in aqueous solution	Reagents																								
		Alcohol	Alcohol-ether	NaCl	$MgSO_4$	$(NH_4)_2SO_4$	Na_2CO_3	NH_4Cl	$CaCl_2$	$HgCl_2$	Pb Acetate (a)	Pb Acetate (b)	$FeCl_3$	$CuSO_4$	$HgNO_3$	K_2CrO_4	NaOH	$Ca(OH)_2$	Sodium acetate	HCl	H_2SO_4	Acetic acid	Phosphotungstic acid	Phosphomolybdic acid	Picric acid	Trichloracetic acid	Tannic acid
1. Goose-blood (12).	NaCl ppt.	P*	P	P[1]	P[1]	P[1]	P[1]	P[1]	N*	N	N	N	—	—	—	—	P[2]	P[2]	P	N	N	N	P	P	P	P	P
2. Goose-blood (2) .	NaOH ppt.	P	P	P[1]	P[1]	P[1]	P[1]	P[1]	N	N	N	N	—	—	—	—	P[2]	P[2]	—	N	N	N	P	P	P	P	P
3. Thymus.....	Histon-HCl	N	N	P[1]	P[1]	P[1]	—	N	N	N	N	N	N	N	N	—	P[2]	P[2]	N	N	N	N	P	P	P	P	P
4. Salmin (22).....	Histon-HCl	—	—	P[1]	—	P[1]	—	—	—	P	—	—	—	—	—	—	P[2]	—	—	—	—	—	—	—	—	—	—
5. Arbacin (21).....	Alc. ppt.																										
	Histon-H_2SO_4	P	P	—	—	—	—	—	—	—	—	—	—	—	—	P	—	—	—	—	—	—	P	—	P	—	—
6. Globin (23).....	NH_4OH ppt.	P[3]	P	P[1]	P[1]	P[1]	P[2]	N	N	—	N	—	N	N	—	—	P[2]	P[2]	—	P[4]	P[4]	N	P	P	P	P	P
7. Scombron (2)....	NaOH ppt.	—	—	P[1]	—	P[1]	—	N	—	P	—	—	—	—	—	—	P[2]	P[2]	—	—	—	—	P	P	P	P	P
8. Para-histon (9)...	Alc. ppt.	P	P	N	—	—	P[2]	—	—	N	—	—	—	—	—	N	N	—	—	—	N	—	P	—	—	—	—
9. Gadus (16)[5].....	NaCl ppt.	P	P	P[1]	P[1]	P[1]	P[1]	—	—	N	N	N	—	—	—	—	P[2]	P[2]	—	N	N	N	P	P	P	P	P
10. Lota (8).....	Histon-HCl	P[6]	P[6]	P[1]	—	P[1]	—	—	—	—	N	P	N	N	N	—	P[2]	P[2]	—	N	N	N	—	—	P	—	—
11. Hen-blood (1) and *Centrophorus* (15).	—	No data given.																									

* P = precipitate; N = no precipitate.

[1] Complete on saturation.

[2] Soluble in excess of reagent.

[3] Ammonia solution of globin is less easily precipitated by alcohol.

[4] In cold, not in hot.

[5] No details are given in the paper but the statement is made that it is like other histons and hence may be assumed to have the properties of goose-blood histon.

[6] Alcohol precipitates the hydrochloric acid but not the sulfuric acid solution.

TABLE 5

Data pertaining to the responses of histons to protein color tests

Kind	Nature of product tested	Color test				
		Biuret	Xanthoproteic	Millon	Molisch	Adamkiewicz
Goose-blood .	NaCl ppt.	P (red)[1]	P	Weak	N[1]	—
Thymus.....	NaCl and NH_4OH ppt.	P	P	Weak	N	N
Salmin.....	Histon-HCl	P	P	P	—	—
Arbacin.....	Histon-H_2SO_4	P	P	P	—	—
Globin.....	NH_4OH ppt.	P (violet)	Weak	Weak	N	Weak
Scombron...	NaOH ppt.	P	P	Weak	N	—
Para-histon..	Alc. ppt.	P	P	Weak	—	—
Gadus.....	NaCl ppt.	P	P	Weak	N	—
Lota.....	Histon-HCl	P (violet)	P	P	P	Weak

6. **A summary of characteristic properties of histon** (2). Histons are precipitated from aqueous solutions by ammonia, and are insoluble in excess of the reagent. Bang (2) claims that the presence of salt is necessary for complete precipitation. Huiskamp (10) claims that the presence of salt hinders the ammonia-precipitation of thymus histon.

Histons are coagulated by boiling only in the presence of salts. This coagulum is soluble in dilute hydrochloric acid. The protein is not reprecipitated when the acid solution is neutralized.

Histons are precipitated by concentrated nitric acid solution in the cold. The precipitate dissolves on heating and reappears on cooling.

Histons are precipitated by the "alkaloidal reagents" from neutral or weakly alkaline solutions.

Neutral solutions of histon yield precipitates with neutral solutions of egg albumin and with blood serum; also with such solutions of caseinogen and serum albumin, if these are poor in salts.

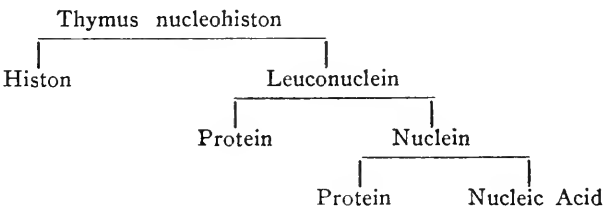
IV. THYMUS HISTON

1. **The nature of the histon complex.** The most exhaustive studies of histon developed from a discussion of the form in which histon occurs in the tissues. Most of the investigation in this connection dealt with thymus histon. The researches of Malengrau (20), Lilienfeld (19), Huiskamp (10, 11), and Bang (3, 4, 5, 6),

[1] P = positive; N = negative.

bring out the points at issue, which may be briefly summarized as follows:

Lilienfeld first advanced the proposition that a water extract of thymus glands contains nucleohiston, precipitable by acetic acid. From his study of this substance he suggested a constitution that is indicated by the appended tabular sequence of decomposition products:



Malengrau (20) repeated Lilienfeld's work and claimed that the latter's precipitate was a mixture of at least two substances. Malengrau called these "nucleoalbumins *A* and *B*." He obtained histon from each. Bang (3) entered the discussion in 1900 and, while agreeing with Malengrau that Lilienfeld's nucleohiston was a mixture, maintained that a water extract of thymus contained at least three substances: Histon, nucleic acid, and a nucleoprotein free from histon. He interpreted Lilienfeld's results as follows:

Acetic acid plus water extract of thymus yields a precipitate which is a mixture of histon nucleate and nucleoprotein (Lilienfeld's *nucleohiston*). This precipitate, in 0.8 per cent. hydrochloric acid solution, yields histon hydrochlorid plus a mixture of nucleic acid and nucleoprotein (Lilienfeld's *leuconuclein*). In 1901 Huiskamp (10) stated his belief that Lilienfeld's nucleohiston is a mixture of nucleoprotein and nucleohiston. The latter he separated by adding calcium chlorid to an extract of thymus, to a strength of 0.2 per cent., obtaining a precipitate of calcium nucleohiston and a solution of nucleoprotein. The former yielded histon hydrochlorid with extraction in 0.8 per cent. hydrochloric acid solution and, according to Huiskamp, a nuclein but not a nucleic acid as was maintained by Bang.

In 1903–4 Bang (6) reported an extensive study of the whole problem. He summarized the results to this point as follows: *Huiskamp* claimed that thymus contains and yields to water, a

nucleoprotein free from histon and a *nucleohiston*. *Malengrau* claimed that thymus contains and yields to water, *A-nucleoalbumin and B-nucleoalbumin*, both containing histon and the latter comparable to Bang's complex. *Bang* claimed that thymus contains a *nucleoprotein*, free from histon and extractable by water or 0.9 per cent. sodium chlorid, and a *histon nucleate*.

Commenting generally on these views, Bang held that the thymus gland yields either a multitude of nuclein-containing substances or the nucleoprotein is changed by the various processes involved in its separation, or the different methods of preparation produce mixtures of pure and impure substances. Bang therefore considered it necessary to reinvestigate all methods. In brief his conclusions are: Malengrau's A-nucleoalbumin is an *albuminate*, not a histon. It is identical with Bang and Huiskamp's *nucleoprotein*. Huiskamp's nucleoprotein is identical with Bang's according to the following percentage analytic data:

Analysts	C, per cent.	H, per cent.	N, per cent.	P, per cent.	Ash, per cent.
Huiskamp.....	50.09	7.18	16.11	0.97	3.11
Bang.....	49.5	6.35	16.51	1.22	2.36
Bang (2d).....	——	——	15.89	0.91	2.18

The essential difference, therefore, lies between Bang's histon nucleate theory and Huiskamp's nucleohiston theory. Bang reported extensive studies to confirm his point of view. For comment on these views see p. 436.

V. EXPERIMENTAL

1. **The cause of the water-insolubility of ammonia-precipitated histon.** A. COMPARISON OF AMMONIA AND SODIUM CHLORID-PRECIPITATED HISTON. Many preparations of thymus histon were made involving variations in the process and the use of many different sets of glands from calves. All the glands used were, with one exception, obtained immediately after slaughtering and were extracted as soon as they could be brought to the laboratory.

The methods of preparation embraced the following variations:

Precipitation [after Lilienfeld (19)] of water extracts with

acetic acid and extraction of the purified precipitates with 0.8 per cent. hydrochloric acid solution.

Precipitation (modification of Lilienfeld's process) of water extracts with a few drops of conc. hydrochloric acid solution and extraction of the purified precipitates with 0.8 per cent. hydrochloric acid solution.

Acidification of water extracts [after Kossel and Kutscher (16)] with hydrochloric acid, to 0.8 per cent. strength, and filtering.

Precipitation of water extracts with alcohol and extraction of the washed precipitates with 0.8 per cent. hydrochloric acid solution.

Precipitation of water extracts [after Huiskamp (10)] with calcium chlorid, to 0.2 per cent. strength, and extraction of the purified calcium chlorid-precipitates with 0.8 per cent. hydrochloric acid solution.

In every case the ammonia-precipitated product from the histon hydrochlorid solution was insoluble in water, and addition of ammonia to the acid solution gave a precipitate which differed in no respects from that obtained by first removing *free* acid by dialysis and then precipitating with ammonia. The best precipitation results were obtained by first carefully neutralising the acid solution with ammonia and then adding the excess a few drops at a time, with stirring. It was demonstrated that ammonia-precipitation takes place in the absence of salts (contrary to Bang's contention), but care must be taken to add the ammonia slowly and in small amounts.

The insolubility of the ammonia-precipitated product was shown to be due neither to the action of the alcohol or ether used in washing the product, nor to abnormalities in the glands used. It was found to increase with the length of the period in distilled water. Precipitates allowed to stand for several weeks in distilled water, with toluene, failed to dissolve or putrefy and ultimately became completely insoluble in 0.8 per cent. hydrochloric acid solution.

In contrast with these results, the histon obtained by saturating a histon-hydrochlorid solution with sodium chlorid was invariably soluble in water. This solubility was not altered by washing with alcohol and ether, by drying at 45°, or even when the product was dried to constant weight at 105° C.; the sodium chlorid-saturation products from both *acid* and *neutral* histon-hydrochlorid solutions

were the same in this respect. Furthermore the precipitate obtained with sodium chlorid was always readily soluble in 0.8 per cent. hydrochloric acid solution.

The above mentioned results suggested different constitutions for histons precipitated by sodium chlorid and by ammonia. To determine this point pure preparations of each kind were made and analysed.

B. Methods of preparation and purification. Histon hydrochlorid was obtained by the Kossel-Kutscher (16) method. A portion of the clear bluish filtrate containing it was saturated with sodium chlorid and filtered, and the resulting precipitate washed with alcohol, dissolved in water and the solution dialyzed in a parchment bag for several days against running water. At the end of that time the bag was transferred to a tall jar of distilled water and the latter renewed daily until no trace of chlorin could be detected in the histon solution with silver nitrate. The solution was then removed from the bag, filtered and evaporated in thin layers to dryness at 45° C. Light yellow flakes were obtained. (During the dialysis and evaporation toluene was used as a preservative.) The flakes were ground to a white powder and dried to constant weight —first *in vacuo* over sulfuric acid and finally at 105° C. A *second* portion of the histon-hydrochlorid solution was treated with ammonia and the resulting precipitate washed free from all traces of ammonia with water. It was then washed with alcohol and ether, and dried to constant weight at 105° C. A *third* histon product was obtained by adding a few drops of ammonium hydroxid solution to a portion of the chlorin-free solution of the sodium chlorid-precipitate. The precipitate thus obtained was insoluble in water. It was washed with water, alcohol and ether, and dried to constant weight at 105° C. These three preparations[1] were used in the quantitative studies.

In the tabulation on page 434 these preparations are designated as follows: (a) sodium chlorid-precipitated histon, free from chlorid admixture; (b) sodium chlorid-precipitated histon, reprecipitated with ammonia; (c) ammonia-precipitated histon.

[1] Inability to find a solvent for ammonia-precipitated histon which would not hydrolyze it made it impossible to attempt the preparation of a sodium chlorid-saturation precipitate of the ammonia-histon.

C. Quantitative procedure. The methods employed in the quantitative analysis were the following: Total nitrogen was determined by the Kjeldahl process. For the chlorin determination 0.15 gm. of the material was placed in a casserole with 100 c.c. of water, 30 c.c. of nitric acid solution and 10 c.c. of $n/20$ silver nitrate solution (containing 1.52 mg. of chlorin per c.c.). The liquid was boiled gently for two hours to effect complete decomposition. The solution remained pale yellow, with silver chlorid at the bottom of the casserole. After cooling, the liquid was filtered and the silver chlorid thoroughly washed. To the filtrate and washings, 1 c.c. of ferric alum solution was added, the mixture decolorized with nitric acid, and titrated with $n/20$ potassium sulfocyanate solution of such strength that 1 c.c. equalled 1 c.c. of silver nitrate solution. The ash was obtained by cautiously incinerating 0.2 gm. of the dry sample in a small porcelain crucible until all carbonaceous matter disappeared. Six hours was usually sufficient. The crucible was then cooled in a desiccator and weighed. The moisture was determined by heating 0.5 gm. of the sample in a weighing bottle at 105° C. for 24 hours. All samples had been previously dried at 45° C. and powdered. The amide nitrogen, diamino nitrogen, monamino nitrogen and humin nitrogen were determined by the Osborne-Harris method.[1]

D. Quantitative results. The quantitative data in these experiments are summarized in Tables 6 and 7.

E. Discussion of the quantitative results. It will be seen that the lower nitrogen content of the sodium chlorid-precipitated histon is not accounted for by the ash difference (sodium chlorid-product averaged 1.33 per cent. and the ammonia-precipitated product, 0.76 per cent). This slight difference, combined with the observation that ammonia separates from an aqueous solution of the sodium chlorid-precipitated product, a water-insoluble mass of higher nitrogen content with a filtrate that responds to the biuret test, suggests the presence (in the salt-precipitated product) of a protein fraction which is absent from the ammonia-precipitated product and contains relatively little nitrogen. The differences between the monamino and diamino fractions tend to confirm this

[1] Osborne, T. B., and Harris, J. F., *Jour. of the Amer. Chem. Soc.*, 1903, xxv, p. 323.

view. The data for the amide fractions also tend to show that the difference in nitrogen content cannot be due to combination of the ammonia nitrogen with the ammonia-precipitated histon.

TABLE 6

Data pertaining to percentage elementary composition

Preparation	Histon A. Sodium chlorid-precipitated product, free from chlorid admixture			Histon B. Sodium chlorid-precipitated product, reprecipitated with ammonia			Histon C. Ammonia-precipitated product		
	N,[1] per cent.	Cl, per cent.	Ash, per cent.	N,[1] per cent.	Cl, per cent.	Ash, per cent.	N,[1] per cent.	Cl, per cent.	Ash, per cent.
1	16.05	0.52	—	16.85	0.00	—	17.47	0.00	—
	15.87	0.56	—	16.84	—	—	17.61	0.00	—
2	15.96	0.62	1.46	16.86	0.00	0.78	17.37	—	0.84
	16.09	0.52	1.20	16.70	0.00	0.88	17.29	—	0.72
3	16.52	—	—	16.75	—	—	17.20	—	—
	16.47	—	—	16.86	—	—	17.16	—	—

TABLE 7

Data in duplicate pertaining to nitrogen partition

Preparation	Histon A		Histon B		Histon C	
	1	1	1	2	1	2
Material taken (gm.)..	0.8035	0.6742	0.6111	0.4995	0.5112	0.4327
	%	%	%	%	%	%
Amide N.....	1.04	1.30	1.01	0.70	0.99	1.02
Humin N.....	1.03	0.91	1.03	1.12	1.18	0.99
Diamino N.....	13.14	12.28	13.46	13.54	13.61	14.17
Monamino N.....	0.87	1.03	1.19	2.01	1.42	1.19
Total.....	16.08	15.52	16.69	17.37	17.20	17.37

Aside from the difference in nitrogen content the only other striking difference shown by these results is the presence of chlorin in the sodium chlorid-precipitated product and its absence from the ammonia-precipitated substance. That this is not due to failure to sufficiently purify the sodium chlorid-precipitated material was shown qualitatively in the following experiments:

A portion of the water-solution of the sodium chlorid-precipitated product was treated with a few drops of ammonium hydroxid.

[1] Nitrogen calculations on an ash-free basis—Histon A, preparation 2: 16.20 per cent. and 16.28 per cent.; Histon B, preparation 2: 17.00 per cent. and 16.88 per cent.; Histon C, preparation 2: 17.52 per cent. and 17.41 per cent.

The clear filtrate from the resultant precipitate, after acidification with nitric acid, gave a good chlorin test with silver nitrate. It therefore seems correct to assume that ammonia actually separates chlorin from the sodium chlorid-precipitated substance and that chlorin is present in the latter histon product in an adsorbed or combined condition, not as an impurity.

A further investigation of the validity of these conclusions is in progress.

F. Conclusions. The results of this work justify the following conclusions: Both sodium chlorid and ammonia precipitate, from histon-hydrochlorid solutions, products having characteristic histon properties but differing in water-solubility, and in nitrogen and chlorin contents. The filtrates from both the ammonia- and sodium chlorid-precipitates give strong biuret reactions and are precipitable by alcohol-ether and saturation with ammonium sulfate.

The latter precipitates are not identical in properties and, while parahiston is possibly admixed with them, they also contain fractions of other protein matter which makes them different from para-histon and from one another. At present the nature of this protein admixture, and hence the exact nature of the difference between sodium chlorid- and ammonia-precipitated histons, is still uncertain.

2. **An improvement in the method of preparing thymus histon.** In following out the various methods of preparing the hydrochlorid solution of thymus histon it was difficult to obtain a water extract of the glands which would filter clear. The glands are also particularly prone to putrefaction at the beginning of the extraction process. The latter difficulty was overcome by conducting the extractions in a refrigerator rather than by depending upon the use of preservatives such as chloroform and toluene. Both difficulties can however be avoided by the following modified method which is recommended as more practical than any of those outlined in the general literature.

After freeing the glands from fat with a knife, pass them through an ordinary meat grinder and pour the hash directly into a comparatively large volume of 95 per cent. alcohol. All the protein material is precipitated and all chance of putrefaction is thus

avoided. Filter off the alcohol and extract the precipitated material directly with 0.8 per cent. hydrochloric acid solution. A clear, bluish white, readily filterable, extract will result. Extraction with hydrochloric acid solution should be continued for several days. In our experiments it was found necessary to repeat the extraction with hydrochloric acid solution several times to obtain all the histon. Preliminary extraction of the glands with alcohol removes none of the histon (as was determined by study of the extract) and, by precipitating all the proteins in the glands, the alcohol prevents the presence of protein impurities in the acid extract. The histon can then be precipitated from the acid extract by whatever method is desired, as already outlined.

3. **Proteins left after removal of histon from thymus extract.** Some experiments were conducted to determine if possible the merits of the contentions of Lilienfeld, Huiskamp and Bang as to the nature of the histon complex in the cell. These views were outlined in detail on page 428. The procedure was as follows: Thymus glands were minced and extracted with water for 48 hours in a refrigerator. The extract was decanted, filtered, precipitated with a few drops of conc. hydrochloric acid solution and the supernatant fluid treated with hydrochloric acid until a strength of 0.8 per cent. was present; after standing for several days, the acid solution was filtered off and the precipitate extracted with 0.8 per cent. hydrochloric acid solution. This process was repeated until the hydrochloric acid extract failed to give an ammonia-precipitate or a biuret reaction. The histon-free *residue* was then washed with alcohol until it was free from acid, treated with 0.3 per cent. potassium hydroxid solution, in which it dissolved slowly but completely, and the solution filtered, placed in a parchment bag and dialysed free from hydroxyl ions. No precipitate resulted, the solution remaining clear. (Toluene was used as a preservative during this process.) To the neutral solution 10 per cent. calcium chlorid solution was now added (2 c.c. of 10 per cent. calcium chlorid solution to each 100 c.c. of liquid). A copious precipitate resulted, the filtrate from this mass giving a good biuret test. Addition of more calcium chlorid to the filtrate failed to produce further precipitation.

The *precipitate* was next completely dissolved in water with the addition of a little 10 per cent. potassium hydroxid solution and the liquid again dialyzed free from hydroxyl ions in a parchment bag. This neutral solution was then reprecipitated with calcium chlorid as before, yielding again a sharply separating precipitate and a clear filtrate which gave neither biuret reaction nor alcohol precipitate. The precipitate was then washed with alcohol, and ether, and dried to constant weight at 105° C. A solution in dilute potassium hydroxid solution, dialysed free from hydroxyl ions, gave characteristic protein tests. The dried product was then analysed for nitrogen, ash and calcium.

The *filtrate* from the calcium chlorid-precipitated product was treated with a few drops of conc. hydrochloric acid solution, yielding a copious precipitate and a water-clear filtrate, which gave no biuret test or alcohol precipitate. The precipitate was purified by solution in 0.3 per cent. potassium hydroxid solution and reprecipitation with hydrochloric acid; washing with water, alcohol, and ether, and finally drying to constant weight at 105° C. The dry product was analyzed for total nitrogen and ash. Both precipitates were rich in phosphorus.

	A. Product precipitated with calcium chlorid, Per Cent.	B. Product precipitated with hydrochloric acid in the filtrate from A Per Cent.
Total N	7.65	15.97
Ash	7.30	5.96
Calcium	None	Not determined

These results agree with Huiskamp's views in certain respects. Assuming that water extracts both a nucleoprotein and a nucleohiston, treatment of the product with hydrochloric acid may be assumed to separate the histon from the nucleohiston, and leave a mixed residue of histon-free nucleoprotein and a nuclein. Both of these substances dissolve in 0.3 per cent. potassium hydroxid solution, but the latter is precipitable by calcium chlorid while the former is not. Furthermore, the view that calcium chlorid precipitates a nuclein rather than nucleic acid is borne out by the protein reactions of the product precipitated by calcium chlorid. Huiskamp, however, claims that his nuclein forms a calcium salt with

calcium chlorid and comes down as calcium-nucleinate. In our analyses we were unable to detect even a trace of calcium in the ash. It is possible, of course, that a nucleic acid fraction was separated from the histon product by the acid treatment, which, in the presence of other protein, may have formed a protein-nucleate precipitable by calcium chlorid. It might be true therefore, as Bang (6) claims, that histon exists in the cell as a histon-nucleate. The failure to obtain any nucleic acid in these tests, and the different nitrogen figures for the product precipitated with calcium chlorid, together with the agreement between the values for nitrogen content of the nucleoprotein and those obtained by both Bang and Huiskamp, suggest that histon occurs in cells as a true nucleohiston and not as a nucleate.

VI. SUMMARY OF CONCLUSIONS

These studies seem to show that histon obtained from aqueous extract of thymus gland by precipitation with ammonia is essentially different from histon obtained by saturation with sodium chlorid. The histon precipitated by ammonia is insoluble in water and has a higher nitrogen content than the histon precipitated with sodium chlorid. The latter product is readily soluble in water, even after drying at 105° C., and contains an appreciable amount of chlorin in combined form. The difference relates apparently both to the content of chlorin and to the presence of a protein fraction in the salt-precipitated histon which is absent from that obtained with ammonia.

The preliminary use of alcohol to precipitate histon and other protein materials in the glands seems to offer marked advantages over the direct water-extraction method, both in obviating putrefaction and in facilitating filtration of the aqueous extract.

Bang's contention that ammonia does not precipitate histon in the absence of salts is incorrect. It is true, however, that their presence facilitates the process.

The data pertaining to the residues insoluble in 0.8 per cent. hydrochloric acid solution seem to confirm Huiskamp's views rather than those of Bang, but in view of the power of nucleic acid to combine with protein and the absence of calcium from the ash of the calcium chlorid-precipitated fraction, the matter cannot be regarded as settled.

Histon prepared by the salt-precipitation method offers excellent material for the study of protein-salt formation.

The necessity of knowing the method of preparation of a given histon product, in order to understand the properties ascribed to it, is obvious from these studies.

The quantitative data on page 434 were obtained with the assistance of Mr. E. G. Griffin and those on page 437 with the aid of Mr. W. F. Hume, to whom I am greatly indebted for the effective coöperation they have given. I wish also to acknowledge my indebtedness to Dr. Gies for facilities and suggestions throughout the entire research.

VII. SELECTED BIBLIOGRAPHY OF HISTON STUDIES

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DID VON WITTICH ANTEDATE OSTWALD IN THE DEFINITION OF ENZYME ACTION?

WILLIAM N. BERG

It seems to be the consensus of opinion that enzyme action was first properly understood and defined by Wilhelm Ostwald about 1893. That this is a reasonable inference is evident from the following typical quotations from the literature of the subject:

The word catalysis was introduced about eighty years ago by Berzelius. It grouped together phenomena that had up to that time remained unconnected. But while a great and increasing number of new catalytic phenomena were discovered in subsequent years, the concept itself remained vague until Ostwald introduced his well known definition based on the conception of velocity of chemical change—a conception which was born with modern chemical kinetics and was unknown to Berzelius. According to Ostwald, a catalyzer is a substance whose presence *hastens* a given chemical reaction, although the reaction would also take place in its absence. . . .[1]

Wir werden also Kontaktwirkungen der Katalysatoren und der Enzyme am besten unter die von Ostwald gegebene (1893) Definition bringen können: *'Katalyse ist die Beschleunigung eines langsam verlaufenden chemischen Vorganges durch die Gegenwart eines fremden Stoffes.'*[2]

Ein wirklicher Inhalt ist diesem Begriff erst von OSTWALD gegeben worden. Er bezeichnete als *Katalysator 'jeden Stoff, der, ohne im Endprodukt einer chemischen Reaktion zu erscheinen, ihre Geschwindigkeit verändert'* . . . (S. 16). Es ist das anzuerkennende Verdienst W. OSTWALDS, eine der indessen ausgebildeten Thermodynamik gerecht werdende Definition der Begriff Katalyse und Katalysator gegeben zu haben.[3]

[1] Rosanoff: "Outline of a theory of homogeneous catalysis," *Jour. Amer. Chem. Soc.*, 35, p. 173, 1913.

[2] Bredig: "Die Elemente der chemischen Kinetic, mit besonderer Berücksichtigung der Katalyse und der Fermentwirkung," *Ergebnisse d. Physiologie*, 1, p. 139, 1902.

[3] Oppenheimer: *Die Fermente und ihre Wirkungen*, 3 Aufl., p. 159; Leipzig, 1910.

Partly on account of their obscure places of publication, and partly on account of faulty references in the literature, Ostwald's original definitions were found with some difficulty:

Katalyse ist die Beschleunigung eines langsam verlaufenden chemischen Vorganges durch die Gegenwart eines fremden Stoffes. . . .[4]

Man nennt die Stoffe, welche solche Änderungen der Geschwindigkeit bewirken, *Katalysatoren,* und zwar positive und negative, je nachdem sie Beschleunigungen oder Verzögerungen hervorbringen. Der Begriff der Katalysatoren hat erst in neuerer Zeit diese bestimmte Definition erfahren (Ostwald 1894). . . .[5]

Since Ostwald himself dates the definition of catalysis at 1894, it would naturally be assumed that that date is the correct one.

While engaged, about five years ago, in a study of enzyme action, the attention of the writer was drawn to a paper publisht by von Wittich,[6] not alone because of the ingenuity of the experimental work described therein, but especially because of von Wittich's striking conclusion (p. 469) regarding the nature of peptolytic action:

Es bleibt daher nichts anderes übrig, als anzunehmen, *dass die Säure allein hinreicht, um jene bekannte Umwandlung des Fibrins einzuleiten, dass aber die Gegenwart des Pepsins letztere wesentlich beschleunigt.*

The "Umwandlung" referred to is the transformation of fibrin into peptone.

von Wittich had observed that fibrin was slowly transformed into peptones by hydrochloric acid and that this transformation took place much more rapidly in the presence of pepsin. Other investigators had made the same observation, but without correctly understanding the rôle of the enzyme, as von Wittich apparently did.

These facts were mentioned about four years ago by the writer, who believed that *von Wittich had antedated Ostwald in the definition of catalysis.* Until recently, lack of opportunity prevented a sufficiently careful search of the literature on this particular point.

[4] Ostwald: *Ztschr. f. physikal. Chemie,* 15, p. 706, 1894.

[5] Ostwald: *Grundriss der allgemeinen Chemie,* 3 Aufl., p. 515; Leipzig, 1899.

[6] von Wittich: "Weitere Mittheilungen über Verdauungsfermente," *Archiv f. d. gesammte Physiologie,* 5, p. 435–469, 1872.

It was for this reason that the name of Ostwald was held in reserve when the following statement was made:

A rather early insight into the nature of peptolysis was given by von Wittich, who concluded that the pepsin simply accelerates a reaction which the acid alone will bring about more slowly.[7]

In his conclusions, von Wittich plainly states that the transformation of fibrin into peptone by pepsin-hydrochloric acid is a reaction similar to the transformation of alcohol into ether by sulfuric acid. This is evidence of the fact that when he defined peptolytic action, he did so not unknowingly, but with a full understanding of the facts. It is reasonable to suppose that von Wittich had a broad, general understanding of enzyme action as the basis for his definition, because he had previously publisht work on diastatic, proteolytic, glucosid-splitting and various other enzymes.[8] In one of these papers (1870, p. 352) he mentions the "katalytische Wirksamkeit" of a diastase in such a way as to leave no doubt that he understood enzyme action to be catalytic. Two years later (1872, p. 465) he stated his belief in the similarity between the action of pepsin in peptic digestion and the action of sulfuric acid in the transformation of alcohol into ether, in the following terms:

Der Vorgang erscheint mir dem ganz analog bei der Ueberführung des Alcohol durch Schwefelsäure in Aether. Wie sich hier zunächst Aethylschwefelsäure bildet, wie diese bei 140° C. auf ein zweites Molecul Alcohol wirkt und dasselbe in Aether und Wasser zerlegt, wie die sich bildenden Wasser und Aether überdestilliren und die Schwefelsäure in ungeschwächter Wirksamkeit zurücklassen, so dass sie immer neuzufliessenden Mengen Alcohol in denselben Verhältnissen in Aether und Wasser zu spalten vermag, so bildet sich bei der Pepsinwirkung zunächst dessen Verbindung mit der freien Säure, die ihrerseits durch Contact das Fibrin in jene leicht lösliche und diffusible Form die Peptone umwandelt. Wie aber bei allen Contactwirkungen die Mengen der durch sie gebildeten Stoffe von der Grösse der Contactfläche abhängt, so bedingt auch hier die letztere, d. h., die Menge des verwendeten Pepsins die Menge der entstehenden Peptone. Wie aber die

[7] Berg: "A comparative study of the digestibility of different proteins in pepsin-acid solutions," *Amer. Jour. of Physiology,* 23, p. 423, 1909.

[8] von Wittich: "Ueber eine neue Methode zur Darstellung künstlicher Verdauungsflüssigkeiten," *Archiv f. d. gesammte Physiologie,* 2, p. 193, 1869. Also, Weitere Mittheilungen über Verdauungsfermente, *Ibid.,* 3, p. 339, 1870.

Schwefelsäure keine dauernde Verbindung mit dem Aether eingeht, kein integrirender Theil des sich neubildenden Stoffes wird, wie sie unter günstigen Bedingungen sich von jenem wieder trennt, um immer neue Mengen Alcohols in gleicher Art zu verändern, so geht auch bei dem Verdauungsvorgang weder die Salzsäure noch das Pepsin in eine bleibende Verbindung mit der Peptone ein und entfernt man letztere, so vermag dieselbe Menge der Säure dieselbe Menge Pepsin, immer neues Fibrin in ähnlicher Weise zu zerlegen. Man kann die Peptone von dem Pepsin und den noch vorhandenen Parapeptonen scheiden, . . .

On the third page after this paragraph, von Wittich concludes his paper (1872, p. 469)—full of interesting and suggestive experiments—with the statement:

Es bleibt daher nichts anderes übrig, als anzunehmen, *dass die Säure allein hinreicht, um jene bekannte Umwandlung des Fibrins einzuleiten, dass aber die Gegenwart des Pepsins letztere wesentlich beschleunigt.*

In view of the fact that von Wittich was probably the first to correctly define enzyme action, it seems strange that his works should have received so little attention. Several of his publications are indext and very briefly discust in Oppenheimer's[9] book, but the important points in the above quotations from von Wittich are not mentioned. Cohnheim[10] and Höber[11] both mention von Wittich once, in a few words, the former to the effect that von Wittich was one of the early investigators who prepared glycerol extracts of gastric mucosa, etc.; the latter mentioned von Wittich's name on a subject other than enzyme action! In short, a reference to the fact that von Wittich defined enzyme action in 1872, twenty or more years before Ostwald, could not be found, altho it was looked for in many places besides those mentioned above.

von Wittich was probably the first to show that when fibrin is immersed in pepsinogen-glycerin solutions (1872, p. 443), or in pepsin-hydrochloric acid solutions (1872, p. 444), the enzyme is rapidly adsorbed by the fibrin. He used the term pepsin for both pepsinogen and pepsin.

He also showed, *most ingeniously,* that while pepsinogen would not diffuse from a glycerol extract thru a Graham dialyzer into

[9] Oppenheimer: *Loc. cit.* [10] Cohnheim: *Enzymes,* p. 2, New York, 1912.
[11] Höber: *Physikalische Chemie der Zelle und der Gewebe,* 3 Aufl., p. 537, Leipzig, 1911.

water, it would pass thru, *if fibrin were placed in the water.* He explains this as follows (1872, p. 443):

Der Vorgang erklärt sich, wie ich glaube, durch die Annahme, *dass das Fibrin das Pepsin sehr energisch absorbirt, dass auch beim Fehlen jenes minimale Mengen diffundiren, die Diffusion aber durch die Absorptionsfähigkeit des Fibrins beschleunigt und verstärkt wird.*

It is quite possible that the principle of this experiment might have a wide application, *i. e.*, that the diffusibility of many substances (now regarded as indiffusible) might be greatly increased by placing material in the diffusion medium which was not soluble in the medium but which could combine with the substance whose diffusibility was under investigation.

The above mentioned observations by von Wittich, on the diffusibility and absorption of pepsin by fibrin, have recently been used by several investigators. Beginning with the work of Abderhalden and Steinbeck,[12] Abderhalden and his co-workers have publisht a long series of researches on the adsorption of enzymes by proteins. In none of these publications that the writer has seen is there any allusion to the fact that the principle involved was not new. The name of von Wittich was not mentioned. From their papers one might justly infer that Abderhalden and Steinbeck believed that they had discovered the adsorption of pepsin by proteins, when they were, in fact, using a principle discovered in 1872 by von Wittich. To a lesser extent this criticism applies to Hedin,[13] in whose several publications von Wittich is not mentioned, altho many of the experiments and conclusions of Abderhalden and his co-workers, and of Hedin, can be found in von Wittich's paper.

In a very interesting manner Chodschajew[14] discusses the work of von Wittich on the diffusibility of pepsin, some of which Chodschajew repeated. Dauwe[15] mentions von Wittich as the first to observe the adsorption of pepsin by fibrin.

Washington, D. C.

[12] Abderhalden and Steinbeck: "Beitrag zur Kenntnis des Pepsins und der Salzsäure," *Ztschr. f. physiolog. Chemie,* **68**, p. 293, 1910.

[13] Hedin: "Observations on the action of trypsin," *Jour. of Physiol.,* **32**, p. 468, 1905; *Biochemical Journal,* **2**, p. 81, 1907.

[14] Chodschajew: "Les enzymes sont-elles dialysables?" *Archives de physiologie normale et pathologique,* 1898, p. 241.

[15] Dauwe: "Ueber die Absorption der Fermente durch Kolloide," *Beiträge zur chemischen Physiologie und Pathologie,* **6**, p. 427, 1905.

THE BIOCHEMICAL SOCIETY, ENGLAND

SCIENTIFIC PROCEEDINGS

RESEARCH INSTITUTE OF THE CANCER HOSPITAL, BROMPTON ROAD, LONDON, S. W., *February 5*, 1913, at 5 p. m.—*S. B. Schryver:* Notes on bile acids; Investigations on phenomena of clot formations: (a) clotting of calcium cholate, (b) clotting of milk.—*G. Barger and A. J. Ewins:* The identity of trimethylhistidine (*histidine betaine*) from various sources.

CHEMICAL DEPARTMENT OF THE LONDON HOSPITAL MEDICAL COLLEGE, *March 13*, 1913, at 5 p. m.—*W. H. Hurtley:* The old and a new test for aceto-acetic acid.—*J. H. Ryffel:* A sensitive modification of Gmelin's test for bile pigment in urine.—*W. M. Bayliss:* The combination of amino acids with neutral salts.—*R. H. A. Plimmer:* The separation of cystine and tyrosine.

PHYSIOLOGICAL LABORATORY, CAMBRIDGE, *May 10*, 1913, at 4 p. m.—*C. Singer and S. B. Schryver:* Some investigations on the gastric juice.—*E. Graf von Schönbein and S. B. Schryver:* Some properties of the bile salts.—*S. Walpole:* On the use of litmus paper as a quantitative indicator of reaction.—*C. Funk:* A complete chemical and physiological investigation of the vitamines from rice polishings and yeast.—*F. W. Foreman:* Esterification of amino acids from proteins.—*A. Neville:* The fatty acids of yeast.—*A. Neville and E. T. Halman:* Feeding experiments with Bastol.—*C. G. L. Wolf:* A note on the estimation of lactic acid.

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Hurtley, F. Keeble, B. Moore, W. Ramsden, E. J. Russell, J. Lorrain Smith and T. B. Wood.

PROVISIONAL SCHEDULE OF MEETINGS, 1913–14

May 10—Physiological Laboratory, Cambridge.

June 11—Institute of Physiology, University College, London.

July 12—Rothamsted Experimental Station, Harpenden.

Oct. 10—Pathological Department, St Thomas's Hospital, London.

Nov. 13—Physiological Laboratory, King's College, London.

Dec. 9—Lister Institute, London.

Feb. 11.—Guy's Hospital, London.

March 12—Botany Department, Imperial College of Science, London, S. W.

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[2] A copy of the official register published after the "annual general meeting" on March 13, 1913.

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SCIENTIFIC MEETINGS OF THE COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION, AT THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK*

PROCEEDINGS REPORTED BY THE SECRETARY,

ALFRED P. LOTHROP

I. NINTH MEETING

The *ninth scientific session* of the Columbia University Biochemical Association was held at the Columbia Medical School, at 4:15 p. m., on February 7, 1913.[1] Abstracts of the papers are here presented (pages 453–461) in two groups: (*A*) Abstracts of the papers on research by non-resident members[2] and (*B*) abstracts of papers from the Columbia Biochemical Department and affiliated laboratories. The appended summary facilitates reference to the abstracts (63–72).[3]

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS (63–72)

A

JACOB J. BRONFENBRENNER AND W. H. MANWARING. Resistance in tuberculosis. (63)

BURRILL B. CROHN. The enzymic power of duodenal contents as a means of diagnosis of the functional activity of the pancreas. (64)

K. GEORGE FALK (by invitation). The occurrence of a urease in castor bean. (65)

K. GEORGE FALK AND MARSTON L. HAMLIN. The action of manganous sulfate on castor-bean lipase. (66)

E. NEWTON HARVEY. The temperature limits of phosphorescence of luminous bacteria. (67)

A. HYMANSON (by invitation). Metabolism studies of amaurotic family idiocy. (68)

MAX MORSE. A micro-Kjeldahl apparatus. (69)

* Scientific meetings are held regularly on the first Fridays of December, February and April, and on the first Monday in June.

[1] Proceedings of the eighth meeting were published in the last number of the BIOCHEMICAL BULLETIN, 1913, ii, p. 284.

[2] Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the research was conducted.

[3] For abstracts 1–44 see BIOCHEMICAL BULLETIN, 1912, ii, p. 156; for abstracts 45–62, *Ibid.*, 1913, ii, p. 285. See also page 462.

B

MAX KAHN. The calcium content of tuberculous areas in lung tissue. (70)

CHARLES C. LIEB. On the localization of the convulsive action of potassium sulfocyanate. (71)

R. OTTENBERG, D. J. KALISKI AND S. S. FRIEDMAN. Experimental agglutinative and hemolytic transfusions. (72)

A. ABSTRACTS OF PAPERS ON RESEARCH BY NON-RESIDENT MEMBERS[4]

63. Resistance in tuberculosis. JACOB J. BRONFENBRENNER and W. H. MANWARING. (*Rockefeller Institute for Medical Research, New York City.*) Tubercle bacilli, injected into the peritoneal cavities of tuberculous guinea-pigs, occasionally degenerate and develop into the non-acid resistant forms described by Deycke and Much,[5] and others. Under certain conditions, there may be a complete disappearance of the bacilli from the peritoneal fluids within as short a period of time as three hours.

Whether this disappearance is due to an actual lysis of the tubercle bacilli, or to other causes, we have not yet determined. As evidence in favor of lysis we have observed that all of the normal control guinea pigs, injected intraperitoneally with the test suspensions of tubercle bacilli, died from a fulminating type of visceral tuberculosis, within a period of from three to four weeks, while most of the tuberculous guinea pigs, receiving the same test doses, have survived for longer periods of time A few of these tuberculous guinea pigs, however, have died within twenty-four hours after the intraperitoneal tests, suggesting an anaphylactic reaction. We have obtained a similar rapid disappearance of tubercle bacilli from the peritoneal cavities of tuberculous rabbits, from tuberculous rats, and from tuberculous dogs.

The question now arose as to the mechanism of this heightened peritoneal resistance. From the similarity between this phenomenon and the Pfeiffer reaction attempts were made to determine whether or not the specific antibodies, upon which the intraperitoneal

[4] Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the research was conducted.

[5] Deycke and Much: *Beitrag z. Klinik f. Tuberk.*, 1910, xv, p. 277; Much and Leschre: *Ibid.*, 1911, xx, p. 405; Kraus and Hofer: *Deutsch. med. Wochenschr.*, 1912, xxxviii, p. 1227; *Wiener klin. Wochenschr.*, 1912, xxv, p. 1112.

lysis may be supposed to depend, are present in the circulating fluids.

To test this, guinea-pigs, rabbits and dogs were made tuberculous by inoculating them subcutaneously with tubercle bacilli. After intervals of from five to eight weeks, the animals were bled and their blood tested *in vitro* and *in vivo*. In a number of these experiments direct transfusion of the blood was made from the tuberculous animals into normal animals, an amount of blood often as great as three quarters of the total blood-volume being thus passed into the circulating system of the normal animals, the normal animals having been previously bled to free them as much as possible from normal blood. The transfused animals were subsequently tested by intraperitoneal injections of tubercle bacilli.

Neither in the test-tube experiments, nor in normal animals injected subcutaneously, intravenously or intraperitoneally with tuberculous serum, nor even in normal animals directly transfused with large quantities of the unaltered blood of tuberculous animals, has the reaction thus far been obtained. Therefore, the substances responsible for the heightened peritoneal resistance do not, apparently, exist in appreciable quantities as circulating antibodies, at least at the stage of the disease studied. The heightened tuberculous resistance is apparently due to substances held in fixed tissue cells.

Evidences of tuberculolytic substances have, however, been obtained in the peritoneal fluids of tuberculous guinea-pigs, soon after the introduction of tubercle bacilli. If these fluids are withdrawn, centrifuged free from form elements and then introduced into the peritoneal cavaties of normal guinea-pigs, they confer upon the normal peritoneal cavities a slight power of destroying tubercle bacilli. It is suggested, therefore, that fixed tuberculolysins are set free by the peritoneal cells in response to the presence of tubercle bacilli, and that these lysins account for the heightened resistance to intraperitoneal reinoculation with tubercle bacilli.

64. The enzymic power of duodenal contents as a means of diagnosis of the functional activity of the pancreas. BURRILL B. CROHN. (*Pathological Laboratory, Mt. Sinai Hospital, New York City.*) The Einhorn duodenal pump was used, in these studies, to obtain duodenal material. This instrument consists of a small acorn-shaped metallic capsule (perforated), to which is

attached a thin rubber tube, 80 cm. long. The capsule and attached tube are swallowed at night to the point marked 80 cm. In the morning 200 c.c. of milk are drunk by the patient and two hours later the contents of the duodenum are aspirated. During the night the capsule is propelled through the pylorus by peristalsis. The milk acts as a test meal in stimulating pancreatic secretion.

The material obtained is tested quantitatively for the strength of its *pancreatic* enzymes. The methods employed are: For *amylase*, a modification of the Wolgemuth starch test; *lipase*, the ethyl butyrate test; *trypsin*, the Fuld-Gross casein test and tests with Fermi gelatin tubes, Mett tubes and coagulated egg albumen cubes.

Normal values for the strength of the pancreatic enzymes in the duodenum were first noted in repeated tests of a normal adult. Figures were then obtained similarly in pathological cases of interest. The following results were recorded: *Acute pancreatitis*, marked deficiency of the enzymes; *chronic pancreatitis*, partial interference with the strength of the enzymes; *diabetes mellitus*, increased strength of enzymes; *hypertrophic cirrhosis of the liver*, hypersecretion of the pancreas, enzymes very active; *gastric diseases*, enzymes normal; *achylia gastrica*, enzymes normal. In this latter group of cases it was impossible to demonstrate the occurrence of a milk-coagulating enzyme, the conclusion being that also the normal pancreas does not contain rennin or any other milk-coagulating enzyme. Enzymes were absent in cases of *tumor of the head of the pancreas but present in stone impacted in the diverticulum of Vater.*

65. The occurrence of a urease in castor bean.[6] K. GEORGE FALK (*by invitation*). (*Harriman Research Laboratory, Roosevelt Hospital, New York.*) A castor bean preparation, husk and oil-free, when allowed to stand in an aqueous solution of urea, caused the formation of ammonia, as shown by the distillation of the ammonia in a current of air at the ordinary temperature. A castor bean preparation, heated with water and then similarly treated, formed no ammonia.

66. The action of manganous sulfate on castor-bean lipase.[7] K. GEORGE FALK AND MARSTON L. HAMLIN. (*Harriman Re-*

[6] Falk: *Journal of the American Chemical Society*, 1913, xxxv, p. 292.
[7] Falk and Hamlin: *Ibid.*, 1913, xxxv, p. 210.

search Laboratory, Roosevelt Hospital, New York.) The hydrolytic action of castor bean lipase on ethyl butyrate was decreased in aqueous suspension and entirely inhibited by heating the aqueous suspension one to two hours in a water bath. Suspensions that had been partially deactivated by standing showed an increase in lipolytic power when allowed to act in the presence of small amounts of manganous sulfate; those that had been completely deactivated by heating showed a slight but consistent activity in the presence of manganous sulfate, and the activity was still greater if, to the water suspension of lipase, manganous sulfate was added and the solution allowed to stand fifteen to twenty hours before testing.

These results suggest the tentative hypothesis that, in view of the common function of manganese as an oxygen carrier, the lipase in castor bean is formed from a zymogen, by oxidation aided by an oxygen carrier, with or without simultaneous hydrolysis.

67. The temperature limits of phosphorescence of luminous bacteria. E. NEWTON HARVEY. (*Physiological Laboratory, Princeton University, Princeton, N. J.*) Light production by many organisms has been observed at relatively high and low temperatures, above or below which we should not expect biochemical processes to continue. According to Panceri, *Phyllirrhöe* (a naked snail of the Mediterranean) is luminous at 75° C., while *Pyrosoma* (a pelagic ascidian) still glows at 60°. On the other hand tissues of the South American fire-fly, *Pyrophorus,* phosphoresce at —100° C. (Dubois) and *Pseudomonas javanica* (a protozoan) at —20° C. (Eijkmann).

The following temperatures have been recorded for luminous bacteria:

Organism	Observer	Minimum	Maximum
Bacterium phosphorescens.....	Lehmann	−12° C.	39.5° C.
Bacterium phosphoreum.....	Molisch	− 5°	28°
Light bacteria.....	Tarchanoff	− 7°	37° 50°

The temperatures recorded are not excessively high or low, yet the variations in the results suggest that further observations are desirable.

Luminous bacteria isolated from fish were grown on absorbent

cotton saturated with beef-broth-peptone-glycerol culture medium. Free access of air between the cotton fibers supplies the conditions for a brilliant light and at the same time an excellent means of handling the bacteria. A wisp of cotton strongly phosphorescent with bacteria was placed in a very thin walled glass tube about 2 mm. in diameter and attached to a thermometer bulb as for melting-point determinations. On slowly raising the temperature, the light (to a dark-accustomed eye) becomes dim at 30°, very dim at 34°, and disappears at 38°. On lowering the temperature the light weakens at 0°, is very dim at —7° and disappears at —11.5°. These values agree best with those given by Lehmann for *Bacterium phosphorescens* and do not greatly exceed the usual temperature limits of activity of organisms.

Bacteria raised to 38°, and then cooled, phosphoresce only very dimly, but, as first observed by Macfayden (an experiment which I have repeated), glow brilliantly at room temperature even after an exposure to liquid air.

68. Metabolism studies of amaurotic family idiocy. A. Hymanson (*by invitation*). (*Chemical Laboratory, Beth Israel Hospital, New York City.*) Two cases of amaurotic family idiocy were kept under observation until death. The metabolism of nitrogen, sulfur and phosphorus was carefully studied. It was found that both absorption and retention were normal or above normal. The digestive system does not seem to be at all deranged in this fatal disease.

69. A micro-Kjeldahl apparatus. Max Morse. (*Boardman Laboratories, Trinity College, Hartford, Conn.*) This is a combination of the apparatus designed by Fritz Pregl[8] [Plate 6] for total nitrogen determination in small quantities of material, and the fume absorber devised by Folin and Denis.[9] The apparatus may be equally serviceable with that devised by Sy.[10] With such a means of eliminating fumes, the determination of total nitrogen may be carried on wherever the water-pressure is sufficient to maintain an ordinary filter-pump in action; in conjunction with the

[8] Pregl: *Abderhalden's Handbuch der biochemischen Arbeitsmethoden*, 1912, v, p. 1344.

[9] Folin and Denis: *Journal of Biological Chemistry*, 1912, xi, p. 503.

[10] Sy: *Journal of Industrial and Engineering Chemistry*, 1912, iv, p. 680.

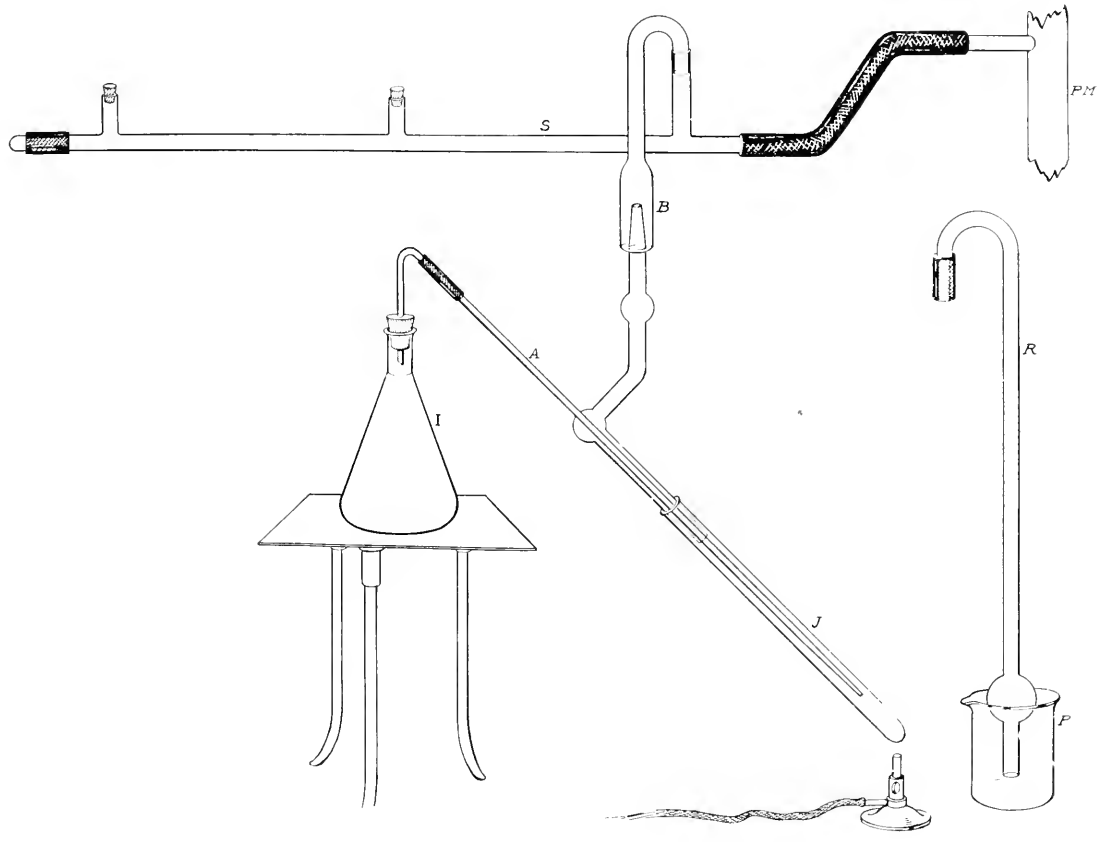
micro-amino-nitrogen apparatus of Van Slyke, it makes an admirable outfit for work with small amounts of protein.

During digestion, the tube with the bulbs is inserted into the absorber, *B*, and the fumes from the 200 mm. Jena test-tube, *J*, are carried to the sink through the pump, *PM*, via tube *S*, which may have a half dozen openings, so that a number of determinations may be conducted at the same time. Since much water vapor is lost during digestion, Pregl passes steam from a flask, *I*, to the bottom of the Jena test-tube, *J*, through the long small-bore tube, *A*, which is fused into the bulb through which it passes. It is quite necessary to pass steam in this way. Neutralization is effected with sodium hydroxid solution poured down tube *A, after the apparatus has cooled;* if the hydroxid is added immediately after digestion, tube *A* will frequently crack. After neutralization, the upper end of tube *A* is closed by a clamp or glass rod introduced into the rubber connection and distillation is effected by connecting tube *R*, which dips into the decinormal acid in the beaker *P*. This should be done, as a matter of fact, before neutralization, so that no ammonia is lost. In place of distilling by heat, Folin's method for urea may be introduced, by driving an air current through *A*, and the only precaution necessary is that the current be sufficiently strong to drive all of the gas into the distillation tube, *R*. The bulb at the lower end of the distillation tube, *R*, prevents back-suction, as sometimes occurs.

The Jena test-tube fits loosely the collar of the apparatus above it, and the water-vapor condenses sufficiently around it to insure an air-tight joint. There is variation in Jena test-tubes of 200 mm. length, but it is small, so that the apparatus fits practically any such tube. The apparatus is made by the Emil Greiner Company, New York City.

B. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEMICAL DEPARTMENT AND AFFILIATED LABORATORIES

70. The calcium content of tuberculous areas in lung tissue. MAX KAHN. Wherever the tubercle bacillus lodges it induces a deposition of calcium salts, which hinders the ingress of other bacilli. The body in general becomes poorer in lime salts. It was



MORSE: A MICRO-KJELDAHL APPARATUS

A combination of the apparatus designed by Pregl, and Folin and Denis.

found that tubercular areas in the lungs contained from two to three times as much calcium as normal lung tissue. The work is in progress.

71. **On the localization of the convulsive action of potassium sulfocyanate.**[11] CHARLES C. LIEB. Potassium sulphocyanate, injected into the anterior lymph sacs of frogs (*Rana pipiens*) in doses of 0.125 to 1.5 mg. per gram of frog, induces convulsions of the strychnine type. Larger amounts cause progressive depression and death without any apparent stimulation. The smaller doses cause first some depression so that the animal corresponds to the decerebrate frog. A little later the croak reflex is lost, and soon the frog is unable to right itself when placed on its back. The spinal reflexes are then depressed or may even be abolished; a few minutes later there is a return of these reflexes, which become more and more active until finally typical tetanic convulsions appear. These are usually of very short duration (two to ten seconds) and are succeeded by a period of relaxation, during which stimulation of skin, tendons, and joints is almost without effect. Then irritability returns and convulsions again appear. This cycle can be elicited repeatedly but eventually recovery from the exhaustion becomes less and less complete and finally all reflexes are lost. Pithing such an animal, or pricking its exposed cord, is usually without effect; *i. e.*, the cord is paralyzed. Direct electrical or mechanical stimulation of nerves and muscle shows that they are still active.

The convulsions are not due to any effect on the muscle or its nerve endings. If the sciatic nerve of a pithed frog be isolated and the rest of the leg ligated, and then potassium sulfocyanate injected below the ligature, no local or general convulsions develop. If at the end of an hour and a half the ligature be cut, typical tetanus appears within forty-five minutes. If one sciatic nerve of a pithed frog be isolated and the rest of the leg ligated *en masse*, and potassium sulfocyanate injected into the anterior lymph sac, then both legs participate in the convulsions. If, after the increased reflexes or convulsions appear, the sciatic be divided low in the

[11] Conducted in the Pharmacological Laboratory of Columbia University as one of a series of researches in collaboration with Drs. Gies and Kahn in this Laboratory under the auspices of the Dental Society of the State of New York. See BIOCHEMICAL BULLETIN, 1912, ii, p. 178.

thigh, the muscles supplied by the cut nerve do not take part in the subsequent convulsions. Successive destruction of the cerebrum, optic lobe, and medulla does not prevent the development of the convulsions nor does it modify them after they have appeared. Destruction of the cephalic half of the cord prevents the tetanus of the arms but does not affect the spasm of the legs. Total destruction of the cord permanently abolishes the convulsions.

A study of the afferent nerves shows that the stimulus must be fairly abrupt, since dilute acid may be applied without inducing any reaction while the application of strong acid is regularly followed by convulsions.

Cocainizing an area of the skin lessens the tendency to convulsions from irritation of that particular area. Provided the stimulation involves the skin only, no convulsion usually results. If the stimulus be a little stronger, so as to cause pressure on a tendon or movement of a joint, a convulsion ensues. If a sensory nerve be cut, and the area of skin supplied by that nerve be stimulated, no reflex or convulsion occurs. Stimulation of the central end of the divided nerve is followed by typical tetanus.

The convulsions, then, are of reflex origin, and apparently can be best explained by assuming that potassium sulfocyanate causes changes in the cord resembling those induced by strychnine. I have repeatedly attempted to perform experiments similar to those of Baglioni but have never succeeded in exposing the cord without destroying the reflexes.

72. **Experimental agglutinative and hemolytic transfusions.**[12] R. Ottenberg, D. J. Kaliski and S. S. Friedman. By a suitable technic, iso-agglutination and iso-hemolysis can be demonstrated to occur between the bloods of different dogs. Iso-agglutinins occur naturally, and it is possible that the immune iso-agglutinins produced by von Dungern and Hirschfeld are merely intensifications of these. No sharp grouping (such as would indicate a limited number of agglutinable substances and of agglutinins) could be made out, however, in the naturally-occurring agglutinins.

[12] Under the auspices of the George Crocker Special Research Fund. Some of this work was done in the Pathological Laboratory of Mt. Sinai Hospital. The authors wish to thank Dr. W. Thalheimer for his assistance with the histological part of the work. *Jour. of Med. Research* (in press).

Natural (as distinguished from immune) iso-agglutination is, however, a relatively weak phenomenon.

Iso-hemolysis and iso-agglutination are closely connected with each other in dogs, as Moss and others have shown them to be in human blood. In our observations hemolysis never occurred without agglutination. Apparently iso-hemolysins may be developed *de novo* by the repeated transfusion of agglutinable cells, but they are never developed by the transfusion of non-agglutinable cells.

Hemolysis in the body of a dog is far more intense than in the test-tube. (The authors have made the same observation in the case of a human transfusion which has not yet been published, and similar experimental observations have been made by Muir and M'Nee.)

The direct transfusion of blood whose red cells can be agglutinated and laked by the recipient's serum is followed by destruction of the transfused blood, with an intense intoxication. It is not yet clear whether agglutination plays any part in this result, or whether it is due entirely to hemolysis.

A very remarkable blood-picture, presenting many of the morphological forms peculiar to pernicious anemia, is produced when the blood of another animal of the same species is destroyed in the circulation. (Similar blood-pictures have been observed by Bunting and others to follow anemia produced by hemolytic poisons). In our experiments this was not due to anemia, as the animal's own blood was not destroyed, and there was no reason to believe they were anemic. The changes must have been due to some peculiar toxic effect, on the bone-marrow, of hemolytic blood destruction.

II. TENTH MEETING

The *tenth scientific meeting* of the Association was held at the Columbia Medical School, at 4.15 p. m., on April 4, 1913. The summary on page 462 facilitates reference to the abstracts (73–85) of the papers presented.

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS (73–85)

A. ABSTRACTS OF PAPERS ON RESEARCH BY NON-RESIDENT MEMBERS

73. On the resistance of various spirochetes in cultures to the action of chemcial and physical agents.[13] J. J. Bronfenbrenner and H. Noguchi. (*Rockefeller Institute for Medical Research, New York City.*) The toxic effect exerted by mercuric chlorid, arsenious oxid, trikresol, phenol, saponin, sodium taurocholate, sodium hydroxid, hydrochloric acid, gentian violet, alcohol and "606" is from twenty to one hundred times greater if tested upon spirochetes than it is against colon bacillus. The toxic effects of salvarsan are increased from two to five times, and possibly more,

[13] Bronfenbrenner and Noguchi: *Journal of Pharmacology and Experimental Therapeutics,* 1913, iv, p. 333.

in the presence of enzymes from the liver and especially from the blood. Spirochetes suspended in physiological salt solution are sterilized by a temperature of 45° C. in from seven to ten minutes.

74. Studies on the chemistry of embryonic growth. I. Certain changes in the nitrogen ratios of developing trout eggs.[14] Ross Aiken Gortner. (*Carnegie Institution of Washington; Biochemical Laboratory of the Station for Experimental Evolution, Cold Spring Harbor, L. I.*) The various nitrogen fractions were determined in fresh trout eggs and in trout eggs at 21 days, 35 days, 51 days and 72 days of development, using Van Slyke's method.

It was found that probably no nitrogen was either lost or gained by the egg up to the time of hatching. After hatching the loss proceeds rapidly, until, twenty-one days afterward, 21.96 per cent. of the total nitrogen in the egg has been eliminated. During seventy-two days of development the eggs lost 25.35 per cent. of their dry weight, 37.26 per cent. of this loss being due to non-protein (fats, etc.), and 62.73 per cent. to protein (N × 6.25). During the process of development the basic nitrogen increases at the expense of the mon-amino acid nitrogen.

There is selective utilization of the various nitrogen fractions by the developing fish, as is shown by the nature of the nitrogen that is lost. Only 25 per cent. of the expected amide nitrogen is eliminated, only 50 per cent. of the expected arginin-reacting nitrogen, only 75 per cent. of the expected lysin-reacting nitrogen, none of the cystin- or histidin-reacting nitrogen, only about one third of the expected basic nitrogen, while the deficit caused by the basic nitrogen is balanced by the elimination of mon-amino acid nitrogen far in excess of the expected quantity—83.30 per cent. of the total nitrogen (expected, 57.65 per cent.).

No appreciable amount of either urea or uric acid is formed in the eggs during development.

It seems probable that some of the energy of development (Entwicklungsarbeit) comes from the shifting of the nitrogen ratios as development proceeds. In the change from mon-amino acid nitrogen to basic nitrogen, the energy relations may be changed and heat liberated, but at present this is only a hypothesis.

[14] Gortner: *Journal of the American Chemical Society*, 1913, xxxv, p. 632.

75. A simple classroom experiment for demonstrating the production of acid by contracting muscle. E. N. HARVEY AND W. E. HOY. (*Physiological Laboratory, Princeton University.*) The experiment is based on the fact that ammonium hydroxid readily penetrates living tissues and hence may be used to neutralize the acid produced in muscle cells during functional activity. The skinned legs of a frog are stained in neutral red and one is electrically stimulated. The stimulated leg becames slightly more red but the difference is not readily detected by student eyes. Both legs are then placed in physiological salt solution containing $n/200$ ammonium hydroxid. The unstimulated muscles are immediately turned yellow by the ammonium hydroxid while the stimulated muscles retain their red color. The experiment can be performed in a very short time, the color change is striking and the reaction a delicate one. The acid produced by ten induced shocks may easily be detected under the proper conditions.

76. The relation of acapnia to shock, and a consideration of the mechanical effects of hyper-artificial respiration upon the circulation. HENRY H. JANEWAY AND EPHRAIM M. EWING. (*Laboratories of Experimental Surgery and Physiology of the New York University and Bellevue Hospital Medical School, N. Y.*) Published in full in this issue of the BIOCHEMICAL BULLETIN, page 403.

77. Metabolism in a case of myotonia atrophica. JACOB ROSENBLOOM and BENSON A. COHOE. (*St. Francis Hospital and Laboratory of Biochemistry of the University of Pittsburgh, Pittsburgh, Pa.*) In a thirteen day metabolism research on an individual suffering from myotonia atrophica, we have determined the nitrogen metabolism and urinary nitrogen partition, the sulfur metabolism and urinary sulfur partition, also the calcium, magnesium, phosphorus, chlorin and fat metabolism. The only striking metabolic anomaly was marked loss of calcium. The creatinine excretion was normal.

78. A method for the determination of tryptophan in protein material. JESSE A. SANDERS AND CLARENCE E. MAY. (*Chemical Laboratories, University of Indiana, Bloomington, Indiana.*) Published in full in this issue of the BIOCHEMICAL BULLETIN, page 373.

79. The production of specific excretion products by Infusoria. LORANDE LOSS WOODRUFF. (*Sheffield Biological Laboratory, Yale University.*) In a previous study[15] it has been shown that the excretion products of *Paramaecium* produce a retardation of the rate of reproduction of *Paramaecium,* and therefore these products may be considered as toxic to this species. The present study shows that the excretion products of another infusorian, *Pleurotricha,* are toxic to *Pleurotricha* and produce a lowering of the reproductive rate. A subjection of *Paramaecium* to the excretion products of *Pleurotricha,* and, *vice versa,* the subjection of *Pleurotricha* to the excretion products of *Paramaecium,* does not produce any characteristic effect on the rate of reproduction of the respective species. This result indicates that these two forms of Infusoria, at least, develop excretion products which are specific in their toxicity, in that the substances are inimical to the form which produces them but not to a closely related form frequently associated with it in its natural environment. Details of the work will appear in the *Journal of Experimental Zoology* (1913, xiv, p. 575).

B. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEMICAL DEPARTMENT AND AFFILIATED LABORATORIES

80. Toxicological studies on the mushrooms, Clitocybe illudens and Inocybe infida.[16] ERNEST D. CLARK AND CLAYTON S. SMITH. When *Inocybe infida* and *Clitocybe illudens* were subjected to processes of extraction and purification for the separation of muscarin from *Amanita muscaria,* we obtained material that exerted a typical muscarin effect on exposed hearts of frogs and turtles. Furthermore, this toxic action on the exposed hearts was completely neutralized by the application of a solution of atropin sulfate. When the toxic material from these fungi was injected into the lymph-sacs of frogs the animals soon became paralyzed, and usually the heart ceased to beat.

It is interesting that experiments on both exposed hearts and whole animals showed that analogous preparations from *Amanita muscaria* did not seem as toxic nor as easily neutralized by atropin

[15] Woodruff: The effect of excretion products of *Paramaecium* on its rate of reproduction. *Journal of Experimental Zoology,* 1911, x, p. 557.

[16] Some of the work was done in the Physiological Laboratory of Columbia University.

as were the *Clitocybe* and *Inocybe* products. This tends to confirm the observations of others that muscarin is not the *only* poison in *A. muscaria.*

The edible *Clitocybe multiceps* yields no toxic material when treated in the same manner as these poisonous fungi, showing that our manipulations were not responsible for the effects observed. The ash constituents of the poisonous fungi were found to have no effect on frogs.

From our studies on *Inocybe infida* and *Clitocybe illudens,* and from Ford's work upon the latter and *Inocybe infelix,* it is plain that these plants should not be eaten, for they contain toxic material not unlike muscarin.

81. The phosphorus content of the blood and serum of normal and parathyroidectomized dogs.[17] ISIDOR GREENWALD. After parathyroidectomy the amount of phosphorus in the blood and serum is increased. The increase is chiefly in that form of phosphorus that may be extracted with dilute hydrochloric acid solution containing picric acid to prevent swelling of the protein.

82. Further metabolism experiments upon parathyroidectomized dogs.[18] ISIDOR GREENWALD. The retention of phosphorus after parathyroidectomy is followed or accompanied, but not preceded, by a retention of sodium and potassium.

83. A study of uroerythrin, with demonstrations. BEATRIX H. GROSS. In the last edition of his mimeographed directions for laboratory work in physiological chemistry, Dr. Gies describes, as follows, a method for the extraction of urochrome from urine.[19]

Treat about 25 c.c. of urine with phenol, little by little, with thoro stirring until the liquid remains decidedly turbid. The pigment is not affected by the phenol. After saturating the urine with phenol in this careful manner, add about 1 c.c. of phenol in excess and then saturate the liquid with ammonium sulfate. As the ammonium sulfate dissolves, the phenol is rendered insoluble. The yellowish turbidity is due to emulsified phenol, which carries urochrome in solution. The yellow

[17] Some of the work was done in the Pathological Laboratory of Columbia University and the Chemical Laboratory of the Montefiore Home, New York. *Journal of Biological Chemistry,* 1913, xiv, p. 369.

[18] See foot note 17. *Ibid.,* p. 363.

[19] This method is based on the findings of Kramm: *Deutsche medizinische Wochenschrift,* 1896, xxv, p. 42.

phenol-globules rapidly collect in a clear oily layer on the surface of the milky aqueous solution. Pour the mixture into a separatory funnel and, after it has remained there undisturbed for about a half-hour, isolate the oily phenolic extract of urochrome by drawing off the underlying liquid. An equal volume of ether is added to the phenolic extract, with which the ether mixes homogeneously. This liquid is then treated with an equal volume of water. Two layers form at once. The mixture is shaken *very gently,* in order to encourage transfer of the urochrome to the water layer but to prevent undue emulsion of the oily extract. Practically all the pigment passes promptly into the underlying aqueous stratum, which is drawn off after a suitable interval.

Under Dr. Gies' guidance I have endeavored to answer his question: Does the aqueous solution of urochrome, as prepared by the foregoing method, contain (or yield) *uroerythrin?* For this purpose colorless sodium urate was dissolved in such urochrome extracts prepared from both human and dog urines, the resulting solutions were acidified for the separation of uric acid, and the deposited crystals of uric acid were examined microscopically for uroerythrin. All the crystals thus obtained were colored in the familiar way with uroerythrin, as when separated from normal urine. Experiments on the use of other solvents than phenol for the extraction of urochrome from urine, on the relationship of uroerythrin to urochrome, and on a number of suggestions from the results, will be described later. (The method was demonstrated.)

84. Comparison of methods for the preparation and determination of cholesterol. Joseph S. Hepburn. Cholesterol, extracted from brain, has been purified by saponification either with sodium ethylate at room temperature or with boiling alcoholic potash, in each case followed by crystallization from ether. Cholesterol has also been prepared from gall stones by extraction with ether and crystallization from that solvent.

The *melting points* of six samples from brain, 148.4–149.1°, of two samples from gall stones, 147.4°, and of various mixtures of two samples (50 per cent. of each sample), 147.7–148.0°, demonstrate the identity of the cholesterol products from the two sources. The melting points of the samples and of their mixtures also show that neither heat in the process of saponification nor alkaline reagents, such as alcoholic potash and sodium ethylate, produce any rearrangement of the cholesterol molecule.

The *iodin reagents* for fat analysis cannot be used in the volumetric determination of cholesterol. The iodin number of pure cholesterol has been determined by the methods of Hübl, Hanus and Wijs. The Hübl method tended to give the lowest values, 70.3–78.0, but even these values are higher than the theoretical value, 65.7, which is based on the assumed existence of one double bond in the molecule of cholesterol. The Hanus method gave higher results, 71.4–81.1. The highest values of all were obtained with the Wijs method, 55.1–158.9, with an average value above 100. There was a marked tendency, especially with the Hanus and Wijs methods, for the iodin number to become higher, the greater the excess of the iodin reagent. However, the iodin numbers were not simple multiples of 65.7, hence the presence of a second double bond in cholesterol is doubtful.

The *gravimetric determination* of cholesterol as the *free alcohol,* by modifications of *Ritter's method,* is unsatisfactory. When carbon dioxide was used to neutralize the excess of sodium ethylate, divergent results were obtained: 99.90 per cent. and 92.43 per cent. of the cholesterol taken was recovered. When hydrochloric acid was used to neutralize the excess of sodium ethylate, the results were still less satisfactory, 64.34 to 89.10 per cent. of the cholesterol taken being recovered. The gravimetric determination as *cholesteryl benzoate* is not quantitative; only 24.29 to 61.79 (average 42.86 per cent.) of the cholesterol taken was recovered. The gravimetric determination of cholesterol as the free alcohol by *Cappenberg's method* gave excellent duplicates; 94.47 and 94.37 per cent. of the cholesterol taken was recovered. The gravimetric determination of cholesterol as *digitonin cholesteride* was the most accurate and most satisfactory of the methods studied. From 93.63 to 103.02, average 97.37 per cent., of the cholesterol taken was recovered.

85. A preliminary study of the resistance of fasting dogs to hemorrhage. Paul E. Howe and William J. Gies. In continuance of the series of investigations in this laboratory on the effects of changes in the volume of circulating blood in normal, undernourished, and overfed animals,[20] we have lately determined,

[20] See Biochemical Bulletin, 1912, ii, p. 186, for the last of the series.

in a preliminary way, the general resistance of fasting dogs to hemorrhage.

Thus far thirteen dogs have been under observation. After preliminary periods (six to eighteen days) on our standard laboratory diet for dogs, each animal was subjected to a *total* fast for from seven to thirteen days. Blood was then drawn from a femoral artery under local cocain anesthesia until the respiratory conditions suggested that further removal might be fatal. The operations were usually performed at about 3 p. m. The fast was continued until the following morning, at 9, when, if the animal survived, the daily ration of the preliminary period was offered. Of the thirteen dogs that have been subjected to comparatively heavy hemorrhages, ten survived and speedily recovered.

After an average loss of 21 per cent. of the normal body weight as a result of fasting, blood equal to an average of 3.3 per cent. of the body weight at the time of *operation* (2.6 per cent. of body weight when the fast was *started*) has been removed from the ten surviving dogs without causing any serious symptoms. The essential data pertaining to the three *fatal* cases are appended.

Days of fast	Body weight		Loss in weight		Blood taken[21]			Remarks
	Before fasting	After fasting			Weight, grams	Original body wt.	Fasting body wt.	
	k	k	k	%		%	%	
10	5.45	4.00	1.45	26.6	144.7	2.7	3.6	Black mongrel, thin, vicious; *died* on table.
10	12.36	9.12	3.24	26.2	256.0	2.1	2.8	Mongrel, bull mixture; *died* during the night.
10	9.05	7.28	1.77	19.6	266.0	2.9	3.7	Mongrel, black and white; *died* in two hours.

Similar experiments on dogs under conditions of *partial* fasting will be conducted prior to the inauguration of the metabolism studies to which these general observations are a prelude.

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New York

[21] The blood was withdrawn in from five to seven minutes.

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2. First quarter, 1913 (January-March) [1]

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I. EXPLANATION OF ABBREVIATIONS, ARRANGEMENT, NOTATION, ETC.

Bibliography. In the appended bibliography *titles of papers* are shortened in a free and easy manner, minor words are ignored, common words are conveniently abbreviated or chemical symbols substituted, *surnames* of collaborators (in italics) are connected by hyphens, and most *punctuation* marks are omitted—all for the sake of condensation. *Volume numerals* are given in Roman at the opening of each paragraph. The Arabic numerals following them, or placed (in bold face type) at the beginning of main sections in the paragraphs, designate respective issues of the volume. Numerals separated by a slanted line indicate month and day of issue. The *bibliographic items* are marked off with em *dashes*. The numeral at the *end* of an item is that of the initial page of the corresponding paper; the numeral at the *beginning* of an item indicates its sequence in the bibliography.

Index. A subject-index is appended to the bibliography (p. 474). The numerals indicate the numbered items in the preceding bibliography. Numerals connected by hyphens are plain abbreviations in accord with the indications of the first numeral in each group. Blanks in the sequence of numerals occur at the end of each journal group, as noted. Abbreviations of words in the index are similar to those in the bibliography. *Each main index item is terminated by a semicolon,* followed by a space; *commas* mark off subdivisions of a general index subject.

Journals included: *Biochemische Zeitschrift, Zeitschrift für physiologische Chemie, Journal of Biological Chemistry, Biochemical Journal, Biochemical Bulletin.*

[1] The first portion of this bibliography and index was published in the preceding issue of the BIOCHEMICAL BULLETIN (1913, ii, p. 298).

II. BIOCHEMISCHE ZEITSCHRIFT (B. Z.)

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[2] See the explanation on page 470.

[3] This series of abbreviations, illustrating all others in the index, represents the following sequence of numerals: 6, 54, 71–72, 210–211–216–221–225–229–235, 401–411–422–423–424–427–435–436, 605–614, 706. The numerals in bold face type here are omitted from the abbreviations above.

BIOCHEMICAL NEWS, NOTES AND COMMENT

I. GENERAL

Necrology. *Manfredi Albanese,* professor of pharmacology and director of the School of Pharmacy at Pavia.—*Philip Hanson Hiss,* professor of bacteriology at Columbia.—*Dr. F. J. A. C. Howitz,* formerly professor of gynecology and obstetrics at Copenhagen; introduced thyroid treatment for myxedema.—*Oscar Oldberg,* dean emeritus of Northwestern Univ. School of Pharmacy, formerly dean of the National College of Pharmacy, Washington (where he was instrumental in introducing the metric system of weights and measurements in the government service), for twenty five years professor of pharmacy and for thirty years a member of the committee on revision of the United States Pharmacopeia.—*John Seeman,* director of the physiological laboratory of the Academy of Medicine, Cologne.—*Prof. G. Vassale,* professor of general pathology at Modena; one of the leaders in the therapeutic utilization of internal secretions.

Honors. ORDERS OF MERIT. Dr. *Alexis Carrel* (Rockefeller Institute) has been appointed a knight of the Legion of Honor.—Drs. *Alexis Carrel* and *Hideyo Noguchi* (Rockefeller Institute), and Dr. *William H. Park* (N. Y. Dep't of Health), have been made knights of the Royal Order of Isabella the Catholic, by King Alfonso of Spain.

AWARDS OF MEDALS. The Chicago Section of the American Chemical Society has awarded the *Willard Gibbs medal* to Dr. Leo H. Baekeland (Yonkers).—The Franklin Institute, Philadelphia, has recently awarded the *Elliott Cresson gold medal* to Prof. Emil Fischer (Berlin) in recognition of numerous contributions of fundamental importance to the science of organic and biological chemistry; also to Sir William Ramsay (London) in recognition

of extended researches of signal importance in chemical science. —The *Helmholtz medal* of the Berlin Academy of Sciences has been awarded to Prof. S. Schwendener (Berlin), for his researches in plant physiology.

Corresponding member. Dr. Alexis Carrel (Rockefeller Institute) has been elected a corresponding member of the Paris Academy of Medicine.

Reception and dinners. A reception was given by the Manhattan Medical Society, February 28, to Dr. Jacques Loeb (Rockefeller Institute), at which he spoke on Some recent experiments in artificial parthenogenesis.—The Bay County Medical Society gave a dinner recently at Bay City, Mich., in honor of Prof. Victor C. Vaughan, who afterward delivered an address on Prevention of disease.—Prof. R. H. Chittenden (Yale) was the guest of his pupils at a dinner at Delmonico's, N. Y., on March 1. (See page 349.)

Award of the Ellen H. Richards research prize. At a recent meeting of the Naples Table Association for Promoting Laboratory Research by Women, the Ellen H. Richards research prize of $1,000, for the best thesis written by a woman on a scientific subject embodying new observations and new conclusions based on independent laboratory research in biological, chemical or physical science, was awarded to Miss Ida Smedley (London, England; D.Sc., London University), who has been working for the past four years in the biochemical laboratory of the Lister Institute of Preventive Medicine. The subject of the winning thesis was: "An investigation into the methods of formation of fatty acids from carbohydrates in the organism." Ten theses were submitted in competition. The examiners for the award of this prize were: Dr. W. H. Howell, of Johns Hopkins Medical School; Dr. Theodore Richards, of Harvard University; and Dr. Henry Crew, of Northwestern University.

Appointments.[1] Breslau: Professor *Henkel* (Königsberg), director of the pathological institute, in succession to Professor Ponfink.

[1] In this summary, institutions from which *resignations* occurred are named in parenthesis. See, also, page 484.

Carnegie Institution, Nutrition Laboratory: Dr. *H. Monmouth Smith* (Syracuse), research chemist.

Idaho State Chemist, Boise: Dr. *H. Louis Jackson* (assistant professor of chemistry, in charge of foods, Univ. of Kansas), state chemist.

Jefferson Medical College, Phila.; department of physiological chemistry and toxicology: Dr. *M. A. Saylor,* demonstrator; Dr. *L. F. Fairhall,* instructor; Dr. *Olaf Bergeim,* instructor; *W. T. Smith,* assistant.

Kiel: Prof. *Otto Lubarsch* (Düsseldorf), director of the pathological institute, in succession to Prof. Arnold Heller.

Königsberg: Prof. *Franz Hoffmann* (Prague), director of the physiological institute, in succession to Prof. L. Hermann.

Marburg: Professor *Jores* (Cologne), director of the pathological institute.

Mass. State Board of Health: Dr. *Milton J. Rosenau,* Harvard, member.

Mich. State Board of Health: Dr. *Victor C. Vaughan,* Michigan, member (reappointment) and president.

N. Y. Agric. Experiment Station, Geneva: Dr. *R. S. Breed* (professor of biology, Allegheny College), bacteriologist. Dr. Breed succeeds Dr. H. A. Harding who becomes head of the dairy department of the University of Illinois.

Tufts College, Medical School: Dr. *Alfred W. Balch,* professor of chemical pathology and toxicology.

U. S. Commission for the Determination of a Standard of Purity for Drinking Water: Prof. *E. O. Jordan,* Chicago, member. This commission has been formed in connection with the enforcement of regulations relative to pure drinking water, and its object is to establish a federal standard which shall be generally applicable.

U. S. Dep't of Agriculture: Prof. *Ralph Hoagland* (head of the division of chemistry, College of Agric., Univ. of Minn.), officer in the Bureau of Animal Husbandry.—*H. B. Humphrey* (head of the department of botany, State College of Washington), pathologist in charge of cereal-disease investigations, Bureau of Plant Industry.

Univ. of Illinois: Dr. *M. J. Prucha* (Cornell Univ.), assistant

professor of dairy bacteriology in the College of Agric., and assistant chief in dairy bacteriology at the Agric. Experiment Station. He will be associated with the new head of the dairy department, Dr. *A. H. Harding* (N. Y. Agric. Experiment Station).

Univ. of Minnesota: Dr. *R. W. Thatcher* (director of the Washington Agric. Experiment Station and head of the department of agric., Washington State College), professor of agric. chemistry and soils.—Dr. *Richard O. Beard,* former head of the department of physiology, will be assistant to the dean of the reorganized medical school.

Univ. of Virginia, Medical School, Richmond: Dr. *Wortley F. Rudd,* professor of chemistry; Dr. *Francis W. Upshur,* professor of pharmacology and therapeutics; Dr. *E. C. L. Miller,* associate professor of physiological chemistry; Dr. *C. Howard Lewis,* associate professor of physiology; Dr. *Leslie B. Wiggs,* associate professor of materia medica and pharmacology.

Washington Univ.: Dr. *David F. Houston,* secretary of agriculture, will retain the chancellorship, on leave of absence. Prof. F. A. Hall, dean of the college, has been appointed acting chancellor.

Wellcome Research Laboratories, Khartoum: Dr. *A. J. Chalmers* (Ceylon), director, in succession to Dr. Andrew Balfour, appointed chief of the Wellcome Bureau of Scientific Research, London.

Lectures. HARVEY LECTURES: Mar. 22, by Prof. *Franz Knoop* (Freiburg), on Modern problems of nutrition; March 29, by Prof. *John Howland* (Johns Hopkins), on The scientific basis for the artificial feeding of infants.—WEIR MITCHELL LECTURE, before the College of Physicians, Philadelphia, April 4, by Dr. *H. P. Armsby* (Penn. State College), on Animal calorimeters and the study of nutrition.—MISCELLANEOUS ITEMS: Dr. *F. K. Cameron* (Bureau of Soils, U. S. Dep't of Agric.) lectured, Mar. 6, before the Phi Lambda Upsilon Society, Columbia Univ., on The solution of the potash problem in America.—Prof. *Martin H. Fischer* (Univ. of Cincinnati) delivered the address at the third winter commencement of St. Louis Univ. School of Medicine, January 30, on Principles of treatment of edema and nephritis.—Prof. *Lafayette B. Mendel* (Yale) addressed the students of Pratt Institute, April 11,

on Nutrition and growth.—Prof. *H. C. Sherman* lectured, Jan. 16, before the Society of the Sigma Xi, Columbia Univ., on Progress and problems in food chemistry.

Societies, associations, etc. NATIONAL ACADEMY OF SCIENCES. The National Academy of Sciences celebrated, on April 22, 23 and 24, the semi-centennial anniversary of its foundation, exactly fifty years after its first meeting. Prof. Wm. H. Welch (Johns Hopkins) was elected president. Prof. Lafayette B. Mendel (Yale) was elected a member.

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. Officers elected: *Section C:* Dr. Carl L. Alsberg (U. S. Dep't of Agric.), vice-president and chairman; *Section K:* Dr. Theodore Hough (Univ. of Va.), vice-president and chairman, and Dr. John R. Murlin (Cornell Univ. Med. College), secretary.

AMERICAN CHEMICAL SOCIETY: *47th annual meeting, Milwaukee, Wis., Mar. 25–28.* An account of the proceedings, including abstracts of papers, appears in *Science,* 37: pp. 674–690. "Although the Amer. Chem. Society changed its time of meeting from winter to spring there was no falling off in the attendance at the Milwaukee meeting." The next meeting will be held in Rochester, N. Y., early in September. *Divisional officers*—Agricultural and food chemistry: chairman, *H. E. Barnard,* secretary, *Glen F. Mason;* biological chemistry: chairman, *Carl L. Alsberg,* secretary, *I. K. Phelps;* pharmaceutical chemistry: chairman, *B. L. Murray,* secretary, *F. R. Eldred;* fertilizer chemistry: chairman, *Paul Rudnick,* secretary, *J. E. Breckenridge.*—Final organization of the *Division of Biological Chemistry* did not occur at the Milwaukee meeting for the reason that this meeting corresponded to the old summer meeting, and it had been voted, at a previous meeting of the Biological Section, that the final organization should take place at one of the annual meetings. It is, therefore, expected that the final organization of the Division will be consummated at the September meeting, which is an annual meeting.

ASSOCIATION OF AMERICAN MEDICAL COLLEGES. Officers elected: *President,* Dr. E. P. Lyon (St. Louis Univ.); *vice-president,* F. F. Wesbrook (Univ. of Minn.); *secretary-treasurer,* F. C. Zapple (Univ. of Ill.).

HARVEY SOCIETY. Officers-elect, 1913–'14: *President,* Frederic S. Lee; *vice-president,* W. G. MacCallum; *treasurer,* E. K. Dunham; *secretary,* A. B. Wadsworth; *additional members of the council*—Graham Lusk, William H. Park and George B. Wallace.

ILLINOIS WATER SUPPLY ASSOCIATION. The fifth annual meeting of the Illinois Water Supply Association was held at the Univ. of Ill., Mar. 11 and 12. The membership of the association consists of waterworks engineers, superintendents, chemists, and others interested in obtaining and conserving an abundant supply of pure water. Officers elected: *President,* C. H. Cobb (sup't, Kankakee Waterworks); *first vice-president,* H. M. Ely (sup't and manager, Danville Water Company); *second vice-president,* W. J. Spaulding (commissioner of public property, Springfield); *state vice-president,* V. E. MacDonald (sup't, Lincoln Water and Light Company); *secretary and treasurer,* Prof. Edward Bartow (director, State Water Survey).

SOCIETY OF THE SIGMA XI. Prof. John H. Long has been elected vice-president of Sigma Xi and president of the Northwestern chapter. Prof. A. P. Mathews is vice-president of the Chicago chapter; Prof. F. G. Novy is president of the Michigan chapter.

Miscellaneous items. HENRY PHIPPS PSYCHIATRIC CLINIC. On April 16, a new psychiatric clinic was accepted by Johns Hopkins University as a gift from Henry Phipps, Esq. The clinic has a capacity of ninety beds and includes a laboratory of internal medicine in charge of Dr. Sidney R. Miller, where such investigations of a clinical and experimental nature will be made as may seem of value for the betterment of the patient and the advance of scientific knowledge. The opening exercises (April 16, 17, and 18) consisted chiefly of sixteen addresses: (16) Sir *William Osler,* Specialism in general hospitals; Dr. *Stewart Paton,* The clinic and the community; (17) Prof. *W. McDougall,* The sources and direction of psycho-physical energy; Prof. *E. Bleuler,* Autistic thinking; Prof. *A. Hoch,* Personality and psychosis; Dr. *L. F. Wells,* The personal factor in association reactions; Dr. *F. W. Mott,* A study of the neuropathic inheritance in relation to insanity; Prof. *O. Rossi,*

Pellagra; Prof. *H. Cushing,* Psychic derangements associated with ductless gland disorders; (18) Dr. *S. Paton,* Primitive mechanisms of individual adjustment; Prof. *Heilbronner,* Demenz probleme; Dr. *E. Jones,* The interrelation of the biogenetic psychoses; Dr. *G. H. Kirby,* The prognostic significance of the biogenetic psychoses; Dr. *C. B. Dunlap,* Anatomical borderline between the socalled syphilitic and metasyphilitic disorders; Prof. *A. M. Barrett,* Disorders connected with anemia. The closing address was given by Dr. Adolf Meyer, the director of the Clinic. Mr. and Mrs. Phipps and three sons were present. A dinner was given in Mr. Phipps' honor, on Apr. 16, to express the community's appreciation of his gift. At this dinner Prof. Wm. H. Welch was toastmaster; the governor of Maryland and the mayor of Baltimore were among the speakers.

Institute for dietetics. There exists in Paris a *Société scientifique d'hygiène alimentaire et d'alimentation rationnelle de l'homme.* In pursuance of the purpose for which it was chartered, this society will build an institute for the study of the hygiene of nutrition, which will be the center not only of research and of teaching of the applied sciences of nutrition, but also of popular education, where instruction will be given in simple terms, and principles necessary for the solution of practical dietetics will be taught. The department of research will comprise laboratories necessary for the study of all the sciences bearing on the general purposes of the society. A bulletin will be published, which will be a permanent record of all the transactions of the society, and experiments done in connection with dietetics. The constitution of the society prevents it from considering industrial and commercial questions, and enjoins its members from using, naming or recommending in any way, commercial products.

Turck Institute, New York. A research laboratory has been established at 428 Lafayette Street, N. Y., under the directorship of Fenton B. Turck, M.D. Gastro-intestinal problems will be investigated. Two departments are now in operation: Chemistry, Dr. A. R. Rose, chemist; bacteriology, Dr. Otto Maurer, bacteriologist. A veterinarian, pathologist, physiologist, histologist and clinician are about to be appointed. Dr. Turck conducted the Turck Institute of Chicago, a research institution of similar scope. Both

the old and new institutions are purely scientific in purpose and conduct.

CARNEGIE GRANTS. The report of the president of the Carnegie Institution of Washington, for the year ending October 31, 1912, contains the following summary of grants in "chemistry"; S. F. Acree, $2,000; G. P. Baxter, $1,000; T. B. Osborne and Lafayette B. Mendel, $15,000; H. C. Jones, $2,200; H. N. Morse, $4,000; A. A. Noyes, $3,000; T. W. Richards, $3,000; H. C. Sherman, $1,200.

INTERNATIONAL PHARMACEUTICAL COMMISSION. The Section on Pharmaceutical Chemistry, Eighth International Congress of Applied Chemistry, has appointed an international commission to continue the inquiry on variations in the activity of certain toxic drugs, and to report at St. Petersburg in 1915. The commission: *Austria,* Prof. Wilhelm Mitlacher; *France,* Prof. E. Bourquelot; *Germany,* Prof. H. Thoms; *Great Britain,* Francis Ransom, F. C. S.; *Netherlands,* Prof. L. van Itallie; *Switzerland,* Prof. A. Tschirch; *United States,* Dr. Rodney H. True. Three secretaries for the commission were also appointed: *European Continent,* George P. Forrester, F. C. S.; *Great Britain,* Peter MacEnau, F. C. S.; *United States,* Otto Raubenheimer.

UNIVERSITY OF ILLINOIS AGAIN GETS MEDICAL SCHOOL. The College of Physicians and Surgeons, Chicago, again passes under the control of the Univ. of Ill. This time it is a gift to the state institution, partly by the stockholders and partly by the alumni who purchased the stock not donated. For several years the medical school has held a contractual relationship with the Univ. of Ill., but it was cancelled last spring. By the present transfer of all the stock, however, the medical school becomes an organic department of the Univ. of Ill. The formal transfer of the college to the university occurred on March 6.

PERSONALIA. Dr. *W. D. Bigelow* of the U. S. Dep't of Agric., lately a member of the "pure food board," has resigned to take charge of the laboratory of the National Canners' Association to be established in Washington, D. C.

Dr. *David Marine,* assistant professor of experimental medicine, Western Reserve Univ., is spending the year in Europe, travelling and visiting various laboratories.

Dr. *Roy G. Pearce,* demonstrator of physiology, Western Reserve Univ., has sailed for Berne, Switzerland, where he will spend some time in the physiological laboratory of Prof. Leon Asher.

Dr. *George B. Rigg,* instructor in botany, Univ. of Washington, and special agent of the U. S. Dep't of Agric. in kelp investigation in 1911 and 1912, is directing an expedition to western Alaska for the purpose of investigating the kelps of that region as a source of potash fertilizer.

Dr. *Alfred Vivian,* professor of agric. chemistry, Ohio State Univ., is making a tour of the world, and is now in India. Professor Vivian will deliver a course of lectures on soil fertility at the agric. school at Allahabad.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

1. General notes

Professor Chittenden ill. Dean *Russell H. Chittenden* is recovering, we are glad to report, from the effects of a recent operation. He will be unable to resume his duties for the remainder of the academic year.

Professor Smith will go to Princeton. Prof. *Alexander Smith* has accepted the professorship of chemistry on the Wyman Foundation, at Princeton University, and the headship of the department of chemistry. By the desire of the president and trustees of Columbia Univ., as well as his own, Professor Smith will complete three years of service at Columbia Univ., and will accept the Princeton appointment to take effect at the beginning of the academic year 1914–15.

Appointments.[2] Dr. *Donald B. Armstrong* (Mass. Inst. of Technology), executive secretary of the N. Y. Association for Improving the Condition of the Poor; Bureau of Public Health and Hygiene.—Dr. *Louis E. Bisch* (Manhattan State Hospital), member of the Medical Board and visiting neurologist, N. Y. City Chil-

[2] See footnote, page 477.

dren's Hospitals and Schools, Randall's Island; also clinical assistant, N. Y. Post Graduate Medical School and Hospital and clinical assistant, Neurological Institute. Dr. Bisch gave two lectures during February, on Prevention of insanity, in the evening series of lectures, under the auspices of the N. Y. City Board of Education. —Dr. *Josephine T. Berry* (Washington State College), chief of the department of home economics, and *Louise McDanell* (Washington State College), assistant professor of foods and cookery, College of Agric., Univ. of Minn.—Dr. *C. C. Lieb,* assistant professor of pharmacology, Columbia Univ. (promotion).—*Hermann J. Muller,* assistant in zoology, Columbia Univ.—Dr. *A. R. Rose,* chemist, Turck Research Laboratory, N. Y. City.—Dr. *Hans Zinsser* (Leland Stanford, Jr., Univ.), professor of bacteriology, Columbia Univ.

Conference on the prevention of infant mortality. A conference on the prevention of infant mortality will be held in Caxton Hall, Westminster, London, August 4–5, under the auspices of the National Association for the Prevention of Infant Mortality and the Welfare of Infancy. The conference will convene immediately before the opening of the International Medical Congress. Dr. *Philip Van Ingen* is secretary of the American committee.

Personalia. Dr. *Carl L. Alsberg* has been elected an honorary member of Phi Lambda Upsilon by the Columbia chapter.—Dr. *Herbert S. Carter* was one of the charter members of the N. Y. Gastro-Enterological Society (p. 315).—Dr. *John Howland* delivered a Harvey lecture, Mar. 29, on The scientific basis for the artificial feeding of infants.—Prof. *Raymond C. Osburn* has been reëlected president of the N. Y. Entomological Society.—Dr. *Jacob Rosenbloom* has resigned his assistant professorship in biochemistry at the Univ. of Pittsburgh (p. 324).—Dr. *F. J. Seaver* is one of the spring lecturers at the N. Y. Botanical Garden, subject: The scenery and flora of Colorado (May 3).—Prof. *E. A. Spitzka* and Dr. *D. C. Twichell* have suffered from general nervous breakdown. Dr. Spitzka has gone to Europe, and Dr. Twichell to the Pacific Coast, to recuperate. Our best wishes attend them.—During the fall of 1912, Dr. *William H. Welker* carried out an investigation on blood in Prof. John Marshall's laboratory at the University of Penn.

Later he conducted research on colloidal solutions of metals at the Amer. Oncologic Hospital, in Phila. This spring he has been, and at present is, studying hydrocarbons with Prof. Ullmann at Lehigh University.

2. Proceedings of the Association

Ninth and tenth meetings. Abstracts of the communications at the ninth and tenth meetings are presented in this issue, on pages 453–469.

Eleventh meeting. The association and its guests were highly favored, on the evening of April 25, by Dr. Warren Coleman, professor of clinical medicine and applied pharmacology at Cornell Univ. Med. College, who delivered a public lecture, at the College of Physicians and Surgeons, under the auspices of the association, on *Diet and metabolism in typhoid fever.* The lecture was a clear, concise and convincing presentation of important findings in a very difficult field of investigation. At the conclusion of the lecture, in expressing the Association's appreciation of Dr. Coleman's kindness in addressing the members, Professor Gies congratulated those present on their "having heard, from the guiding spirit himself, so interesting and instructive an account of the results of an American classical research in nutrition."

ALFRED P. LOTHROP, *Secretary*

EDITORIALS

Our Amerian readers will doubtless be most agreeably surprised when they scan the list of members of the Biochemical Society, England.[1] Who has expected the development, in so short a time, of an organization of such evident vigor![2] We have long appreciated the great ability of our English confrères; their eminent achievements have interested and stimulated us greatly, and have taught us very much. But it is a revelation to find that so many very able men among them have so promptly united effectively to advance biochemical science. We follow the proceedings of the Biochemical Society with fraternal interest and professional expectancy; and we hope to present to the readers of the BIOCHEMICAL BULLETIN such information, from time to time, as may be available for publication. Meanwhile, we recommend careful attention to the fact that, as the official organ of the Biochemical Society, the *Biochemical Journal* will mirror hereafter, even more perfectly than heretofore, the activity, influence and achievements of British biochemical investigators. A biochemical laboratory without the *Biochemical Journal* must be a pretty poor place, indeed!

Biochemical Society, England

The St. Louis Court of Appeals has reversed the lower court in "the bleached flour case." This decision is not final, the Government having the right and the intention to carry the case to the Supreme Court. There is no question that from the point of view of both the common people and physiologists, this decision of the court is one of the most injurious to the whole people which has been rendered by a Court in some time, since, if upheld, it makes a dead letter of a most important part of the pure food law. The fifth section of the Food and Drugs act reads: "An article shall be deemed adulterated," etc., (5) "if it contains any added poisonous or other added deleterious ingredient which may render such article injurious to health."

The bleached flour decision

[1] See the BIOCHEMICAL BULLETIN, this issue, p. 447.

[2] *Ibid.*, 1912, ii, pp. 128 and 209.

Judge McPherson of the lower court held that the meaning was to be interpreted in the light of the plain purpose of the act and that the intention was to prevent the addition of *any* poisonous substance, and in *any* quantity, which might render the article injurious. According to the new ruling the government must prove that the added poisonous substance is in sufficient quantity so that it would render the food injurious. To a plain man this reading appears directly contrary to the purpose of the act, which clearly was to protect the people against the addition of any substance of a known poisonous character to foods.

This is another example of courts deciding against the people and in the interest of "big business." The bleaching of flour profits no one but the millers; no one claims that it improves the flour. Its taste is injured as the Government showed. If it is not to conceal inferiority so that a higher price can be had for the flour, why do the millers use the process? Such decisions furnish additional argument for the recall of judges and decisions. There can be no question as to the verdict of the people on this case were it submitted to them.

There would seem to be no reason, so far as this decision is concerned, why small amounts of arsenic, strychnin or other poisons could not be added to food with impunity. But what shall we say of physiologists and physicians who testify, for a price, that a food is not rendered injurious by the addition to it of small amounts of poisons? Is it more charitable to believe that they lack character or ability?

M. C.

Occupational diseases in chemical trades

A Committee on Occupational Diseases in the Chemical Trades was appointed by the New York Section of the American Chemical Society in February 1912. The objects of the Committee may be specifically stated as follows:

1. To hold itself ready to advise the Legislature of the States of New York and New Jersey in reference to matters pertaining to occupational diseases in the chemical trades;

2. To study various bills presented in the Legislatures in an effort to avoid unwise legislation; especially that which might be

inoperative or ineffective from one or many reasons resulting from lack of technical knowledge at the time of writing the laws;

3. To inaugurate and superintend such investigations as might be decided upon which look toward improvement of conditions of labor in the chemical trades.

The personnel of the Committee may be seen from the appended list of membership: Dr. *Charles Baskerville,* Professor of Chemistry and Director of the Laboratory, College of the City of New York, Chairman; Mr. *E. C. Uhlig,* Chief Chemist, Brooklyn Union Gas Co., Brooklyn, N. Y., Secretary; Dr. *Geo. P. Adamson,* Baker & Adamson Chemical Co., Easton, Pa.; Mr. *W. H. Bassett,* Technical Sup't and Metallurgist, American Brass Co., Waterbury, Conn.; Dr. *Wm. F. Doerflinger,* Consulting Chemist, 52 Beaver St., N. Y. C.; Dr. *H. M. Kaufman,* Gen'l M'g'r Mutual Chemical Co. of America, 55 John St., New York; Dr. *Chas. F. McKenna,* Chemical Engineer, 50 Church St., New York; Dr. *A. C. Langmuir,* Chief Chemist, Marx and Rawolle, 9 Van Brunt St., Brooklyn, N. Y.; Dr. *Chas. L. Parsons,* Mineral Chemist, Bur. Mines, Washington, D. C.; Dr. *Geo. A. Prochazka,* Gen'l M'g'r, Central Dye Stuff and Chemical Co., Newark, N. J.; Dr. *Geo. D. Rosengarten,* Powers, Weightman and Rosengarten, Phila., Pa.; Mr. *A. M. Sabin,* Consulting Chemist, National Lead Co., 129 York St., Brooklyn, N. Y.

To date the following work has been undertaken or completed:

1. Dr. *Charles L. Parsons,* Mineral Chemist of the Bureau of Mines, Washington, D. C., reports that the Bureau's experts engaged in studying mineral resources and utilization under instructions from Dr. J. A. Holmes, Director of the Bureau of Mines, are noting health conditions and collecting information on occupational diseases in chemical trades in so far as they relate to mining and metallurgy. When funds are available, bulletins will be issued on the subject from time to time.

2. Dr. *Charles F. McKenna* was retained by the Factory Investigating Commission of the State of New York, as chemical adviser to Dr. Charles M. Price, Medical Director, who supervised the investigation of the Chemical Trades in New York State. Dr. McKenna's work was associated with the manufacture of "commercial acids."

3. Dr. *Charles Baskerville* prepared a report for the Factory Investigating Commission of the State of New York on "Wood alcohol."

These reports, which are now in press and will soon be available, contain suggestions for new legislation. Bills have been introduced carrying these suggestions. Among them is one which involves the appointment of a Chemical Engineer as one of the four members of the Division of Industrial Hygiene of the proposed reorganized Department of Labor.

N. Y.

The Mathews plan for an American Biological Society

In an editorial in the January number of the BIOCHEMICAL BULLETIN, we referred briefly to the recent organization of the *Federation of American Societies for Experimental Biology,* and commended to the attention of our readers the "Mathews plan for the organization of an *American Biological Society.*" Our attitude toward the establishment, and our feeling regarding the consequences of the logical development, of the Federation were indicated in that editorial. In pursuance of our purpose to facilitate consideration and removal of the difficulties in the way of more effective biological organization in this country, we addressed, late this month, the following circular note to the members of four of the leading biological societies:

The BIOCHEMICAL BULLETIN invites your attention to the Mathews plan for the organization of the American Biological Society (*reprint herewith*)[2] and solicits a brief expression of your opinion regarding the feasibility of the plan, for publication, with similar comment, in an early issue of the BIOCHEMICAL BULLETIN.

As we go to press, replies to this letter are arriving with every mail. *We append copies of some of the responses that are typical of the entire group already received.* The remaining letters, together with all others received meanwhile, will be published in succeeding issues of the BULLETIN, in the hope that through this agency the problems involved in more perfect *federation* or *consolidation* of biological societies will be fully exposed for discus-

[2] Reprint of the paper by Dr. Mathews on pages 261–268 of this volume (1913, ii).

sion and solution at the next annual meetings of the organizations most directly concerned.

JOHN HENDLEY BARNHART, *N. Y. Botanical Garden.* It is an unpleasant task to criticize adversely any scheme which has for its avowed object the advancement of biological science in America. I must confess, however, that I can see nothing in Prof. Mathews' proposal except a new society and a new journal, and I believe that we have already too many of both. There may be a few men in America who are broad enough (without being too shallow) to wish to receive currently journals occupying such widely separated fields as the *Journ. of Infec. Diseases,* the *Psychological Rev.,* and the *Botan. Gaz.;* but their number must be few indeed. If ability to read all three of these journals appreciatively is the criterion of a "biologist," I greatly fear that there would not be a sufficient membership to support an "American Biological Society."

D. H. BERGEY, *Univ. of Penn.* I am not in favor of the organization of any new societies at the present time unless it can be done through the amalgamation of the societies in existence to-day. Even if this can be done, I fear that it would make the new society so large and unwieldy as to render the amalgamation undesirable because of its size. The expense connected with membership in the societies already in existence forbids the encouragement of the organization of new societies, because the burden is greater than it should be. In fact, in the last two years I have felt obliged to resign from several societies in which I had long held membership in order to accept membership in newer societies, which I felt might be more beneficial to me; but I do not feel that I would care to resign from any additional societies for that reason, unless those societies were to amalgamate in one large organization, such as is proposed for the Amer. Biolog. Soc'y.

H. BUNZEL, *U. S. Dep't of Agric.* I am strongly in favor of the plan Professor Mathews suggests for the organization of an Amer. Biolog. Soc'y.

THEO. C. BURNETT, *Univ. of Cal.* The idea of an Amer. Biolog. Soc'y is a good one. Can you be sure of the financial scheme? It looks to me a little doubtful.

A. J. CARLSON, *Univ. of Chicago.* A greater coördination of the biological interests of the country is certainly desirable. The only question at issue is the most efficient or practical way of bringing it

about. I think we must face the fact that the *special societies* have not only come to stay, but that specialization will increase as the years go by. There is strength in smaller organizations of men of similar training and aim not found in larger and more heterogeneous societies. There would be little gained and much lost by converting the present special societies into sections of a general organization. In my opinion the *Biolog. Sec.* of the Amer. Chem. Soc'y is not as effective as the *Amer. Soc. of Biolog. Chemists.* It seems to me that the essentials hoped for by the organization of an all-inclusive Biolog. Soc'y, namely coöperation and encouragement in biological research, would be secured—so far as mere organization will secure it—by a *Federation* similar to that recently effected between the physiologists, the biochemists, and the pharmacologists. Such a federation would leave the present societies intact, but it would bring us all together at our annual meetings, thus affording opportunities for personal control and facilitating concerted action in matters of general scientific or public interest.

I do not favor the starting of a new Biolog. Abstract Jour. The *Centr. f. Biochem. u. Bioph.* is already on the ground. To duplicate *abstract journals* is waste of energy and money.

The figures showing how we may, by increasing the subscription list from 500 to 1,500 or 2,000, cut the price of some thirteen of our biological journals in half seem too good to be true. I would have been more convinced by these figures, had they been submitted by the respective publishers. Most biologists, undoubtedly, feel obliged to subscribe for journals that they actually cannot afford to take. But I do not think that even a considerable increase in the subscription lists would go far enough as a remedy. The number of subscribers to the *Amer. Jour. of Physiol.* is increasing, and so is the price of the journal. *Our research journals must be subsidized or endowed.* The attempt to run them as self supporting (or paying) propositions retards scientific progress.

The suggestion that men of no standing as biologists, but who have sufficient means and public spirit to pay annual dues of $25.00–$40.00, be elected to membership in the proposed Biolog. Soc'y is contrary to the best tradition of all the present societies, with the possible exception of the anatomists. These societies are primarily organizations of research men. The qualification for membership is not willingness or ability to pay the dues, but scientific attainments. I think a reorganization involving the abandoning of this ideal would be fatal. We see the practical results of such a policy in the virtual demise of several *Sec-*

tions of the A. A. A. S. Personally, I would rather ask men of means and public spirit to endow our research journals, than invite them to "pay the freight" in the form of society dues.

A. CARREL, *Rockefeller Institute.* Your letter of April 30th was duly received. I was very much interested to hear about Dr. Mathews' scheme. I shall give the matter ripe consideration and will write you again when I have had the time to do so.

E. G. CONKLIN, *Princeton Univ.* I have read with very great interest the plan for the organization of the Amer. Biolog. Soc'y, proposed by Albert P. Mathews, a reprint of which you recently sent me. I am in hearty accord with the plan at almost every point. It seems to me that some such organization as this is necessary, not only to avoid the extremely narrow specialization into which biology is now falling, but also for the promotion of biology in general, and of its various subdivisions in particular. If there is any serious objection to the loss of autonomy on the part of the various societies which are asked to coöperate, this difficulty might be overcome by granting the societies full autonomy in the matter of their membership and meetings, the general Biolog. Soc'y being a federation of the existing special societies. I shall be very glad to do all in my power to advance this or some similar plan which is greatly needed at present and which I am convinced the future will find indispensable.

C. B. DAVENPORT, *Station for Experimental Evolution, Cold Spring Harbor, L. I.* Before starting the Biolog. Abstract Jour. it would seem to be desirable to figure upon what it would involve in scope, number of pages per year and editorial work. As science does not recognize international boundaries, it would seem necessary to include abstracts of all papers published in all countries, and this would lead to overlapping of work, as we have already excellent abstracts in *Physiol. Centr., Zool. Anzeig., Zool. Record, Just's Bot. Jahresb.,* etc. If there is any part of the field not covered, it might be better to concentrate on it.

BRADLEY M. DAVIS, *Univ. of Penn.* It seems to me that the biologists are far more diversified in their interests than are the chemists and that it would be correspondingly more difficult to organize them satisfactorily into a single society. Many men would not care to pay the heavy dues when their interests are chiefly centered in one journal or at most a small group of journals. It would be very difficult to hold the interests together and what is now well done by the enthusiasm of each group separately would be poorly done when brought under an organization in common.

We are working definitely towards a closer affiliation of the biological societies as witnessed by the arrangements for the meeting next winter in Philadelphia when all of the societies will be together except the Bacteriologists and Botanists. This is distinct progress and it would seem to me best to give the present arrangements a longer trial before considering a plan so complicated as that proposed by Dr. Mathews. I am most heartily in favor of a close affiliation of the biological societies but believe that we shall make our best progress along the present lines of development.

MARTIN H. FISCHER, *Univ. of Cincinnati.* In answer to your letter relative to the organization of an Amer. Biolog. Soc'y permit me to say that I think the plan as suggested in Dr. Mathews' article is not alone a necessary one, but a feasible and good one. I hope that you will be successful in bringing about such needed reform.

G. W. FITZ, *Peconic, Suffolk Co., N. Y.* I am heartily in favor of the Mathews plan of coöperation to reduce the cost of the various journals to a more reasonable basis.

R. A. GORTNER, *Station for Experimental Evolution, Cold Spring Harbor, L. I.* I am heartily in favor of the Mathews plan for the amalgamation of the existing biological societies into one great organization. Any means by which greater coöperation can be obtained is to be approved. The Biolog. Abstract Jour. would be a most welcome addition to our library, and would supply a need that I have often felt. Personally I should prefer to see the membership fee $10 or $12 and to receive the Abstract Jour. and say two others, with the privilege of securing more journals by a graduated membership fee.

CHAS. W. GREENE, *Univ. of Mo.* I have read over again Dr. Mathews' suggestion for a larger Biolog. Soc'y. Without entering into all the details I must say that the relations to journal publications especially appeal to me. American biologists are paying too dearly for their scientific publications. Any scheme such as this that will largely increase the subscription lists with the corresponding decrease in cost per volume is to be commended. In three of the leading biological societies to which I belong I pay for membership and journals, by the present plan, from $23.00 to $28.00 per year, receiving four volumes. The journals do not allow plates outside of the cheaper process type. Lithography is out of the question and even color process plates must be paid for extra. There is also unnecessary duplication in the administrative expense of the societies. I am heartily in favor of any plan

in which the membership of the society must subscribe for its technical journals.

WINFIELD S. HALL, *Northwestern Univ. Med. School.* I have read Dr. Mathews' plan for the organization of the Amer. Biological Soc'y and consider it: (1) Desirable on general grounds; (2) feasible and practical; (3) advisable.

V. E. HENDERSON, *Univ. of Toronto.* I very strongly approve of the Mathews plan of reorganization of the biological societies, and feel quite sure that some such arrangement as proposed should be made. I am a little dubious as to whether the estimated costs of printing the journals are not underestimated, but feel sure that it should be possible to so increase the subscription lists as to very greatly diminish their cost. I have strongly urged, on several occasions, that the societies to which I belong should insist upon all their members subscribing to the journal which represents the society. I think that until the journals are more widely taken by the members, it will be quite impossible to develop a feeling of *comaraderie* which should prevail in the societies. I would be very willing to do anything I can to help in this movement.

YANDELL HENDERSON, *Yale Univ.* I am inclined to regard the plan favorably, providing it will reduce the burden of dues and subscriptions; otherwise I should oppose it.

A. W. HEWLETT, *Univ. of Mich.* Your communication in regard to the organization of an Amer. Biolog. Soc'y received. I would personally favor a federation of the biological societies of the country similar to the federation recently established of societies for experimental biology. The societies in the federation could hold meetings at the same time and in the same city, the secretaries could equalize the programs, and possibly the federation agree to support a certain number of journals.

R. G. HOSKINS, *Starling-Ohio Med. College.* I am in receipt of your request for an expression of opinion regarding the "Mathews Plan." The plan as a whole does not appear feasible in that it proposes to amalgamate interests too widely diverse—for instance, paleontology and pharmacology. The proposed society would have little to recommend it that is not shared by the Amer. Assoc. for the Adv. of Science and there is no need for two such organizations. Indeed, signs of dissolution of the latter are not lacking—and from just such causes as would be operative in the Mathews plan. As an effective working organization the proposed Federation of Experimental Biologists seems

preferable. Two of Mathews' proposals do, however, appeal to me: those to establish a biological abstract journal, and to seek endowments for it and various existent periodicals devoted to the publication of biological research. If the matter were properly brought to the attention of philanthropists who desire to support scientific research the necessary money would doubtless be forthcoming.

THEODORE HOUGH, *Univ. of Va.* I have been greatly interested in the proposals of Dr. Mathews for the organization of a Biolog. Soc'y of Amer. Naturally there are many details which have a very material bearing on the carrying out of such a scheme and these would have to be worked out very carefully before the success of the plan can be assured. The two great questions raised by Dr. Mathews' paper, as I see them are, (1) the advisability and feasibility of organizing all lines of biological work in the same manner as all kinds of chemical work have been organized; and (2) the advisability and feasibility of the journal feature of the plan.

I fully believe in the importance of organization of the various specialties which are sufficiently cognate in their subject matter to form a sufficiently homogeneous group. This condition of success was realized in the Amer. Chem. Soc'y and also in the Amer. Med. Assoc. Whether it can be realized among the biologists I am not quite so sure. The biological scientists have, perhaps more than the chemists or physicians, double allegiances. Anatomy, physiology, pharmacology, neurology are as closely related to the medical group as they are to zoology, botany, and psychology. Psychology is as closely related to pedagogics as to botany or infectious diseases. When this is the case I think it an open question whether the "chaos" of which Dr. Mathews speaks is an undesirable condition. Sometimes it is desirable—despite disadvantages, of course; sometimes it is not desirable. How it is in the present case *at the present time* I am unable to decide. But I do think the question should be very seriously considered by representatives of the various groups; for I think that the establishment of larger groups of sufficiently homogeneous subjects should go on as rapidly as possible to increase the influence of science in our national life.

As to the journal feature, I cannot quite see how the proposed scheme will successfully finance it. The estimate is that 500 copies of each of the journals mentioned will cost $50,000.00 to publish, while the scheme of dues calls for an income of some $60,000.00. But for each of the 2000 members to receive all these journals will mean the publication of 2000 copies of each, not 500. The additional 1500 copies

of each journal would mean at least an average of $700 to $900—for each journal—and the higher figure would probably be the only safe average one, since many of these journals publish more than one volume per year. This alone would add $15,000.00 to the expense of publication, and make a deficit of $5000.00 on the journal scheme alone, leaving nothing for the salaries of officers, and office expenses of the association as a whole or of its constituent societies.

It may be a contribution to the question to say that of the journals listed I subscribe at present to two, the annual cost of these being approximately $25 per year. At times in the past I have subscribed to four others, but have discontinued these subscriptions because I had to use that money to *get foreign physiological journals which our University library does not supply*. I imagine that many members of these societies are in the same position as myself; $25.00 or $30.00 per year is all that they can afford on American journals. They cannot subscribe to those outside their own line of work, because they must have the foreign journals in that line of work and their universities do not furnish them a complete set. I may add that my own expenditure for physiological journals (including the *Ergebnisse*) exceeds $120.00 per year, and of the assumed 2000 members I doubt whether 200 could afford this expenditure. If this is at all representative, the dues cannot be placed at a higher figure and, as I have shown above, these dues do not seem to cover the cost of publication which would be required.

May I add that it seems to me that there is presented here a most attractive field to men of means who desire to aid in the advancement of American science; namely, that of furnishing to the libraries of the universities of the country the journals in the subjects studied in those universities. Many of the great universities of course have these journals; many others doing good work, and subscribing to all they can afford, are unable for lack of means to subscribe to all. The university subscription might be supplemented so that each American university could offer its faculty and students the original work done in the past. Such a gift would have permanent value as few others would; it would advance science, and it would make the results of scientific work accessible to a far greater number of students.

W. H. Howell, *Johns Hopkins Univ.* I have read the Mathews plan for the organization of the Amer. Biolog. Soc'y with great interest. The plan to publish a Biolog. Abstract Jour. seems to me well worth while, but I should think that this end might be accomplished without constructing the machinery of a new society with annual meetings,

reading of papers, etc. Most of us I believe realize that we belong to too many societies. It is not possible to put a genuine interest and coöperation into all of them, except in the matter of paying dues, and the result I fancy is that a selection is made of a few, perhaps one or two, in whose work one can actively participate. So far as the encouragement of productive work is concerned, I am convinced that more good is done by the small specialized societies than by the more general ones, the congresses, etc. The latter may be useful in improving the public at large, but they do not permit the same opportunities for intimate and informal and stimulating contact of one worker with another. We have so many general societies now, that I do not contemplate with pleasure the formation of another. Therefore, as I said at the beginning, I should much prefer to see an organization formed to launch the Abstract Jour. and perhaps to finance the other journals, which shall be simply a business affair rather than a society for meetings.

Ida H. Hyde, *Univ. of Kan.* A properly conducted "Amer. Biolog. Abstract Jour." published biweekly, similar to the *Zentr. f. Physiol.*, would prove a valuable aid. It might be the organ of several societies; not only for abstracts of papers, but notices and advertisements of a purely scientific nature. The plan as I understand it, as outlined by Dr. Mathews, is too expensive to appeal to the majority of scientists, I fear.

D. E. Jackson, *Wash. Univ. Med. School.* I am deeply impressed not only with the feasibility but also with the very great desirability that some such plan as that proposed by Prof. Mathews should be put into effect in this country. The vastly increased momentum and penetrating power which much of our scientific work would receive would certainly yield valuable results. It seems to me that arrangements might be made whereby at least one foreign publication, probably the *Jour. of Physiol.*, might be included in the subscription list.

Edwin O. Jordan, *Univ. of Chicago.* I am heartily in favor of some such plan of federation as that proposed by Professor Mathews. The need for (1) the union of all biological interests, (2) a Biolog. Abstract Jour. and (3) some method of reducing the cost of scientific periodicals and the cost of management of scientific societies is more urgent today than it was five years ago.

C. F. Langworthy, *U. S. Dept. of Agric.* Perhaps I do not appreciate the needs of the situation, but it seems to me that it is more desirable to strengthen existing societies than to form new ones. As the

matter stands, any person whose interests are at all broad has the opportunity to join a fairly large number of societies and associations, each of which has something to offer. Local associations, which will bring men together, are of undoubted value, but I have a feeling that multiplying the number of national societies is less desirable than strengthening and enlarging the scope of existing bodies.

J. J. R. MACLEOD, *Western Reserve Univ.* I am heartily in favor of the formation of some such Biological Society as Mathews suggests. Before further steps are taken, however, I think that a very comprehensive canvass should be made of those who would likely be members, in order to ascertain (1) What annual subscription they would be willing to guarantee; (2) what journals they would take; (3) whether they think the scheme advisable.

W. J. MACNEAL, *N. Y. Post-Grad. Med. School.* The plan outlined by Professor Mathews for the organization of the Amer. Biolog. Soc'y deserves careful consideration, which I am unable to give to it at present. The biological societies and publications are already so numerous that the problem is much more complex than that of organizing the Amer. Chem. Soc'y. I hope that the project may meet with success.

GUSTAV MANN, *Tulane Univ.* In reply to your letter regarding the Mathews plan for the organization of an Amer. Biolog. Soc'y, I wish to express my complete sympathy with this movement, as the spreading of interest in biological problems will make people realize that biology is a question of chemistry and physics and of nothing else, and thus will allow the substitution of knowledge for dogma. I understand the two main objects to be (1) the establishment of an Abstract Jour. which will help not only the members of such a society but also many universities which at present are not in a position to subscribe to every journal, and thereby to make it possible for teachers to keep abreast with the work which is being done all over the world. (2) The second aim of consolidating the different journals and so obtaining them at a cheaper rate is one of almost equal importance.

I should like to make the following suggestions: that if the Amer. Biolog. Soc'y be organized, let there be some arrangement, with the consent of each society, whereby, for example, more purely chemical questions should be taken out of the *Jour. of Physiol.* and placed in the *Jour. of Biolog. Chem.*, and that papers dealing with neurological problems should be taken out of the *Jour. of Anat.* and be placed in the *Jour. of Compar. Neurol.*, etc.

Since Professor Loeb and myself started applying the principles of physical chemistry and colloidal chemistry to biology as a whole and to physiological chemistry in particular, so much work has been done along these special lines, that a separate chapter should be devoted to these subjects in the Jour. of Abstracts, if it is not possible to run a journal along the same lines as the *Zeit. f. Chem. u. Ind. d. Kolloide.*

I realize the importance of getting money for putting the project on a sound financial basis. I do not, however, like the idea of discriminating against foreign subscribers by making them pay the same rate as libraries pay (see page 265 of Mathews' article, fifth line from the bottom). It should be possible for foreigners to join the proposed Amer. Biolog. Soc'y and to share all the advantages of this society. Special stipulation should be made whereby libraries will be excluded from membership.

I do not quite understand what is meant by a "Jour. of Biolog. Industries." This might mean anything from the making of vaccines, or filters, or patent medicines to shoe leather.

On the whole I believe that the Wistar Institute of Anatomy should be given full charge of all the publications of the proposed Amer. Biolog. Soc'y and that all the journals mentioned on page 264, including the Biolog. Abstract Jour., should be available for $25.00 a year.

E. G. MARTIN, *Harvard Med. School.* I approve the Mathews plan for a common Biolog. Soc'y in its general outline, and in the proposed feature of a Biolog. Abstract Jour. If, incidentally, the cost of the other journals can be decreased, so much the better. I do not believe, however, that the plan of a combined subscription for all the journals is good. No biologist who has access to a general library has shelf room to give to thirteen journals, half of which are wholly out of his line, and most of the others of only occasional use.

J. F. MCCLENDON, *Cornell Univ. Med. College.* I would like to see the "Mathews Plan" for an Amer. Biolog. Soc'y in operation.

A. R. MOORE, *Univ. of Cal.* The "plan for the organization of the Amer. Biolog. Soc'y" seems to me an excellent one. I shall be glad to support such a scheme most heartily.

MAX MORSE, *Trinity College.* The fact that other scientific groups such as the chemists have been successful in organizing must not be taken as a basis for believing that the biologists would be likewise successful. The attempt which has been made in the past to correlate biological societies has not been successful. This is due to the fact,

mainly, that the "pure" biologists will not coöperate with the workers in applied lines and to a strong tendency, also, to subdivide the field and segregate the investigators into their several societies. The chemists are successful in that they associate the practical with the pure aspects of their science and it is principally the former group which maintain the organization, owing to their financial abilities being enhanced over those of the "pure" chemist. Could there be a Biologists' Club maintained as successfully as the Chemists' Club on 41st St., N. Y.? There might if the pure and applied departments would coöperate. In much the same way, the biologists could maintain a national organization if they coöperate with their "applied" brethren, who are often more fully equipped with this world's goods than others. The first thing to do is to found a national organization with minimum dues, after which the problem of an abstract and other journals could be taken up. As to the former, I should like to see the *Zent. f. Zool. allg. u. exper. Biolog.*, of the press of B. G. Teubner, subsidized and adapted to Amer. readers, for it is already founded and organized. Whatever is done, it is important to establish a paid permanent secretary-treasurer with adequate office assistance to correlate the various interests and to take care of the financial and business end of the venture; then the journal question could be worked out when the data as to subscriptions, dues, etc., are in.

Raymond C. Osburn, *N. Y. Aquarium.* I am heartily in favor of any scheme that will bring the workers in the various fields of biological research into closer touch. The Amer. Chemical Soc. is no longer an experiment and a biological society based on a similar plan of organization ought to be equally successful. Especially does the plan for the *Abstract Journal* seem commendable, as there is nothing covering this field and nothing could be more useful. Even if the plan were carried no farther than the issuing of such a journal it would be worth while, and very much so, in my mind. Moreover, coming from the ranks of the biological chemists, I believe there is more chance of such a plan being successful than if it had emanated from the zoologists or botanists, since biochemistry is more and more the common meeting ground for all investigators.

William H. Park, *N. Y. City Dep't of Health.* I have read over the Mathews plan for the Biolog. Soc'y. If it could be carried out, I think it would make a most useful society. It seems to me, however, that it will be very difficult to get the individual societies to merge themselves in the new one. Such a society as the Amer. Assoc. of Pathol.

and Bacteriol. would probably prefer to keep separate, although it might be better to unite. It certainly is a most interesting proposition that you submit for expression of opinion.

G. H. PARKER, *Harvard Univ.* I approve of Mathews' plan in general for the establishment of an Amer. Biolog. Soc'y and I would couple with that the suggestion that such a society should replace the "Naturalists," in that this society might well be abolished and the Biolog. Soc'y be made a new center for the smaller societies to gather round.

RICHARD M. PEARCE, *Univ. of Penn.* I do not feel that I can truthfully say that I favor the plan which Dr. Mathews suggests for an Amer. Biolog. Soc'y. The men in pathology, clinical medicine, and surgery, who are interested in experimental pathology, are now forming an organization to be known as the Soc. of Exper. Pathology. This will meet at Christmas with the Amer. Physiol. Soc., the Soc. of Biolog. Chem. and the Soc. for Pharmacol. and Exper. Therap. It will look forward eventually to becoming a constituent member of the Federation of the Amer. Societies for Exper. Biology.

This affiliation will give all these groups a point of contact with the physiologists, chemists and pharmacologists at Christmas, and, on the other hand, in the Spring it will have a point of contact with the Assoc. of Pathol. and Bacteriol. and the Assoc. of Amer. Physicians. Thus, all the needs of experimental pathology will appear to be served. You see that with this arrangement there is little need for the formation of other affiliations.

RAYMOND PEARL, *Maine Agric. Exper. Station.* It seems to me that the plan of Prof. A. P. Mathews for the organization of an Amer. Biolog. Soc'y, to which attention was called in your circular letter, has much to commend it. Personally I should very much like to see such a consolidation of the various scattered biological societies accomplished. The point made by Professor Mathews that the present condition of affairs renders the science of biology as a whole less effective in the community than it ought to be is a strong argument in favor of affiliation.

Although in entire sympathy with the general features of the plan, as outlined by Professor Mathews, I feel somewhat uncertain as to whether it will be possible practically to bring about at the present time any affiliation of the biological societies, which shall be at once widely inclusive in its scope and closely articulated in its organization—and both of these things seem to be necessary if the plan is to have any real

success. The primary reason for scepticism as to the possibility of bringing about a successful affiliation of the sort proposed is that, historically, it is a fact that various earlier attempts in the same direction in this country have either failed at once, or at best had only a short life. If the present plan, with its extensive publication program, is to succeed, a reasonable assurance of permanency is necessary before even a beginning can be made. Can a sufficiently close agreement on matters of general and special policy be obtained in the different biological societies to guarantee the necessary permanence to the undertaking?

There is one matter of detail to which attention should be called. On page 262 of the Mathews paper stands this sentence: "All persons sufficiently interested in the progress of biology to pay the dues of the Society should be eligible for membership." It should be noted that this proposal is directly contrary to the rules of admission of many of the existing biological societies which it is hoped to affiliate. To speak more particularly of the Amer. Soc. of Zool., I think it altogether unlikely that a majority of that society would favor making its only qualifications for membership "interest in the subject" and "ability to pay the dues." This society has consistently maintained a high standard in regard to the qualifications necessary for membership. The reason for this policy is, I take it, that the society is an organization of professional zoologists desirous of meeting together to discuss the more technical phases of their subject. There are a vast lot of people in the country who are decidedly interested in one phase or another of zoology who would neither be able to get any particular profit themselves out of the meetings of the Amer. Soc. of Zool., nor to contribute anything of especial interest or value to those meetings (so far as concerns the present professional members). Yet it is on just that class of "generally interested" membership that the main *financial* foundation of the Amer. Biolog. Soc'y would rest, if I correctly interpret Professor Mathews' fiscal policy.

If it be urged that the Amer. Chem. Soc'y is an example of the successful operation of a scientific society without special requirements for membership beyond an interest in the subject, it is fair to point out that there is a real difference between chemistry and biology in regard to this point. To be a chemist of whatever sort or degree, or even to be interested in chemistry, implies some technical knowledge and experience with the fundamentals of the science. Interest in biology carries no such implications. There are a great many people who are, or think they are, interested in biology who have not the slightest real knowl-

edge of the fundamentals (speaking in a technical sense) of any one of the biological sciences. For these reasons it seems to me that the organization of an Amer. Biolog. Soc'y cannot proceed on quite the same basis as the organization of the Amer. Chem. Soc'y.

I am in heartiest sympathy with the general features of the proposed plan and should very much like to see an arrangement worked out whereby there could be a closer affiliation between the various biological societies of the country than now exists.

"PHARMACOLOGIST." I feel that there is a great need for an abstract journal of a somewhat different field from that suggested. One, namely, that would mention or abstract all articles dealing with the administration of drugs to animals (and man), including in its scope, pharmacology, toxicology, therapeutics and veterinary medicine. Articles dealing merely with the treatment of disease and not treating of the physiologic action of the drug should merely be indexed. Pharmacy and the chemistry of drugs should be included as far as such articles were of scientific interest. The action of antiseptics on germs *in vitro* could better be left out as they are handled elsewhere. Local action of drugs need not be considered when it is merely action on a parasite, or cleansing, etc. Salt action should be included as well as articles dealing with the physics of absorption, etc. (where absorption of drugs is implied). None of the *Centralblatts* or other journals cover this field in a way which is at all satisfactory and such a journal should have a widespread demand.

E. W. ROCKWOOD, *State Univ. of Iowa.* While I should certainly favor any plan which would advance biological interests, I am not certain that the Mathews plan would be the best. The case of the Amer. Chem. Soc'y is not a parallel one. When that was organized, and for a great many years afterwards, there were no strong societies occupying any part of the field. There are a number of them in biology and the field is not only covered but perhaps more than covered, that is, their work overlaps in many instances. In the Amer. Chem. Soc'y, membership in the society carries with it membership in all sections and divisions. I do not think there is any chance of such an arrangement prevailing in a Biolog. Society. In other words the present organizations would keep their identity and we should have a rather loose affiliation.

I do not think there is any possibility of the majority of the membership of 2000 being willing to pay $25 to $30 per year to receive all

the journals listed. Most of the men eligible for membership are probably connected with some institution where the journals are on file and while many would like all of them, more would regard it as an unnecessary expense. At $10 for a good Abstract Jour., with one or two others, more men would think the object worth while.

I should want the estimated costs of journals worked out by experts in such lines. From a number of years' experience in the Council of the Amer. Chem. Soc'y I have found that it is the easiest thing in the world to overestimate resources in advance, and that, where in advance it seems certain that a certain membership at a certain rate will leave a comfortable margin, in retrospect it is apt to be found that the expenses have an uncomfortable way of mounting faster than the income.

All this does not mean that I am opposed to a general society. If the majority, or a large number, of biological workers do want it, I think it should be delegated to a larger committee to go more into detail, utilizing the experience of others, like the Amer. Chem. Soc'y.

TORALD SOLLMANN, *Western Reserve Univ.* In reply to your inquiry as to Mathews' plan, it seems to me that object 1 is already accomplished by the formation of the Federation of Amer. Societies for Exper. Biology. I believe that this is as far as it is necessary to go at present. In my opinion it would be a great mistake to increase the expense of the societies to their members. Every such increase would make membership, and attendance at the meetings, more difficult to some men. It seems to me more desirable that the members should attend the meetings than that they should receive additional journals, to which they generally have access in the departmental libraries.

COLIN C. STEWART, *Dartmouth Med. School.*—I do not know how I can any better show my hearty approval of the Mathews plan than by promising my full subscription as soon as the scheme may go into operation. The need for something of the nature of "Biolog. Abstracts," the burden of subscription expenses (or what is worse, the necessity of doing without journals), and the number of journals, grow greater year by year.

EDWARD L. THORNDIKE, *Teachers College, Columbia Univ.* I think some such plan as the Mathews plan is desirable. You should include the new *Jour. of Animal Behavior.* Also I think $5,000 a year should be allowed to pay for the actual reviews written for the "*Abstracts.*"

J. L. TODD, *McGill Univ.* If the enormous difficulties in the way of consolidating and operating the societies with biological interests could

be satisfactorily overcome, the proposed merger would doubtless prove useful and economical.

H. W. WILEY, *Bureau of Foods, Sanitation and Health, Washington, D. C.* I have read with interest the plans for the reorganization of the American biological societies, or perhaps better the organization of an Amer. Biolog. Soc'y. Of course the term "Biological" is a most comprehensive one and would practically include every science or activity relating to live organic processes. More particularly I suppose it would include those sciences enumerated under "Details of Organization." If all these societies could be united into one great organization, it would be highly desirable.

The scheme proposed is very much like that which has made the Amer. Chem. Soc'y the great organization which it is. I am especially favorable to the establishment of local sections and the scheme of affiliation of the present societies. I should think that all the journals that are published ought to be collected into one journal as has already partly been done with the chemical publications of the country.

This method is favorably commented upon by Dr. Mathews on page 262 of the proposed plan of organization. I believe that the organization of such a society would promote efficiency and economy. I think the two thousand members would come over easily if the plan of affiliation were agreed upon; and especially I believe that the cost of the literature would be materially reduced. Upon the whole I am quite favorably impressed with the proposed plan.

F. C. WOOD, *Columbia Univ.*—In answer to your request for a discussion of the Mathews plan for the organization of an Amer. Biolog. Soc'y, I would say that anything which will lead to a concentration of the widely scattered interests in biology will have my hearty approval. We already have too many small societies and do not give them sufficient support. There would be many advantages in the proposed Biolog. Soc'y becoming the Biolog. Sec. of the Assoc. for the Adv. of Science, but I think there would have to be some line of cleavage inside the Biolog. Soc'y; that is, experimental medicine, pharmacology, pathology, and bacteriology might form one group, taking over the membership of the Assoc. of Amer. Pathol. and Bacteriol. and the Soc. of Amer. Bacteriol., and of a recently formed Soc. for the Promotion of Scientific Med., and preventing the formation of a proposed Soc. for Exper. Pathology.

Anatomy, physiology, and biochemistry could form another group; and zoology, botany, and psychology (?) a third. No one wants to

wade through or pay for journals in which he is not interested, and the zoologists and botanists are such prolific gentlemen that the journal might be swamped with their productions. The Assoc. of Amer. Pathol. and Bacteriol. already has an excellent journal, the *Jour. of Med. Research,* which it partly supports, and which might serve as a nucleus for further expansion. I do not doubt that you would have very considerable financial support from societies like the New York, Philadelphia, and Chicago Pathological Societies, if a good journal were published under the auspices of the Biolog. Soc'y in which their proceedings could appear. We have at present in this country no journal which can afford to take important papers with many illustrations on purely morphological pathology, and the time is coming when we shall need a good abstract journal on physiology, pharmacology, bacteriology, and pathology; but there would be absolutely no profit in it and it would be very expensive to run. The German situation is, as you know, perfectly hopeless. New journals are appearing every few months and no one can afford to subscribe for them all. Many of the articles published are of poor quality and, as the good ones are scattered through many journals, it is almost impossible to have access to them all except through a library.

Anything which would lead to a fusion of journals, and increase the interest in society meetings would meet with my hearty approval. Anything which would lead to the establishment of new journals paralleling those already in existence would, I think, be distinctly a step backwards, and would postpone the time when America can stand on its own feet in those special phases of biology such as experimental pathology, bacteriology, and pharmacology.

ROBERT W. YERKES, *Harvard Univ.* I have read with keen interest both your letter and the Mathews plan for the organization of an Amer. Biolog. Soc'y. Some three or four years ago I discussed this general subject with Dr. Mathews and at that time, as now, I was enthusiastically in favor of attempting to do something in the directions indicated by your circular.

I desire to express myself as eager for the carrying out of some such plan as Dr. Mathews has outlined, and I should hope that we might go even further than he has suggested in that we should organize a scientific press for the handling of our biological journals. I stand ready to subscribe thirty dollars ($30) a year at any time as membership dues, and I think I might be willing to pay even fifty dollars ($50), supposing that all of the Amer. biolog. journals were supplied and I

were relieved from membership dues in several biological societies to which I belong. There would, I am sure, be somewhere between fifteen and twenty journals that would have to be included in our complete list.

I wish you would regard me as an enthusiastic supporter of the idea and one who is willing to further it.

Antigens

Woman's cause is man's; they rise or sink together, dwarfed or godlike, bond or free.—*Tennyson.*

In healing men, as in other lines of industry, the first requisite is to know how. To know how is the essence of science.—*Jordan.*

The great object in trying to understand history—political, religious, literary or scientific—is to get behind men and to grasp ideas.—*Acton.*

The only important difference between the practical doctor and the scientific doctor is that the patients of the practical doctor are more likely to die.—*Minot.*

The most urgent problems of medical education to-day relate to the teaching of the clinical subjects. It is the so-called theoretical or laboratory subjects which are now taught most practically, whereas the practical branches are taught most theoretically.—*Welch.*

It is the little fellow who struts, the minor actor who is worried about the spot light, the man of small caliber who demands the chief place at the feast. The gang foreman walks with an air; the superintendent of the plant is too busy to give thought to the appearance that he is making.—*C. H. Esty.*

The pursuit of fame is purely a gambling enterprise. If any one has a mind to be famous, by all means let him "go to it"—this is a free country; but he ought clearly to keep in view the fact that he is not engaged in legitimate or honest work but in an affair that is wholly luck—as much so as if he were pursuing fortune at the gaming tables of French Lick or Monte Carlo. There are no known laws for becoming noted, even after the human race has been experimenting for generation after generation. If your card turns up you win; if the little ball stops on your number, you are it. That's all.—*Crane.*

BOOKS RECEIVED

The BIOCHEMICAL BULLETIN promptly acknowledges here the receipt of publications presented to it. From time to time, selections will be made for review on pages of the volume to be appropriately indicated here. Reviews will be matter-of-fact statements of the nature and contents of the publications referred to, and will be intended *solely to guide possible purchasers.* The wishes or expectations of publishers or donors of volumes will be disregarded, when they are incompatible with our convictions regarding the interests of our colleagues. *The sizes of the printed pages are indicated, in inches, in the appended notices.*

Diabetes: Its pathological physiology. (*One of the International Medical Monographs.*) By John J. R. Macleod, professor of physiology, Western Reserve University, Cleveland, O. Pp. 224—4 × 7; $3.00 net. Edward Arnold, London; Longmans, Green & Co., New York, 1913.

Practical physiological chemistry. By Sidney W. Cole, demonstrator of physiology, Trinity College, Cambridge. Third edition. Pp. 230—4 × 6½; 7s. 6d. net. W. Heffer & Sons, Ltd., Cambridge, Eng., 1913.

Glycosuria and allied conditions. By P. J. Cammidge. Pp. 467—4×6¾; $4.50 net. Longmans, Green & Co., New York; Edward Arnold, London, 1913.

The chemical constitution of the proteins: Part II, Synthesis, etc. 2d ed. (One of the *Monographs on Biochemistry.*) By R. H. A. Plimmer, Univ. reader and ass't prof. of physiological chem., University Coll., London. Pp. 107—4⅜ × 7½; $1.20 net. Longmans, Green & Co., 1913.

Studies from the Rockefeller Institute for Medical Research. Volume XVI; 1913. (29 reprints.)

Collected papers: Institute of Physiology, University College, London. Edited by Ernest H. Starling, Jodrell professor of physiology. Volume XVII; 1912–13. (32 reprints.)

Collected papers: Physiological Laboratory, Kings' College, University of London. Edited by W. D. Halliburton, professor of physiology. Volume XII; 1913. (12 reprints.)

Sigma Xi Quarterly. No. 1, Vol. 1 (March, 1913). Pp. 30. Editorial committee: J. McK. Cattell, D. C. Miller, H. B. Ward, S. W. Williston. Published by the Society of the Sigma Xi, H. B. Ward, corresponding secretary, Champaign, Ill.

Bulletin of the American Home Economics Association. No. 1, Series 1 (Nov., 1912). Published quarterly by the American Home Economics Association, Benjamin R. Andrews, secretary, 525 W. 120th St., New York City.

Collected papers: Laboratory of physiological chemistry, Sheffield Scientific School, Yale University. 1911–1912. (35 reprints.)

Practical physiological chemistry. *A book designed for use in courses in practical physiological chemistry in schools of medicine and of science.* By Philip B. Hawk, professor of physiological chemistry and toxicology in the Jefferson Medical College of Philadelphia. Fourth edition, revised and enlarged. Pp. 475—4½ × 8; $2.50 net. P. Blakiston's Sons & Co., Philadelphia, 1912.

The protein element in nutrition. (One of the *International Medical Monographs.*) By Major D. McCay, professor of physiology, Medical College, Calcutta. Pp. 216—4 × 7, with 8 full page portraits of human subjects; $2.00 net. Longmans, Green and Co., New York; Edward Arnold, London, 1912.

Oxidations and reductions in the animal body. (One of the *Monographs on Biochemistry.*) By H. D. Dakin, The Herter Laboratory, New York. Pp. 135—4½ × 8; $1.40 net. Longmans, Green and Co., 1912.

Researches on cellulose. III (1905–1910). By C. F. Cross and E. J. Bevan. Pp. 173—3½ × 6; $2.50 net. Longmans, Green and Co., 1912.

OFFICERS OF THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY, 1912–1913*

OFFICIAL REGISTER, MAR. 31, 1913

WILLIAM J. GIES: *Professor and Chairman of the Staff;* Consulting chemist, New York Botanical Garden; Pathological chemist, First Division, Bellevue Hospital; Member of the Faculties of N. Y. Teachers College and N. Y. College of Pharmacy. [B.S., Gettysburg College, 1893 and M.S., 1896; Ph.B., Yale University, 1894 and Ph.D., 1897. Instructor, 1898–'02; adjunct professor, 1902–'05; professor, 1905–.]

PAUL E. HOWE: *Assistant Professor.* [B.S., University of Illinois, 1906; A.M., 1907 and Ph.D., 1910. Assistant Professor, 1912–.]

ALFRED P. LOTHROP: *Associate and Departmental Registrar.* [A.B., Oberlin, 1906 and A.M., 1907; Ph.D., Columbia, 1909. Assistant, 1908–'09; instructor, 1909–'12; associate, 1912–.]

NELLIS B. FOSTER: *Associate;* Associate Physician, New York Hospital; Chemist, St. Luke's Hospital. [B.S., Amherst College, 1898; M.D., Johns Hopkins University, 1902. Instructor, 1906–'08; associate, 1908–.]

WALTER H. EDDY: *Associate and Secretary of the Staff.* [B.S., Amherst College, 1898; A.M., Columbia, 1908 and Ph.D., 1909. Assistant, 1908–'10; associate, 1910–.]

HERMAN O. MOSENTHAL: *Associate;* Assistant Attending Physician, Presbyterian Hospital; Assistant Physician, Vanderbilt Clinic; Instructor in medicine. [A.B., Columbia, 1899 and M.D., 1903. Assistant, 1908–'09; instructor, 1909–'12; associate, 1912–.]

EMILY C. SEAMAN: *Instructor.* [B.S., Adelphi College, 1899; A.M., Columbia, 1905 and Ph.D., 1912. Tutor, 1909–'10; instructor, 1910–.]

MAX KAHN: *Instructor;* Director of the chemical and physiological laboratories of Beth Israel Hospital. [M.D., Cornell University Medical College, 1910; A.M., Columbia, 1911 and Ph.D., 1912. Instructor, 1912–.]

LOUIS E. WISE: *Instructor.* [A.B., Columbia, 1907 and Ph.D., 1911. Instructor, 1912–.]

EDGAR G. MILLER, JR.: *Assistant,* 1911–. [B.S., Gettysburg College, 1911.]

FREDERIC G. GOODRIDGE: *Assistant,* 1912–. [A.B., Harvard University, 1897; M.D., Columbia, 1901.]

ARTHUR KNUDSON: *Assistant,* 1912–. [A.B., University of Missouri, 1912.]

ETHEL WICKWIRE: *Assistant,* 1912–. [A.B., Tri-State College, 1909.]

TULA L. HARKEY: *Assistant,* 1912–. [A.B., Colorado College, 1909.]

BENJAMIN HOROWITZ: *Assistant,* 1913–. [B.S., Columbia, 1911 and A.M., 1912.]

CHRISTIAN SEIFERT: *Laboratory assistant,* 1898–.

STELLA WALDECK: *Recorder,* 1908–.

BLANCHE E. SHAFFER: *Laboratory assistant,* summer session, 1912.

JOSEPH S. HEPBURN: *University fellow,* 1912–'13. [A.B., Central High School, Philadelphia, 1903 and A.M., 1908; B.S., University of Pennsylvania, 1907 and M.S., 1907.]

* The work of the department was inaugurated in October, 1898, by Prof. R. H. Chittenden (lecturer and director), Dr. William J. Gies (instructor), Messrs. Alfred N. Richards and Allan C. Eustis (assistants), and Christian Seifert (laboratory assistant).

COURSES OFFERED BY THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY, 1912–13

(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. ELEMENTARY ORGANIC CHEMISTRY. (*First half year. Medical School.*) Introductory to course 102 (52). (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Drs. Wise and Goodridge, and Messrs. Miller and Knudson.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101(2)—Grad. GENERAL BIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Prof. Gies, Dr. Lothrop and Messrs. Miller and Knudson.

101(2)—B. T. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* (*All year. Medical School.*) L, 1 hr. Lw, 4 hr. Dr. Eddy.

101:102—T. C. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Each half year. Teachers College, School of Practical Arts.*) L, 2hr. R, 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Dr. Seaman and Misses Wickwire and Harkey. (This course is designated "Chemistry 51" and "Household Arts Education 125" in the Teachers College Announcement.)

This course is designated "Chemistry s 51" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies, Dr. Seaman and Miss Shaffer.

102 (52)—Med. GENERAL PHYSIOLOGICAL CHEMISTRY. (*Second half year. Medical School.*) *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Dr. Wise, and Messrs. Miller and Knudson.

This course is designated "*S*—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Dr. Smith.

104. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (*Second half year. Teachers College, School of Practical Arts.*) L, 1 hr. Prof. Gies. (This course is designated "Chemistry 52" in the Teachers College Announcement.)

209–210. CHEMISTRY OF NUTRITION. (*All year. School of Pharmacy. Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

213–214. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (*All year. Teachers College, School of Practical Arts.*) L, 1 hr. Lw, 5 hr. Prof. Howe, Dr. Seaman and Mr. Horowitz. (This course is designated "Household Arts Education 127" in the Teachers College Announcement.)

217–218. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS AND URINARY ANALYSIS IN GENERAL. (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Profs. Gies and Howe, Dr. Lothrop, and Messrs. Miller and Hepburn.

219–220. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* (*All year. Medical School.*) L, 2 hr. Lw, 14 hr. Profs. Gies and Howe, and Dr. Lothrop.

221–222. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* (*All year. Medical School.*) L, 2 hr. Lw, 14 hr. Prof. Gies.

223–224. NUTRITION IN DISEASE. (*All year. Medical School.*) L, 1 hr. Profs. Gies and Howe, and Drs. Foster, Mosenthal, Kahn and Goodridge.

225–226. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. (*All year. Medical School.*) Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Howe, and Dr. Lothrop.

TOXICOLOGY

231–232. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. (*All year. Medical School.*) Lw, 6 hr. Prof. Gies and Mr. Miller.

BOTANY

235–236. CHEMICAL PHYSIOLOGY OF PLANTS. (*All year. New York Botanical Garden.*) L, 1 hr. Lw, 5 hr. Prof. Gies.

BACTERIOLOGY

241–242. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Prof. Gies.

SANITATION

105. SANITARY CHEMISTRY. (*Second half year. Teachers College, School of Practical Arts*). L, 1 hr. Lw, 3 hr. Dr. Seaman and Miss Harkey. (This course is designated "Chemistry 57" and "Household Arts Education 129" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301–302. BIOCHEMICAL SEMINAR. (*All year. Medical School.*) 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance, in any of the departmental laboratories.

LABORATORIES FOR ADVANCED WORK IN BIOCHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College, New York Botanical Garden and Bellevue Hospital. Each laboratory is well equipped for research in nutrition and all other phases of biological chemistry.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all past and present workers in the Department.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds scientific meetings regularly on the first Fridays in December, February and April, and on the first Monday in June. These meetings are open to all who may be interested in them.

SUMMER SCHOOL COURSES

Summer session courses are mentioned in the foregoing references to Courses 101–102 and 102 (52). Prof. Gies will have charge of both courses next summer. He will also conduct a special lecture course in nutrition. The laboratories will be open for research throughout the summer.

ANNOUNCEMENTS

Professional Assistance Offered to Biological Chemists

The Columbia University Biochemical Association will be glad to coöperate confidentially with all who desire the services of biological chemists and with all who seek positions in biological chemistry.

Address inquiries to William J. Gies, 437 West 59th St., New York.

Journalistic

NEW JOURNAL. *Physiological Researches.* To appear at irregular intervals. Edited by *Burton E. Livingston,* Manager, Johns Hopkins University; *Daniel T. MacDougal,* Carnegie Institution of Washington; *Herbert M. Richards,* Columbia University. "The recent rapid advance of physiological science has been accompanied by a realization of the community of interest and uniformity of method which characterize the physiology of plants and of animals, and it has seemed highly desirable that the general physiological field thus indicated should possess an organ of publication in which its more comprehensive and technical papers might appear. This need is emphasized by the fact that present facilities for publication in physiology are generally taxed beyond their capacity and papers are consequently subject to long delays in appearance. It has therefore been decided to inaugurate a new series of scientific papers which will embrace contributions towards the advance of fundamental physiological knowledge."

"The plan of publication of the new series, for which the title of *Physiological Researches* has been adopted, is one in which practical ownership is vested in the contributors. It is hoped that the project will receive the interest and support of biologists of all classes. (Each volume will contain about 450 pages; each number will contain but a single contribution; and the numbers will be issued irregularly). Publication of the first contribution may be expected in a short time. Subscriptions will be received by the volume, the price being $5.00 per volume, payable in advance. Subscriptions to volume I, which are received prior to the date of publication of the first research, may be made at the reduced price of $4.00. At the date of the appearance of the first research the price will automatically become the regular one. Remittances should be made payable to *Physiological Researches,* and *all correspondence should be addressed to Physiological Researches, Station N, Baltimore, Maryland, U. S. A.*" (Editors' announcement.)

REDUCED SUBSCRIPTION PRICE OF THE JOURNAL OF BIOLOGICAL CHEMISTRY. The directors of the *Journal of Biological Chemistry* have announced that "beginning with the February issue of 1913 (Vol. 14, No. 1) the subscription price of the *Journal* to domestic subscribers will be reduced from $4.00 to $3.00 per volume; to foreign subscribers, $3.25. Any one engaged in biochemical work who subscribes for the *Journal* at this rate (beginning with Vol. 14) may secure Volumes 1–13 for $20.00, plus cost of transportation. The price at which a complete set has hitherto been sold is $50.00. Subscribers for the Journal who wish to complete their files may secure early volumes for $1.50 each, plus cost of transportation. Address: Alfred N. Richards, Secretary, University of Pennsylvania.

Meetings of Societies and Congresses

TENTH INTERNATIONAL CONGRESS OF AGRICULTURE: Ghent, Belgium, *June 8–13*. Secretary-general, Dr. P. de Vuyst, 22 Avenue des Germaines, Brussels. *American committee:* Dr. L. O. Howard, member of the International Commission on Agriculture and chief of the Bureau of Entomology; and Dr. A. C. True, director, Mr. John Hamilton, specialist in farmers' institutes, Dr. C. F. Langworthy, chief of nutrition investigations and Dr. J. I. Schulte, assistant agriculturist, of the Office of Experiment Stations.

SECOND INTERNATIONAL CONGRESS FOR THE TEACHING OF HOUSEHOLD ECONOMY: Ghent, Belgium, *June*. General Secretary, Miss Delcu, 19 Rue Willems, Brussels.

THIRD INTERNATIONAL CONGRESS OF THE ASSOCIATIONS OF AGRICULTURAL WOMEN: Ghent, Belgium, *June*. General Secretary, Miss Van Aarschot, 38 Rue du Pépin, Brussels.

AMERICAN MEDICAL ASSOCIATION, Annual meeting: Minneapolis, Minn., *June 17–20*. General secretary, Geo. H. Simmons, 535 Dearborn Ave., Chicago.

GENERAL MEETING OF THE INTERNATIONAL ASSOCIATION OF BOTANISTS: Copenhagen, *June 27*. Secretary-general, J. P. Lotsy, Haarlem, Holland.

CONGRÉS INTERNATIONAL POUR LA PROTECTION DE L'ENFANCE: Brussels, *July 23–26*. General Secretary, Henry Jaspar, 93 Avenue de la Toison d'Or, Brussels.

SEVENTEENTH INTERNATIONAL CONGRESS OF MEDICINE: London, *Aug. 6–12*. General secretary, Dr. W. P. Herringham, 13 Hinde St., London, W.

FOURTH INTERNATIONAL CONGRESS ON SCHOOL HYGIENE: Buffalo, N. Y., *Aug. 25–30*. Secretary-general, Prof. Thomas A. Storey, College of the City of New York.

NINTH INTERNATIONAL PHYSIOLOGICAL CONGRESS: Groningen, Holland, *Sept. 2–6*. American Secretary, Prof. W. T. Porter, Harvard Medical School.

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BIOCHEMICAL BULLETIN

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AN INVESTIGATION TO DETERMINE THE ACCURACY OF A MODIFIED MEIGS METHOD FOR THE QUANTITATIVE DETERMINATION OF FAT IN MILK, WITH A DESCRIPTION OF AN IMPROVED FORM OF APPARATUS

WALTER LEWIS CROLL

(WITH PLATE 7)

(Robert Hare Chemical Laboratory, University of Pennsylvania)

Owing to the importance of determining accurately and quickly the amount of fat in a given quantity of milk, special efforts have been made to devise a method that would embody both these requisites. These efforts have resulted in methods based upon many principles, and some are brilliant examples of chemical and mechanical ingenuity. The separation of the fat has been accomplished by the use of differences in specific gravity, by saponification, extraction with ether, colorimetry, and absorption; but, to the present time, no rapid method, which is sufficiently accurate for legal, pediatric and biological work, has been devised. Today the most accurate method and, indeed, the only one suitable for most legal work, is the Adams paper-coil method, with the Soxhlet extraction apparatus. This method, while extremely accurate, requires at least twenty-four hours for its execution and considerable care in its manipulation.

A COMPARISON OF WELL KNOWN METHODS

The Adams method. In this investigation the Adams method was carried out as follows: First a homogeneous mixture was

obtained, then some of it was placed in a small Erlenmeyer flask having a 5 c.c. bulb-pipette fitting snugly in its mouth. About 4 or 5 gm. were removed with the pipette and dropped into the center of a coil of specially prepared, fat-free, absorbent paper. The sample was weighed by difference. The paper coil containing the milk was transferred to a hot-air oven, the temperature of which was constantly below 100° C., and permitted to dry for three or four hours, after which it was removed to a glass desiccator, kept there at least twelve hours, over conc. sulfuric acid, and then transferred to a Soxhlet extraction apparatus, where it was extracted for twelve to sixteen hours with absolute ethyl ether which had been redistilled after standing over metallic sodium for from five to six days. After completing the extraction, the excess of ether was distilled into the upper part of the apparatus and the ethereal solution of fat quantitatively transferred to a weighed evaporation dish, using redistilled ether as a rinsing fluid. The ether was evaporated over a safety water-bath (the dish being protected from dust by an inverted funnel), at a temperature below 33° C., to prevent loss from ebullition of the ether. After the ether had been evaporated, the fat was placed in a hot-air oven and dried for three hours at a temperature ranging between 95°–100° C., then dried over sulfuric acid to constant weight, which usually required about twenty-four hours. The weight was then recorded and the percentage of fat calculated.

Of the above method there are many modifications, but none of them are rapid methods.

Miscellaneous methods. In the method devised by Soxhlet,[1] the drying of the milk is accomplished by the use of gypsum. Froidevaux[2] precipitates the protein and fat by means of acetic acid and calcium phosphate. The precipitate is collected quantitatively on a filter paper, dried and extracted in a Soxhlet apparatus with absolute ether. Le Comte[3] suggests the use of sodium sulphate as desiccating material. In the modification suggested by Rieter,[4] gypsum and later some Fehling solution are used to precipitate the

[1] Soxhlet: *Polytech. Jour.*, 1879, ccxxxii, p. 461.
[2] Froidevaux: *Jour. de Pharm. et Chem.*, 1897 (6), vi, p. 485.
[3] Le Comte: *Ibid.*, 1901 (6), xiii, p. 58.
[4] Rieter: *Schweiz. Wochschr. Pharm.*, 1903, xli, pp. 39 and 53.

protein and fat. The precipitate is collected quantitatively on filter paper, dried, and extracted with absolute ether.

"RAPID" METHODS. Nearly all the so-called rapid methods depend upon centrifugation. In these methods accuracy is sacrificed for speed and the results obtained are, at best, only approximate. For commercial and pediatric uses they are of sufficient precision to answer most requirements. An example of these methods is the widely used Babcock process, with the special scale for reading off the contained fat, and various other modifications differing only in the shape of the tube or the scale. Another rapid but cruder method is the well known Feser lactoscope process.

Woosnam,[5] following the idea of Schmid, suggests the following method: 25 c.c. of milk and 28 c.c. of conc. hydrochloric acid sol. are placed in a special apparatus and heated on a water-bath until a slight browning occurs, when the flask and contents are cooled, an ether extraction made, and the volume of fat-ether mixture is read. A definite portion of this mixture is then taken, evaporated to dryness in a weighed glass dish and the dry fat weighed.

Following the method of Krug and Hampe[6] as a basis, Arndt[7] describes a process involving special extraction apparatus. The milk is desiccated with kaolin and dry sodium sulfate, and then extracted with ether in the apparatus.

As a modification of the usual butyrometer method, Gerber and Craandijk[8] recommend the following: Into a beaker of 5.5 c.c. capacity place 4–5 gm. of previously weighed, well mixed milk, introduce the beaker and contents into the butyrometer, add 10 c.c. of warm water, place the butyrometer in a water-bath at 60°–70° C. until complete solution has occurred. To the liquid add 1 c.c. of amyl alcohol, 10 c.c. of sulfuric acid sol. (sp. gr., 1520–1525), close the butyrometer, shake until the contents are well mixed, and then place it in a water-bath until the greater part of the fat separates. Finally, centrifuge twice and read the scale.

[5] Woosnam: *Analyst,* 1897, xxii, p. 91.

[6] Krug and Hampe: *Ztschr. f. angew Chemie,* 1894, p. 683.

[7] Arndt: *Chem. Centr.,* 1897, ii, p. 636 (*Forsch.-Ber. üb. Lebensm. u. ihre Gez. z. Hyg.,* etc., iv, p. 231).

[8] Gerber and Craandijk: *Milch-Ztg.,* 1898, xxvii, pp. 35 and 273.

Richmond and Rosier[9] advise treatment of milk with definite proportions of 90–91 per cent. sulfuric acid sol. and amyl alcohol, and, after cooling to about 25° C., extracting with 20 c.c. of petroleum ether. This method is not very accurate because of the fact that petroleum ether itself frequently leaves an evaporation-residue, probably an isomeric substance, hence the percentage by this method is likely to be too great.

Utilizing the tragacanth process devised by Rusting,[10] Bonnema[11] describes an ether-extraction method,—following preliminary treatment with potassium hydroxid sol. Tragacanth is added to facilitate the separation of the water and the ethereal solutions. The results are fairly good but not as accurate as those obtained with the paper-coil method.

In the Ram-Fouard[12] method, milk is treated with a special reagent containing potassium hydroxid, ethyl alcohol, amyl alcohol and ammonium hydroxid. The milk is placed in a special 50–60 c.c. flask with 10 c.c. of this reagent, the flask is immersed in boiling water for ten minutes, then removed, and enough distilled water added to carry the liquid into the neck of the flask, when the flask is placed in water at 40° C., and the fat allowed to separate. The average milk fat has a specific gravity of about 0.90 at 40° C.; hence, one-quarter the amount of fat recorded volumetrically gives the number of grams of fat per liter of milk.

Manget and Marion[13] speak favorably of a method in which they use a special reagent consisting of 100 c.c. of *n*/6 ammonium hydroxid sol. neutralized to litmus by the addition of lactic acid and made up to 150 c.c. with distilled water, and 435 c.c. of absolute ether, 420 c.c. of absolute alcohol and 40 c.c. of a solution of 1 gm. of methyl violet (5B) in 1,000 c.c. of absolute ether. After contraction has occurred, the volume is measured and 38.9 c.c. of absolute ether are added per liter. A special apparatus is used, by the aid of which the percentage of fat is read off on the scale at 37° C.

[9] Richmond and Rosier: *Analyst,* 1899, xxiv, p. 172.

[10] Rusting: *Chem. Centr.,* 1898 (ii), p. 393 (*Nederl. Tijdschr. v. Pharm., Chem. en Tox.,* 1899, x, p. 163).

[11] Bonnema: *Chem-Ztg.,* 1899, xxiii, p. 541.

[12] Lézé (Ram-Fouard method): *Ann. de Chim. anal. appl.,* 1899, iv, p. 371.

[13] Manget and Marion: *Ibid.,* 1902, vii, p. 297.

The refractometer is utilized by Wollny[14] on an ethereal solution of the fat obtained by extraction after the application of a special reagent. The refractive power of the ethereal solution of fat is determined at 17.5° C.

Maccagno and Mizzi[15] have devised a special apparatus in which they treat the milk with a definite quantity of a solution containing ethyl alcohol, amyl alcohol and ammonium hydroxid. The percentage of fat is read off on the scale of the apparatus at a temperature near the boiling point of water.

By the Sichler "sinacidbutyrometer method,"[16] the casein and lactalbumin are dissolved by means of a solution of sodium phosphate containing a small amount of sodium tri-citrate. The fat is dissolved in isobutyl alcohol, which is called "sinol" in this method.

In the Pilsner method,[17] the butyrometer is filled with 11 c.c. of alkaline solution (5 gm. of sodium phosphate, 15 gm. of neutral sodium citrate, 30 gm. of sodium chlorid, 65 gm. of sodium hydroxid dissolved in 600 c.c. of distilled water and filtered), 0.5 c.c. of isobutyl alcohol and enough sudan III to color, and 10 c.c. of milk and, after thoroughly mixing, the butyrometer and contents are placed in a water-bath at 58°–62° C. and the temperature kept above 55° C. In a half hour the butyrometer is removed, centrifuged and read.

All the foregoing procedures, which include the important methods, are open to criticism either because of the time consumed in making the determinations or because of inaccuracy in the final results.

THE MEIGS METHOD

The original Meigs method. The Meigs method, described in this article, is neither extremely rapid nor simple but, everything considered, it possesses certain advantages over each of the above

[14] Hals and Gregg (Wollny method): *Milch-Ztg.*, 1902, xxxi, p. 433.

[15] Maccagno and Mizzi: *Chem. Centr.*, 1906 (3 qt.), p. 633 (*Rev. intern. falsific.*, 1906, xix, p. 55).

[16] Cornalba (Sichler method): *Chem. Centr.*, 1905 (ii), p. 77 (*Staz. sperim. agrar. ital.*, 1905, xxxviii, p. 227).

[17] Kundrát and Rosam (Pilsner method): *Chem. Centr.*, 1907 (1 qt.), p. 513 (*Milchwirtschaft. Zentr.*, 1907, iii, p. 20).

methods that make it preferable in biochemical and pediatric work. The time consumed is as short as in any of the above methods, except the Babcock or other simple centrifugation methods, the simplicity of the apparatus compares favorably with that of any other method, and the degree of accuracy with ordinary care and skill is so great that it entirely suffices for all except the most precise work.

This method was described by Dr. Arthur V. Meigs[18] before the Philadelphia County Medical Society, on Feb. 22, 1882. It is, in brief, as follows: Approximately 10 c.c. of milk, after being carefully weighed, are transferred to an ordinary 100 c.c. graduated cylinder, 20 c.c. of distilled water added and, after this, an equal amount of ether (0.720 sp. gr.). The ground-glass stopper is inserted and the bottle shaken for five minutes. Then, after carefully removing the stopper, 20 c.c. of ethyl alcohol are added, the stopper re-inserted and the cylinder shaken again for five minutes. This, as soon as settling occurs, gives two distinct strata, the upper of which contains little but ether and fat, the lower contains the other constituents of the milk. The upper stratum is now drawn off with a small pipette and transferred to a weighed glass dish. Then 5 c.c. of ether are added and pipetted off, five successive times, and these 25 c.c. are added to the ethereal solution first removed. This is done to wash off the thin layer of fat and ether which was left behind at the first pipetting. The dish and contents are now transferred to a safety water-bath, protected from dust, the ether evaporated, and the residue placed in a hot-air oven, heated at a temperature below 100° C., and finally dried in a desiccator over sulfuric acid until the weight is constant. Dr. Meigs stated in his report that the idea for this method was gained from a paper by Hallock.[19]

The results we have obtained by this method were fairly accurate but, in order to be absolutely sure that no lactose or protein material was weighed as fat and to be certain that in both this and the Soxhlet method the end-products were identical, we decided to dissolve the first fat product in absolute ether, filter through a small hardened filter paper, as in the Soxhlet method, into a weighed dish, evaporate the ether, dry the fat and weigh.

[18] Meigs: *Philadelphia Medical Times,* 1882, xii, p. 660.
[19] Hallock: *Amer. Jour. of Pharmacy,* 1874, xlvi, p. 477.

Results obtained with the original Meigs method. While the results first obtained by this process seemed to compare very favorably with those obtained by the Soxhlet method, a series of investigations was begun to determine whether this was a mere coincidence or due to the efficiency of the method. For this purpose there were secured twelve samples of human milk from women in various stages of lactation and six of cow milk, from as many dairies so widely separated that in no two cases were the milks from the same herd. In all determinations the milk for both the Meigs and the Soxhlet methods was taken from one specimen, and at the same time, so that the samples were as nearly uniform as it was possible to get them. In every weighing the figures were recorded to 0.01 mg. The results are given in the accompanying table. The average difference in the results of the eighteen determinations recorded in the table is 0.0234 per cent.

The data in the table show that the method is useful for the determination of fat in both cow and human milk, and that it is applicable to all stages of lactation and to all kinds of milk. The degree of accuracy is such that it can be used in practically all work; the differences between the results obtained by this method and those by the Soxhlet, as may be seen from the individual determinations given in the table and from the average difference for the series, are so small that they fall within the limits of experimental error.

A modification of the Meigs process. In the manipulation, it was found that the use of the pipette was exceedingly tedious, required a great deal of skill and practice, and that, even under the most favorable circumstances, the possibility of error was great. To obviate this danger, we devised a simple inexpensive piece of apparatus (Plate 7). If only ordinary care is exercised in its use, the possibility of error is practically eliminated. This apparatus renders use of the pipette unnecessary, there is no need for careful attention to the protein layer or to the end of the pipette, and the speed of the whole process of removing the ethereal solution and washing away the last traces of fat is markedly increased.

Comparative data pertaining to the content of fat in milk

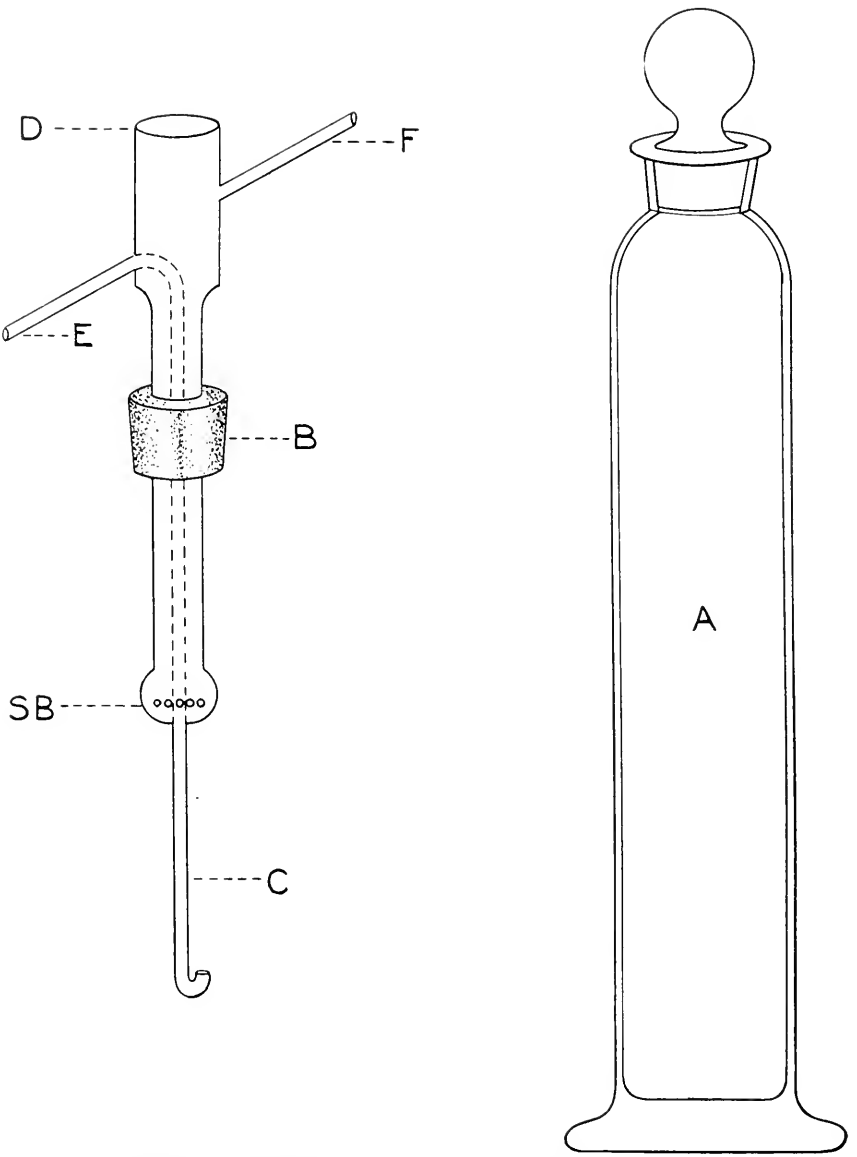
A. Human milk

Days of lactation	Fat by the Soxhlet method %	Mean by the Soxhlet method %	Fat by the Meigs method %	Mean by the Meigs method %	Difference in favor of the Soxhlet method %
10	3.254 3.266	3.260	3.228 3.241	3.234	+0.026
10	5.413 5.422	5.417	5.400 5.398	5.399	+0.018
4	5.278 5.215	5.246	5.221 5.183	5.202	+0.044
33	6.155 6.153	6.154	6.149 6.151	6.150	+0.004
9	3.713 3.701	3.707	3.686 3.690	3.688	+0.019
3	2.391 2.405	2.398	2.401 2.384	2.392	+0.006
2	3.958 3.943	3.950	3.923 3.935	3.929	+0.021
4	3.042 3.041	3.041	3.032 3.020	3.026	+0.015
11	3.910 3.900	3.905	3.892 3.897	3.894	+0.011
5	2.795 2.797	2.796	2.767 2.781	2.774	+0.022
6	3.642 3.627	3.634	3.622 3.618	3.620	+0.014
12	4.363 4.359	4.361	4.333 4.362	4.347	+0.014

B. Cow milk

1*	3.372 3.376	3.374	3.359 3.351	3.355	+0.019
2*	2.478 2.483	2.480	2.422 2.399	2.410	+0.070
3*	3.148 3.146	3.147	3.122 3.130	3.136	+0.011
4*	3.759 3.736	3.747	3.722 3.714	3.718	+0.029
5*	2.954 2.925	2.939	2.900 2.846	2.873	+0.066
6*	3.235 3.249	3.242	3.238 3.220	3.229	+0.013

* Sample number.



CROLL: NEW APPARATUS FOR USE WITH THE MEIGS METHOD FOR THE DETERMINATION OF FAT IN MILK.

In the drawing of the apparatus (Plate 7), **A** indicates a 100 c.c. graduated measuring cylinder with a ground-glass stopper; **B** is a rubber stopper; **C**, a glass tube of small bore, which is welded to another tube, **D**, at the points where it passes through the latter. The lower portion of tube **C** is bent sharply on its length and the open end is ground. Tube **D** is enlarged at the top to facilitate the introduction of ether; at its lower end there is a bulb, **SB**, with small holes in its walls, which allow the ether poured into tube **D** to spray against the sides of the cylinder, thus washing them. **F** is a side arm of small-bore tubing welded to **D** near the upper end. In forcing out the ethereal solution of fat, a finger is held over the upper end, **D**, and the operator blows air through **F**, the solution being ejected through **E** into a weighed glass dish. In case it is desired to increase the force of the washing spray, a finger may be placed over the upper end of **D** and, by blowing into **F** (just as when driving out the ethereal solution of fat), the pressure can be increased as much as may be desired. Before starting to force out the ethereal solution of fat, the opening of the lower end of tube **C** is brought on a level with the surface of the protein layer by raising or lowering the entire glass piece through the rubber stopper, **B**. The shaking is done, of course, with the ground-glass stopper inserted tightly into the cylinder just as when the pipette is used, and, after a few minutes, the stopper is carefully washed with absolute ether and the special apparatus inserted.

Advantages of the modified Meigs method. The improved Meigs method possesses the following advantages over the Soxhlet: (1) The time in weighing out is shortened and the danger of accidental loss is diminished, for no absorption of the milk by fat-free material is necessary. (2) Two or three hours are saved, as no drying is required. (3) The extraction requires ten minutes for its execution against three hours as the minimum for the Soxhlet process. (4) The apparatus is simple and inexpensive, while the Soxhlet is neither.

The advantages over the other processes have already been mentioned and are even greater than those over the Soxhlet method.

The Meigs method has been tested against the Soxhlet process on six samples of cow milk, *with the aid of the special apparatus*

described above, and the differences in favor of the Soxhlet method have been found to range between + 0.007 per cent. and + 0.059 per cent., or about the same as when the pipette was used, *but with much more rapid and less difficult manipulation.*

I wish to express my thanks to Prof. John Marshall and Dr. Wm. H. Welker for suggesting this investigation, and for the kindly interest shown by them throughout its progress.

THE OCCURRENCE OF ARSENIC IN SOILS

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Introduction. Kunkel[1] showed the presence of arsenic in many rocks and waters, while Czapek[2] states that traces are nearly always present in soils. Herzfeld and Lange[3] found arsenic in certain German raw sugars, and traced it to the lime which had been used in the manufacture of the sugar. Headden[4] found some virgin prairie soils relatively rich in arsenic, an observation in accord with my own experience. I have found arsenic to the extent of 4 parts *per million* in virgin soil; and, as in the cases referred to by Headden, it did not result from smelter fumes or any such source, but was derived from the decay of native rocks. On the other hand, Headden found arsenic in some cultivated orchard soils to the extent of 138 parts *per million.* He claims that in many places arsenic is accumulating in sufficient quantities to become injurious to vegetation. Francois,[5] however, considers there is little danger of the earth becoming unfit for vegetation from the proper use of insecticides. Grunner,[6] who found arsenic to the extent of from 0.026 per cent. to 1.426 per cent. in the Reichenstein soil, is not so optimistic. It appears, however, that it is not so much the total quantity of arsenic present as the form in which the arsenic occurs, that determines toxicity. Little work has been done on this phase of the question. I have therefore determined the quantity of arsenic, both total and water-soluble, in many of the orchard soils of Western America, and it is the purpose of this paper to consider briefly a few of these results.

Experiments. The water-soluble arsenic was determined by

[1] Kunkel: *Zeitschr. f. physiol. Chem.*, 1905, xliv, p. 511.
[2] Czapek: *Biochemie der Pflanzen*, 1905, ii, p. 862.
[3] Herzfeld and Lange: *Chem. Abs.*, 1911, v, p. 2342.
[4] Headden: *Proc. Col. Sci. Soc.*, 1910, ix, p. 345.
[5] Francois: *Rev. de chim. ind.*, 1912, xxiii, p. 124.
[6] Grunner: *Landw. Jahrb.*, 1910, xl, p. 517.

extracting, for eight days, 500 grams of soil with 2000 c.c. of carbon-dioxide-free distilled water, and then using an aliquot part, while the total arsenic was determined by extracting the soil with nitric and sulfuric acids, and applying the Marsh method so modified that the iron did not interfere.[7] The results are given in the accompanying table (1) as *parts per million* of dry soil.

TABLE I

Data pertaining to the quantities of arsenic in soils: parts per million

Total arsenic	Water sol. arsenic	*Per cent.* soluble	Total arsenic	Water sol. arsenic	*Per cent.* soluble	Total arsenic	Water sol. arsenic	*Per cent.* soluble
102	0.92	0.9	36	3.48	9.67	16	0.72	4.50
79	6.20	7.85	33	5.16	15.63	15	1.74	11.60
64	4.1	6.41	32	3.92	12.25	13	0.84	6.46
63	6.88	10.92	32	1.07	3.34	12	3.85	32.08
63	1.02	1.62	24	3.56	14.83	12	0.70	5.83
62	3.38	5.45	24	1.32	6.33	11	3.40	30.91
60	1.00	1.67	20	1.08	5.04	11	1.36	12.36
55	8.87	16.13	19	6.08	30.20	10	0.88	8.80
50	4.68	5.36	19	Traces	——	9	3.13	34.78
45	4.3	9.55	18	1.36	7.56	5	3.08	61.60
45	1.8	4.00	18	0.48	2.67	5	1.08	20.80
40	5.2	13.06	17	0.36	2.12	5	1.08	21.60
39	0.68	1.74	16	2.50	15.60			

From the data in the table it may be seen that some orchard soils contain large quantities of arsenic and some carry comparatively large proportions of it in the water-soluble form; but there is no uniform relationship between the total arsenic in soils and the water-soluble arsenic. If, as is most likely the case, the injury to plants is due to the water-soluble arsenic, greater injury would result in a soil containing only 5 parts *per million* of total arsenic with 61.60 per cent. of the arsenic water-soluble, than in a soil containing 102 parts per million, in which only 0.9 per cent. of the arsenic is water-soluble. It may be seen, further, that one of the above soils, which contains 63 parts *per million* of total arsenic, has only 1.62 per cent. in soluble form, while another soil, having exactly the same amount of total arsenic, contains 10.92 per cent. in soluble form.

Arsenic in soil is not confined to the surface. One soil, obtained

[7] Greaves: *Jour. Amer. Chem. Soc.*, 1913, xxxv, p. 150.

at a depth of three feet, yielded 11 parts per million of total arsenic, 30.91 per cent. of which was water-soluble.

It may be safely concluded, from the above data, that some virgin soils contain arsenic in large quantities, but that the proportion in a soil is no index of the amount which is soluble in water. The latter is probably governed by many factors, *e. g.,* kind of soil, water-soluble salts in it, and form in which the arsenic was applied to the soil.

The latter factor has been tested by applying the same quantity of different forms of arsenic to different portions of the same soil and then determining the quantity of water-soluble arsenic present in the soil. The soil used was a typical bench soil—a sandy loam—fairly high in content of calcium and iron, and supplied with an abundance of all the essential elements of plant food with the exception of nitrogen, which was low as is characteristic of the arid soil. The proportion of water-soluble salts in the soil was low. The influence of the soil on the solubility of the arsenical insecticide was determined as follows: Quantities of lead arsenate (21.96 per cent. of arsenic), Paris green (47 per cent. of arsenic), zinc arsenite (31.25 per cent. of arsenic) and arsenic trisulfide (60 per cent. of arsenic), were added to 100 gm. portions of soil in quantities sufficient to give 112 mgm. of arsenic per 100 gm. of soil. The soil and arsenic, together with 2 gm. of dry blood, were placed in sterile tumblers, covered with Petri dishes, the water content made and kept at 18 per cent.; and then each mixture was incubated at 28° C. for three weeks. At the end of this time the soil was transferred with 1000 c.c. of carbon-dioxide-free distilled water, to large acid bottles. The mixture was left in these bottles with occasional shaking for eight days, then filtered and the arsenic determined in an aliquot part. In another set each quantity of insecticide was mixed with a 100 gm. portion of soil and 2 gm. of dry blood, and the water-soluble arsenic determined as above, without incubation. All determinations were made in duplicate. The data are given in Table 2, as mgm. of water-soluble arsenic in 100 gm. of soil either before or after three weeks' incubation (112 mgm. of arsenic had been added to each.)

TABLE 2

Data pertaining to the water-soluble arsenic in soils mixed with arsenical insecticides

Treatment	Lead arsenate	Paris green	Zinc arsenite	Arsenic trisulfide
	mgm.	mgm.	mgm.	mgm.
Incubated three weeks. Water-soluble arsenic determined...	14.3	80.80	36.9	50.0
Water-soluble arsenic determined *direct*.....	20.2	82.00	31.7	5.6
Average.....	17.3	81.40	34.3	27.3

These results show that there may be a great difference in the quantity of water-soluble arsenic existing in the same soil to which various forms of arsenic have been added in equivalent quantities; and that even a soil comparatively rich in iron and calcium, to which arsenic has been added in large quantities, may have a high water-soluble content of arsenic. It is much higher when arsenic is added in the form of Paris green, than when added in the other forms mentioned. The solubility of the compounds, with the exception of arsenic trisulfide, is not greatly changed on standing in a soil containing a large quantity of decomposing organic matter.

The results also show the superiority of lead arsenate over any of the other arsenical insecticides. Any injurious effect of such compounds on plants must be proportional to the available amount of *water-soluble* arsenic, not to the *total* arsenic. In the use of an arsenical insecticide it should be the rule to select a compound which would remain insoluble for the greatest possible length of time. Lead arsenate possesses this advantage in high degree.

Summary. Some virgin soils contain arsenic in appreciable quantities which comes from the decay of the native rocks. Many cultivated orchard soils contain it in large proportions, but there is no uniform relationship between the total quantity of arsenic in different soils, and the water-soluble arsenic of these soils. A soil containing over 100 parts *per million* of total arsenic contained much less water-soluble arsenic than did a soil carrying only 5 parts *per million* of total arsenic. The solubility of the arsenic found in a soil is governed largely by the salts in the soil and the form in which the arsenic is applied. Different portions of the same soil,

to which equivalent quantities of various so-called insoluble arsenical compounds had been added, showed great dissimilarities in water-soluble arsenic content. The portion to which Paris green was added contained four times as much water-soluble arsenic as did a portion of the same soil to which an equivalent quantity of lead arsenate had been applied. Arsenic trisulfide, when first applied to soil, is less soluble than lead arsenate, but as time progresses, at least in some soils, the arsenic trisulfide becomes more soluble. For this reason lead arsenate is probably safer than any of the other arsenical insecticides.

FURTHER NOTES ON THE RELATIONSHIP BETWEEN THE WEIGHT OF THE SUGAR BEET AND THE COMPOSITION OF ITS JUICE

J. ARTHUR HARRIS AND ROSS AIKEN GORTNER

(Biochemical Laboratory of the Station for Experimental Evolution, Cold Spring Harbor, L. I.)

(WITH PLATE 8)

1. **Introductory remarks.** In an earlier paper[1] we discussed in terms of correlation and regression the relationship between the weight of the sugar beet and the composition of its juice (total solids, sucrose and purity). At that time (October, 1912) we were unaware of any previous attempt to deal with the problem by correlation methods. It was with considerable pleasure, therefore, that we found that only a few months before (January, 1912) Andrlík, Bartoš and Urban[2] had considered some of the same problems on the basis of data derived from pedigreed strains, and had even gone so far as to form correlation tables. They have not, however, calculated the constants which are essential to a full understanding of the tabled data. For this reason, and because their conclusions differ from our own, we have thought it worth while to take up the problem again.

2. **Analysis of data.** Although they discuss six series, Andrlík, Bartoš and Urban give only one table from which a correlation

[1] Harris, J. Arthur and Ross Aiken Gortner; On the relationship between the weight of the sugar beet and the composition of its juice: Proc. Columbia Biochem. Assoc., BIOCHEMICAL BULLETIN, 1913, ii, p. 287, and *Journ. Industrial and Engineering Chem.*, 1913, v, p. 192.

[2] Andrlík, K., V. Bartoš and J. Urban; Über die Variabilität des Gewichtes und des Zuckergehaltes der Zuckerrübenwurzeln, und über die gegenseitigen Beziehungen dieser beiden Merkmale: *Zeitschr. f. Zuckerindustrie in Böhmen*, 1912, xxxvi, p. 193.

TABLE I

Weight of root in grams

Sugar content in percents	300 to 350	350 to 400	400 to 450	450 to 500	500 to 550	550 to 600	600 to 650	650 to 700	700 to 750	750 to 800	800 to 850	850 to 900	Totals
16.1–16.5	—	—	—	1	—	—	—	—	—	—	—	—	1
16.6–17.0	—	—	1	—	—	1	—	—	—	—	—	—	2
17.1–17.5	—	3	1	2	—	1	—	—	—	—	—	—	7
17.6–18.0	1	5	4	7	2	5	5	1	—	—	—	—	30
18.1–18.5	4	12	15	13	8	10	7	6	1	—	—	—	76
18.6–19.0	7	20	38	33	48	25	17	17	2	4	—	—	211
19.1–19.5	4	15	35	30	32	23	19	15	3	2	—	1	179
19.6–20.0	1	8	22	28	30	14	14	16	4	1	—	—	138
20.1–20.5	2	2	2	8	17	6	7	2	1	—	—	—	47
20.6–21.0	—	4	—	1	1	2	1	—	—	—	1	—	10
Totals ...	19	69	118	123	138	87	70	57	11	7	1	1	701

coefficient can be determined.[3] From their summary, reproduced in slightly modified form in the above table, we deduce, using the convenient formula

$$r_{ws} = \frac{\Sigma(w's')/N - \bar{w}\bar{s}}{\sigma_w \sigma_s},$$

where $\Sigma(w's')$ denotes the summations of the (mid-ordinate) values of the weight and sugar content of the individual beets, the bars denote the means and the sigmas the standard deviations of the two variables,

$$r_{ws} = .116 \pm .025.$$

The correlation is over four times its probable error and so possibly statistically significant, but certainly is very low. Expressing the relationship in terms of regression, using the straight line equation employed in the preceding paper

$$s = \left(\bar{s} - r\frac{\sigma_s}{\sigma_w}\bar{w}\right) + r\frac{\sigma_s}{\sigma_w}w,$$

we find

$$s = 18.722927 + 0.000796\ w,$$

[3] Throughout their work these authors speak of their first series as containing 699 beets. But the entries in their fundamental table, "Tabelle II *a*," add up to 701, so we have thought it best to follow this. In two other series we cannot make our additions agree with theirs.

where weight is in grams and sugar in percents. Thus, for a difference of 50 grams in weight (the range of the weight classes employed by these Bohemian investigators), one would expect an increase in sugar content of only $0.000796 \times 50 = 0.00398$ per cent. The regression straight line and the empirical means are represented in Plate 8.[4] We note that the observed positive correlation only means a difference of 0.44 per cent. between the lowest and the highest weight grade.

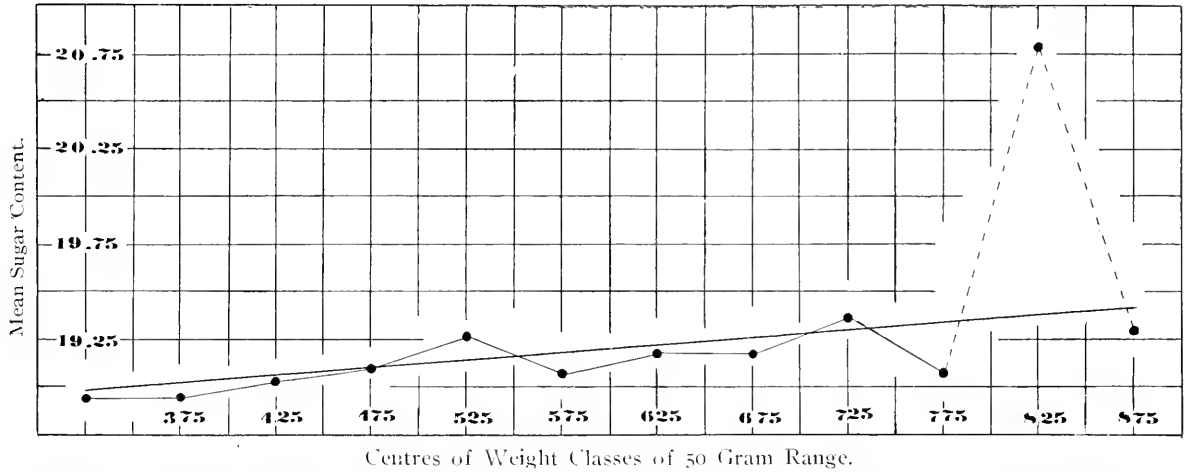
Data from which correlation surfaces might be prepared are not given for the remaining five series.[5] It would have been possible to get the *sign* of the remaining five correlations from the data of "Tabelle I and Tabelle VI" by throwing the above formula for r into the form

$$r_{ws} = \frac{S(n_w w \bar{s}_w)/N - \bar{w}\bar{s}}{\sigma_w \sigma_s},$$

where n is the number of individuals in any weight grade, w, $\bar{s}_w$ is the mean sugar content associated with this given grade, and S denotes a summation for all weight classes. But unfortunately, these two tables are not consistent! Thus, series 2 ranges from 525 to 1475 gm. (mid-ordinates) in one case and from 575 to 1525 in the other. Series 3 ranges from 525 to 1375 gm. in one table and from 575 to 1525 in the other. Series 4 begins at 625 and ends at 1375 in one table while in the other it ranges from 725 to 1425. Series 5 and 6 are equally faulty in this regard; in addition, the number of beets for series 5 is given as 173 in one table and as 1390 in the other; the number of beets for table 6 is given as 426 in one table and as 173 in the other. Even such confusion as this might possibly be straightened out with some probability of certainty, had not the

[4] The two upper weight classes, connected by broken lines, contain each only a single beet, so are of little significance.

[5] The data for the first series are given in ponderous detail. The first table gives sugar in tenths of percents for weight groups of 50 gr. range. Tables showing correlation between weight and sugar (in groups of 0.5 per cent range) and between sugar and weight are extracted from this as Tables II *b* and IV. Both of these tables, in essentials identical, are reduced to percentage frequencies and published as Tables III and V. It is really a great pity that the four or five pages thus needlessly used could not have been devoted to the actual data for the five other series upon which their conclusions might have been critically tested.



HARRIS AND GORTNER: RELATIONSHIP BETWEEN THE WEIGHT OF THE SUGAR BEET AND THE COMPOSITION OF ITS JUICE.

value of the data been completely destroyed by the reduction of all frequencies to percentages. We must confess that these evidences of gross carelessness in the treatment of their numerical data lessen our confidence in the conclusions they and their reviewers have drawn from it.

Turn now to three other series of analyses by Andrlík and Urban made for a different purpose.[6] They give the weight, sugar content and nitrogen[7] content of 36 beets grown from normal-nitrogen-content mothers and the same data for 36 roots grown from high-nitrogen-content parents. The first of these we designate as Series A, the second as Series B. They also give 100 analyses of beets from the same parent individual. We refer to these as C. For weight and sugar the correlations are:

$$\text{For Series A, } r_{ws} = -0.278 \pm 0.104$$
$$\text{For Series B, } r_{ws} = -0.456 \pm 0.089$$
$$\text{For Series C, } r_{ws} = -0.031 \pm 0.067$$

All the relationships are negative. The probable errors are high because of the small number of beets given, but the two first cases may perhaps be regarded as statistically significant in comparison with their probable errors. They are in excellent agreement with the correlations published in our first paper.

We have also deduced the coefficients for weight and nitrogen, n. They are:

$$\text{For Series A, } r_{wn} = -0.234 \pm 0.106$$
$$\text{For Series B, } r_{wn} = -0.393 \pm 0.095$$
$$\text{For Series C, } r_{wn} = +0.054 \pm 0.067$$

One of the constants is positive in sign but less than its probable error. The two negative values are fairly large and possibly significant in comparison with their probable error.

Still another series of data[8] of quite a different sort affords, it

[6] Andrlík, K. and J. Urban; Über die Variabilität des Stickstoffgehaltes in Zuckerrübenwurzeln: *Zeitschr. f. Zuckerindustrie in Böhmen,* 1912, xxxvi, p. 513.

[7] Milligrams of nitrogen in 100 grams of root.

[8] Novotný, K.; Ein Beitrag zur Betrachtungen über die Beziehungen zwischen dem percentuellen Zuckergehalte und dem Gewichte der Rüben: *Zeitschr. f. Zuckerindustrie in Böhmen,* 1912, xxxvi, p. 269.

seems to us, the strongest evidence for a negative relationship between the weight of the beet and its sugar content in commercial cultures. Novotný selected from his laboratory record books analyses of samples of beets which had been divided into two subsamples, one of large and one of small beets. Altogether there are 27 of these pairs of samples, taken from 1892 to 1910. With only two exceptions (in which the sugar percentage was identical in the two cases) the sugar content of the lighter was higher than that of the heavier fraction.[9]

3. Summary and discussion. There is a more or less widespread idea that the percentage sugar-content of large beets is lower than that of small roots. This belief, which has often been opposed, was placed on a scientific basis for American commercial cultures by an earlier study, in which we showed that there is a significantly negative (and sometimes numerically very substantial) correlation between the weight of the root and its total solids, its percentage sugar-content and its coefficient of purity. The strongest evidence in support of our conclusions (so far as sugar content is concerned) is furnished by the data of Novotný. Recently, however, wide currency has been given to the conclusions that there is no necessary negative relationship between weight of root and composition of juice, and that when analyses are made of a series of beets derived from the same mother plant no such correlation is demonstrated.

The data upon which this statement has been made seems to be six series by Andrlík, Bartoš and Urban. Of these the figures for one series only are given in a form really suitable for statistical analysis. This gives a low positive correlation. The tables for the other five series contain so many obvious inconsistencies that they cannot be used. But three other series by Andrlík and Urban, also from beets derived from an individual mother but analyzed for a different purpose, all give negative correlations between weight and sugar content. One of these is numerically insignificant; the other

[9] Notwithstanding the fact that his data bear evidence without a single exception against the statement that there is no negative relationship between weight of root and composition of juice, Novotný attempts, by the use of a formula which seems to us to have no theoretical justification, to show that the difference in sugar content between large and small beets is becoming smaller and that his results are consequently in accord with those of Andrlík, Bartoš and Urban.

two are of the order $r = -0.300$. Thus, of the four series of data which can be scientifically analyzed, three give correlations which are negative in sign while one is positive. One negative and one positive coefficient are very low; two of the negative coefficients are more substantial, agreeing fairly well with the values previously published by us.

Surely such facts as these form a very slender basis for the conclusion (widely circulated by uncritical reviewers) that in beets of the same strain there is no negative correlation between weight and sugar content! Nevertheless one must recognize the possibility of the correctness of the conclusion. Should it prove to be valid, the suggestion follows that the negative correlation demonstrated in commercial cultures has a genetic origin, *i. e.*, that strains characterized by large root size are also characterized by low sugar content, and that when these strains are intermingled and intercrossed in field cultures there results a negative correlation between the weight of the individual beet and the sugar content of its juice. Such a result would be of the greatest interest to breeders.

NOTE ON THE RELATIONSHIP BETWEEN BAROMETRIC PRESSURE AND CARBON-DIOXIDE EXCRETION IN MAN

Higley's application of the product-moment correlation method to the question of the influence of barometric pressure on carbon-dioxide excretion in man[1] seems to deserve some extension. Fortunately, this is possible on his published data.

Instead of inquiring merely whether there is a correlation between barometric pressure and carbon-dioxide excretion, one may profitably consider (*a*) whether the volumes excreted by the same individual at different periods in the day are correlated, and (*b*) whether the amounts excreted by different individuals on the same day are correlated.

The correlation between the volumes respired by the same individual at different times of observation on the same day might be due either to internal physiological conditions,[2] or to variation from day to day in external conditions (barometric pressure, or some other environmental factor). A correlation between the volumes excreted by different individuals on the same day would necessarily be due to some common external condition.

Designating Higley's subjects by *a, b* and *c,* and the three observation periods by m = morning, n = noon and e = evening, I find the following results.[3]

A. For correlations between different observation periods for the same individual:

For *a,*

$$r_{mn} = +0.30 \pm 0.12,\ r_{me} = -0.15 \pm 0.13,\ r_{ne} = +0.00 \pm 0.13$$

[1] Higley: Biochemical Bulletin, 1913, ii, p. 393.

[2] These might conceivably tend to bring about either a positive or a negative correlation.

[3] In determining the correlations, the means and standard deviations were necessarily recalculated for each product-moment, since several observations are wanting. Thus N varies from coefficient to coefficient.

For *b*,

$r_{mn} = -0.53 \pm 0.10$, $r_{me} = +0.19 \pm 0.14$, $r_{ne} = -0.07 \pm 0.13$

For *c*,

$r_{mn} = +0.04 \pm 0.13$, $r_{me} = +0.21 \pm 0.13$, $r_{ne} = +0.07 \pm 0.14$

B. For correlations between the excretions of different individuals on the same day:

For 7 A. M.,

$r_{ab} = -0.00 \pm 0.14$, $r_{ac} = +0.46 \pm 0.10$, $r_{bc} = +0.00 \pm 0.14$

For 12 M.,

$r_{ab} = +0.20 \pm 0.12$, $r_{ac} = +0.31 \pm 0.12$, $r_{bc} = +0.08 \pm 0.13$

For 5 P. M.,

$r_{ab} = +0.15 \pm 0.13$, $r_{ac} = +0.07 \pm 0.14$, $r_{bc} = +0.03 \pm 0.14$

In each case three of the values are negative and six positive in sign. They thus *tend* to confirm Higley's suggestion concerning the relationship between common external conditions and volume of carbon-dioxide excreted. But the probable errors are high (because of both the lowness of most of the coefficients and the smallness of the number of observations) and the constants are very irregular.

For trustworthy conclusions larger series are necessary. When these are available, the correlations here suggested may, I believe, be of supplementary value.

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Cold Spring Harbor, Long Island, N. Y.

THE BLEACHED FLOUR DECISION

May I be permitted to answer the editorial signed "M. C.," on "The Bleached Flour Decision," which appeared in the April issue of the BIOCHEMICAL BULLETIN (p. 487)?

While I was associated with Professor Alway, at the Nebraska State Experiment Station, we had occasion to spend the greater part of a year investigating the subject of the bleaching of flours by the Alsop process, *i. e.,* the use of nitrogen peroxide.[1]

At the start I may state that the work was not done under any grant from the millers, or others interested in the process, but was under a grant from the Adams Fund; and that all conclusions were based solely upon the experimental evidence. I may also add that at this time I have no interest in the matter except to see that *facts* are stated.

1. The "poison" to which "M. C." refers is the nitrite-reacting substance ($NaNO_2$?) which is present in bleached flours and gives the reaction with the Griess-Ilosvay reagent. This "poison" is present in bleached flours not to exceed 6–10 parts *per million,* and usually in much smaller quantities. A greater proportion may be introduced by "over bleaching," but in over bleached flours a dirty yellow color is produced and the flour is ruined commercially.

When a flour which has been bleached is baked, all, or nearly all, of the nitrite-reacting substance is destroyed, and *it very rarely happens that loaves of bread made from bleached flours and unbleached flours can be distinguished from each other by the nitrite test.* This is especially true if the baking has been done in a gas-fired oven, for under such conditions bread made from unbleached flour will give the nitrite test.

Aside from the nitrite test, there is only one other distinguishing feature, since the baking quality of the flour, the expansion of the gluten, and the odor, taste, weight, lightness, and texture of the

[1] See Bull. No. 102, Nebr. Agric. Exper. Sta. (The Effect of Bleaching upon the Quality of Wheat Flour).

bread are the same, whether made from bleached or unbleached flours; but, in all cases, the bread made from high grade bleached flour is whiter and more inviting in appearance. Eating bread made from bleached flour has, as I personally know, produced no ill effects, even when the test lasted for months at a time.

The saliva of normal human beings almost always contains nitrites, and the quantity of nitrites secreted by the average individual in 24 hours in the saliva is far in excess of the quantity which we found in the entire loaf of bread highest in nitrites. *In order to obtain the medicinal dose of the "poison" ($NaNO_2$), it would be necessary to eat at least a pound loaf of bread each day for a year.*

2. "M. C." also adds: "If it is not to conceal inferiority so that a higher price can be had for the flour, why do millers use the process?" In the first place our experiments show that low grades of flour cannot be successfully bleached, and, when bleached, can in no case be confused with a high grade flour, for the color of the small particles of bran in a low grade flour is not affected by the nitric fumes and the bread produced from such a flour has an uninviting color.

It is merely a question as to where the wheat comes from, that causes bleaching. At the last trial in St. Louis one of the witnesses for the Government testified and protested against the use of the bleachers, although he was himself a miller. On cross examination he admitted that he had formerly used the process but had abandoned it. On further questioning it developed that, while he was in the "yellow wheat belt," he had used the bleacher but now, in Kentucky, the wheats gave a white flour, *and he did not need to use the bleacher to get the same result.*

The wheats of Nebraska, Kansas, Iowa, etc., have a dark yellow oil which, when milled, gives a very yellow tint to the flour. This answers the "WHY": it is because the farmers of Nebraska, Iowa, Kansas, etc., wish to produce as saleable a flour as do their neighbors.

The influence of the minute quantities of NO_2 changes this yellow oil into a colorless compound: very probably it is the old reaction of oleic acid changing into elaidic acid.

3. I am heartily in accord with the Pure Food and Drugs Act, even if I do seem to fall in the class mentioned in the final para-

graph by "M. C." But why do we need to go to such extremes? To quote "M. C."—the fifth section reads: "An article shall be deemed adulterated—if it contains any added poisonous or other added deleterious ingredient *which may render such article injurious to health.*" Why not prohibit the addition of salt to the bread? Stefansson tells us that, in the Arctic when he was unable to procure salt, he experienced dizziness and all of the cravings due to the lack of a customary narcotic, but his health was far better after the cravings were over, and that, on returning to civilization, salted foods were decidedly distasteful to him and he had to acquire a taste for them again. Certainly, if the salt taken in bread were increased in amount several hundred fold, physiological disturbances might occur, and poisonous symptoms be observed.

The only way we can use a law is to give it a sane interpretation. I do not protest against the branding of the flour "bleached." That is all right; we have a right to know what we are buying, but I do protest against forbidding its inter-state transportation when no harmful effects have ever been *demonstrated,* even by the widest stretch of imagination.

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EMIL CHR. HANSEN FUND

Pursuant to the last will and testament of the late Professor Emil Chr. Hansen and his wife, a Fund bearing his name has been established, the Statutes of which were ratified by the Danish Government on June 17, 1911. At proper intervals, as a rule every two or three years, *beginning in the year 1914,* a Gold Medal bearing Hansen's effigy and accompanied by a sum of (at least) Five hundred dollars (2000 Kroner) is to be awarded on the donor's birthday, the 8th of May, to the author of a distinguished publication on some microbiological subject that has appeared of late years in Denmark or *elsewhere.*

The Fund is committed to the administration of the Chiefs of the two departments of Carlsberg Laboratory, together with a Danish biologist elected by the governing body of Carlsberg Laboratory.

The person to whom the Medal is to be assigned shall be designated by a Committee composed of the above mentioned Trustees of the Fund, together with at least two foreign microbiologists, who, at the request of the said Trustees, will have accepted appointment to membership in the Committee. Professeur et Dr. Calmette, Lille; Geh. Ober-Med.-Rath Prof. Dr. Gaffky, Berlin; and Professor Theobald Smith, M.D., Boston, have become members of the Committee.

It is proposed to award the medal, in 1914, to a scientist in the field of Medical Microbiology (*comprehending the morphology, biology and mode of action of the microbes generative of disease in the human or animal body*).

All communications regarding the Hansen Fund should be sent to, and all further particulars will be given by, the President of the Board of Trustees.

Board of Trustees:

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Copenhagen, Valby, Denmark,
June, 1913.

BIOLOGICAL CHEMISTRY IN THE PHILIPPINES

There are, at present, no laboratories of biological chemistry in the Philippines, but there are well equipped chemical and biological laboratories at the University of the Philippines in Manila, at the Agricultural College in Los Baños and at the Bureau of Science. The course in biological chemistry in the College of Medicine and Surgery is under the control of the Department of Chemistry in the University, and the small biochemical laboratory is temporarily used for pharmacy. It is planned in the near future to put the biochemistry in the Department of Physiology. Research in the Department of Pharmacology has been in the biochemical field, and this laboratory is well equipped for the work.

Research in the Islands is largely concerned with local and tropical problems. Aron's work on Philippine rice and on beriberi, and Freer's investigations of tropical sunlight are recent examples. Gibbs has studied the chemistry of the mongo bean, an important article of diet in the orient. Coöperative work, by the Board for the Study of Tropical Diseases of the U. S. Army and the chemists in the Bureau of Science, on the protective principle in rice polishings, may be mentioned. Andrews has reported the analyses of samples of milk from native women, whose infants have died of beriberi—puppies, suckled by these women, developed the disease. Shaklee is at present carrying out an extensive series of experiments on low nutrition under tropical conditions.

The satisfactory conditions for work, particularly the large amount of available material out here, should appeal to the biological chemist. The Bilibid prisoners can be used as exactly controlled subjects for metabolism work; and normal fresh human organs are obtainable at the not infrequent prison executions. There is an abundance of autopsy material. Cases in the Philippine General Hospital can be studied experimentally. Animals, such as dogs and monkeys, are easily obtained. The native laboratory assistants are quickly trained, and make good technicians. There is an excellent

library at the Bureau of Science. Finally, living conditions are good and salaries are liberal. It is to be hoped that the near future will find more biological chemists in the Islands.

R. B. Gibson

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DOCTORATES IN BIOLOGICAL CHEMISTRY

Conferred by American Universities, 1912–'13

The names of recent recipients of the Ph.D. degree in biochemical science, with the subjects of the dissertations, are arranged below in university groups:

Columbia University.[1]—*Joseph Samuel Hepburn:* Biochemical studies of cholesterol.—*Benjamin Horowitz:* A study of the action of ammonia on thymol.—*Edgar Grim Miller, Jr.:* Studies in pathological chemistry; (I) Enzymes as possible factors in the development of edema, (II) The lecithin content of the blood in syphilis, (III) Studies on dental caries.—*Anton Richard Rose:* Biochemical studies of phyto-phosphates.—*George Gilmore Scott:* A physiological study of the changes in *Mustelus canis* produced by modifications in the molecular concentration of the external medium.—*Clayton Sidney Smith:* A study of the influence of cold-storage temperature upon the composition and nutritive value of fish.—*Charles Weisman:* Biochemical studies of expired air in relation to ventilation.

Cornell University.—*George Ellsworth Thompson:* An experimental study of photoactive cells with fluorescent electrolytes.—*Eleanor VanNess VanAlstyne:* The absorption of protein without digestion.

Harvard University.—*Chauncey J. Vallette Pettibone:* The quantitative estimation of urea in urine.

Johns Hopkins University.—*Lon A. Hawkins:* The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals toward fungous spores.

University of Chicago.—*Aaron Arkin:* The influence of chemical substances upon immune reactions, with special reference to

[1] Additional information regarding the Columbia doctors (*and masters*) in biological chemistry is given on page 579, where may also be found the names of successful Ph.D. candidates in botany, chemistry and zoology, whose *minor* work was done, in part, in the Columbia department of biological chemistry.

oxidations.—*George Lester Kite:* The relative permeability of the surface protoplasm of animal and plant cells.—*Shiro Tashiro:* Chemical change in nerve fiber during passage of a nerve impulse.—*Arthur Lawrie Tatum:* Studies in experimental cretinism.

University of Missouri.—*Leroy Sheldon Palmer:* Study of the natural pigment in the fat of cow milk.

University of Wisconsin.—*William Harold Peterson:* Forms of sulfur in plants.—*Roy Lee Primm:* Some phenomena associated with cellulose fermentation.—*Nellie Antoinette Wakeman:* Plant pigments other than chlorophyl.

Washington University.—*Jacob Richard Schramm:* A contribution to our knowledge of the problem of free-nitrogen fixation in certain species of grass-green algæ, with special reference to pure-culture methods.—*Charles Oscar Chambers:* The relation of algæ to dissolved oxygen and carbon dioxide, with special reference to carbonate.

Yale University.—*Robert Bengis:* The synthesis of amino acids related to adrenalin.—*Howard Bishop Lewis:* The behavior of some hydantoin and thiohydantoin derivatives in the organism, together with a study of certain related sulfur compounds.—*Ben Harry Nicolet:* Some derivatives of amino-malonic acid and their biochemical interest.—*Ruth Wheeler:* Nutrition experiments with mice.

Universities which conferred Ph.D. degrees in the natural and exact sciences, but at which there were no biochemical candidates, are named below:

Boston University
Brown University
Clark University
George Washington University
Indiana University
Massachusetts Institute of Technology
New York University
Princeton University
Stanford University
Tulane University
University of California
University of Cincinnati
University of Illinois
University of Iowa
University of Michigan
University of Minnesota
University of Nebraska
University of Pennsylvania
University of Pittsburgh
University of Virginia
Vanderbilt University

Comparing the foregoing list with the data published a year ago,[2] we note the main points of interest shown by the appended summary:

Number of Ph.D. degrees awarded by American universities to biochemical candidates, 1912 and 1913.

	1912	1913	Total	Women 1912	Women 1913
Brown University	1	0	1	0	0
Columbia University	11	7	18	1	0
Cornell University	5	2	7	0	1
Harvard University	1	1	2	0	0
Johns Hopkins University	1	1	2	0	0
University of California	5	0	5	1	0
University of Chicago	8	4	12	1	0
University of Illinois	5	0	5	0	0
University of Michigan	2	0	2	0	0
University of Missouri	0	1	1	0	0
University of Wisconsin	4	3	7	0	1
Washington University	0	2	2	0	0
Yale University	6	4	10	1	1
Total	49	25[3]	74	4	3

P. H. D.

[2] Biochemical Bulletin: 1912, i, p. 546.

[3] The decrease from 49 in 1912 to 25 in 1913 accords with a general tendency: the number of doctorates in the "natural and exact sciences" decreased from 273 in 1912 to 231 in 1913, whereas the doctorates in the "humanities" increased from 209 in 1912 to 230 in 1913—totals of 482 in 1912 and 461 in 1913.

TWELFTH SCIENTIFIC MEETING OF THE COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION, AT THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK, JUNE 2, 1913[1]

PROCEEDINGS REPORTED BY THE SECRETARY,

ALFRED P. LOTHROP

The *twelfth scientific session* (*fourth "annual" meeting*) of the Columbia University Biochemical Association was held at the Columbia Medical School, at 8.15 p. m., on June 2, 1913.[2] Abstracts of the papers are presented here (pages 542–558) in two groups: (A) *Abstracts of papers on research by non-resident members*[3] and (B) *abstracts of papers from the Columbia Biochemical Department and affiliated laboratories.* The appended summary facilitates reference to the abstracts (86–107).[4]

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS (86–107)

A

A. F. BLAKESLEE and ROSS AIKEN GORTNER. On the occurrence of a toxin in juice expressed from the bread mould, *Rhizopus nigricans* (*Mucor stolonifer*). (86)

ROSS AIKEN GORTNER. Studies on melanin: V. A comparison of certain nitrogen ratios in black and in white wool from the same animal. (87)

MAX KAHN. Metabolism studies of five cases of endarteritis obliterans ("Hebraische Krankheit"). (88)

DANIEL R. LUCAS. On the content in expired air of protein detectable by the anaphylactic reaction. (89)

MAX MORSE. On the comparative physiology of creatin and creatinin. (90)

MATTHEW STEEL. Influence of electricity upon metabolism. (91)

[1] Scientific meetings are held regularly on the first Fridays of December, February and April, and on the first Monday in June.

[2] Proceedings of the ninth, tenth and eleventh scientific meetings were published in the last (April) number of the BIOCHEMICAL BULLETIN at pages 452, 461 and 486.

[3] Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the researches were conducted.

[4] Previous abstracts were published in the BIOCHEMICAL BULLETIN: 1–44, 1912, ii, p. 156; 45–62, 1913, ii, p. 285; 63–72, 1913, ii, p. 452; 73–85, 1913, ii, p. 462.

B

LOUIS BERMAN and WILLIAM J. GIES. Studies of intracellular chemistry: A differential stain for mucins and mucoids. (92)

WALTER H. EDDY. Two new histons. (93)

WALTER H. EDDY. Histon nucleoprotein: A protein salt. (94)

FRANK R. ELDER. Further experiments on the preparation of modified collodion membranes for use in dialysis experiments. (95)

SAMUEL GITLOW. Comparative studies of the permeability of collodion and collodion-fat membranes. (96)

TULA L. HARKEY. Further studies of edema: On the absorption of water by white lupin seeds. (97)

TULA L. HARKEY. Further studies of edema: On the postmortem absorption of water by tissues from well nourished and fasting animals. (98)

PAUL E. HOWE and WILLIAM J. GIES. A preliminary study of the resistance of fasting dogs to hemorrhage. (99)

V. E. LEVINE. Biochemical studies of selenium. (100)

HELEN I. MATTILL and H. A. MATTILL. The influence of electrolytes on the precipitation of soluble starch. (101)

EDGAR G. MILLER, JR. Determinations of the acidity of fruit juices. (102)

OLIVE G. PATTERSON. A study of the influence of external hemorrhages on the partition of urinary nitrogen. (103)

P. W. PUNNETT. The action of a high frequency current on the activity of pancreatic amylase. (104)

CHRISTIAN SEIFERT and WILLIAM J. GIES. A further study of the distribution of osseomucoid. (105)

A. W. THOMAS. A further effort to prepare a colorless biuret reagent. (106)

CHARLES WEISMAN. Biochemical studies of expired air in relation to ventilation. (107)

A. ABSTRACTS OF PAPERS ON RESEARCH BY NON-RESIDENT MEMBERS[5]

86. On the occurrence of a toxin in juice expressed from the bread mould, Rhizopus nigricans (Mucor stolonifer). A. F. BLAKESLEE and ROSS AIKEN GORTNER. (*Biochemical Laboratory of the Station for Experimental Evolution, The Carnegie Institution of Washington.*) During a series of immunity studies, having as their aim a possible solution of the chemical nature of sex, we observed that the "presssaft" from the aerial filaments of *Rhizopus nigricans* caused almost instant death when injected intravenously into rabbits. Several other species of the *Mucorineae* were tested and no such result has yet been obtained. The mycelium directly in contact with the substratum apparently contains as great a quan-

[5] Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the researches were conducted.

tity of toxin as do the aerial filaments. Furthermore, the toxin is present in both sexes of the fungus in large amount. We have not determined whether one sex contains a greater absolute quantity than the other.

The toxicity of the *Rhizopus* extract may be better understood by saying that a solution containing the water-soluble substances extracted from 0.045 gm. of the dry fungus, when injected intravenously, is sufficient to kill a 1.35 kilo rabbit in less than two minutes. Convulsions begin almost before the needle can be withdrawn, and are followed by great turgidity of the chest and abdomen, then by relaxation of the rigid abdomen, throwing the head backward with cough-like movements of the diaphragm, protrusion of the eyes, and death. When a dose containing the toxin from a greater quantity than 0.045 gm. is injected, the animal is often dead before the needle can be withdrawn, with no convulsive movements and only a sudden turning of the head and the sinking of the body on one side. A sub-lethal dose causes extreme lethargy, lasting for 48 hr. or more, during which time the animal moves only when forced to do so. This may be followed by complete recovery. We have not as yet elucidated the chemical nature of the toxin. Its activity is not diminished by peptic digestion for three hours, nor is it affected when its aqueous solution is heated to boiling for 10 min. Apparently, therefore, it is not a toxalbumin.

One of us (B) has shown that *Rhizopus* is nearly universally distributed and is almost certain to appear as an infection on starchy food under suitable moisture conditions, its occurrence being so common as to have earned for it the name, "Bread-mould." While connected with the Agricultural Experiment Station of the University of Nebraska, one of us (G) coöperated in an investigation of the origin of the "corn-stalk disease," which causes the death of thousands of cattle in the Middle West each year, and which is, in some way, connected with the use of corn stalks as fodder. At that time no known toxin could be detected in the stomachs of the diseased animals, and the direct cause of the disease has never been elucidated.

The method of growth of the fungus, its wide distribution, as well as certain of the symptoms produced by the toxin which it

contains, would seem to indicate that there may be a possible relationship between *Rhizopus* and some of those diseases, such as pellagra, the "corn-stalk disease," and the "horse disease" of the Middle West, the causes of which are at present unknown, but which have been supposed to be due to infected food. We are at present carrying out a series of investigations on the chemical nature of the *Rhizopus* toxin, as well as its possible relation to such diseases, and we hope to be able to make a more detailed report in the near future.

87. Studies on melanin: V. A comparison of certain nitrogen ratios in black and in white wool from the same animal. Ross Aiken Gortner. (*Biochemical Laboratory of the Station for Experimental Evolution, The Carnegie Institution of Washington.*) Black and white wool from a pied lamb were analyzed by Van Slyke's method, with the following average percentage results (corr. for solubilities of the bases):

	White wool	Black wool
Ammonia nitrogen	9.32	9.46
Humin nitrogen	1.20	4.74
Arginine nitrogen	17.46	16.81
Lysine nitrogen	3.90	3.97
Cystine nitrogen	2.70	3.09
Histidine nitrogen	7.00	7.04
Amino nitrogen (filtrate)	54.54	52.04
Mon-amino nitrogen (filtrate)	2.76	2.13
Total	98.88	99.27

The nitrogen content of the black wool was 15.11 per cent. and of the white wool, 16.27 per cent.

By subtracting the humin nitrogen for the white wool from the corresponding fraction for the black wool we have 3.54 per cent. of the nitrogen as due to the melanin present in the black wool. I have already shown[6] that the pigment which remains when the melanin from wool is treated with boiling conc. hydrochloric acid, contains 8.48 per cent. of nitrogen. Using these data I have calculated the percent of nitrogen which would be present in the black wool providing that the nitrogen due to the melanin, *as given by the acid hydrolysis,* were not present, at the same time correcting

[6] Gortner: *Bull. soc. chim. de France,* 1912, xi, p. 498.

the weight of wool taken for the corresponding weight of melanin; and I find that the percent of nitrogen in the keratin structure (white wool) would be only 15.48 per cent. It can be readily seen that this is far too great a divergence from the white wool percentage (16.27) to be an experimental error. It is far more probable that the melanin molecule is broken down by acid hydrolysis and that only a portion of the nitrogen is obtained as humin nitrogen, so that the correction which I used did not take into account a considerable portion of the melanin molecule which has a lower nitrogen content than the keratin structure. This supposition is in agreement with the results of my previous work.

88. Metabolism studies of five cases of endarteritis obliterans ("Hebraische Krankheit"). MAX KAHN. (*Service of Dr. Charles Goodman, Beth Israel Hospital, New York.*) In five male adults suffering from endarteritis obliterans of the vessels of the leg, and fed a Folin diet, the urinary nitrogen partition was normal, and the excretion of ethereal sulfate and calcium was increased.

89. On the content in expired air of protein detectable by the anaphylactic reaction. DANIEL R. LUCAS. (*Chemical Laboratory, College of the City of New York.*) The lethal intraperitoneal dose of dog serum for normal guinea pigs is about 5 c.c. for 300 grams of body weight; 4 c.c. for the same weight is usually followed by recovery, 6 c.c. by death. The lethal dose of human serum is about 10 c.c. for 300 grams of body weight.

Two c.c. of dog serum or 15 c.c. of distilled water, injected intraperitoneally, in normal guinea pigs, caused shivering, twitching and noisy respiration. Such symptoms have been interpreted as evidences of anaphylactic shock but are not reliable indications of it. Proportionate amounts and the production of a lethal effect by a sublethal dose are essential for accuracy when dog serum is used. Intraperitoneal injections (2–15 c.c.) of condensations from the expired air of dogs did not sensitize guinea pigs to dog serum. Guinea pigs exposed to dog exhalations for a week, under conditions of very poor ventilation, were not sensitized. Nor were any evidences of sensitization to human serum obtained when pigs were exposed for a week in the exit of a ventilation system in a college building containing many people daily.

These findings fail to support the conclusions of Rosenau and Amoss,[7] but are in accord with, and extend, the observations of Weisman.[8]

90. **On the comparative physiology of creatin and creatinin.** MAX MORSE. (*Woods Hole, Mass.*) In a study of the absorption of the muscles in the tail of the larva of the common frog, attention was directed to the creatin-creatinin content of the muscle and also of the elimination of these compounds in the excretions. Numerous attempts to determine the amounts led to the general conclusion that, with the color reactions used (Folin-Benedict-Meyers method and the older Jaffé reaction), no creatin or creatinin is demonstrable either in the fresh muscle or in the excretions. The results were negative with Weyl's test and with Kramm's and Salkowski's methods.

An attempt to isolate the crystallin compounds was futile. The following method was used: The larva was weighed and measured; the tail was removed, ground in sand, covered with 95 per cent. alcohol and shaken for two-minute intervals on an International Instrument Company centrifuge-head shaker with four changes of water. The liquids were mixed and made up to 100 c.c. Ten c.c. portions were used for the various reactions. A Duboscq colorimeter was used, but the readings were not recognizable in the instrument. Excretions were caught by placing the larva in a large petri dish with a measured amount of distilled water, the whole being concentrated at low heat by evaporation.

This observation accords with other data showing that the absorption of the tail involving the disappearance of over a gram of tissue within 12 hr. does not concern nitrogen elimination in excess of the normal for non-metamorphosing frogs.

The study of the creatin-creatinin content of tails and excreta of metamorphosing frogs was inspired by the conclusions of several workers on mammalian material, which seemed to show that muscle metabolism, especially the atrophying muscle of involuting uteri, etc., involved quantitative relations of these compounds, but Mellanby has more recently shown that this is not true for mammalian uteri.

[7] Rosenau and Amoss: *Journal of Medical Research,* 1911, xxv, p. 35.

[8] Weisman: BIOCHEMICAL BULLETIN, 1913, ii, p. 295. (See also page 558 of this issue. *Ed.*)

Denis has failed to discover creatin and creatinin in the urine of elasmobranchs, but aside from this work, I know of no determinations of the creatin-creatinin content, and the rôle of these compounds, in the lower chordates.

91. Influence of electricity upon metabolism. MATTHEW STEEL. (*Laboratory of Physiological Chemistry, Long Island Medical College.*) Informal report.

B. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEMICAL DEPARTMENT AND AFFILIATED LABORATORIES

92. Studies of intracellular chemistry: A differential stain for mucins and mucoids. LOUIS BERMAN and WILLIAM J. GIES. From a mixture of five parts of 0.25 per cent. sol. of safranin (Grübler) and three parts of 0.25 per cent. sol. of methyl green (Grübler), *mucins and mucoids select the methyl green and are colored green.* In a 0.25 per cent. sol. of safranin alone, these glucoproteins are stained red. The following types of pure mucin and mucoid preparations, which we prepared by the methods best adapted for the highest purity of the products, stained green in the safranin-methyl green sol.: mucoids from tendon, ligament, cartilage and bone; sodium salt of tendon mucoid; salivary mucin and a sodium salt of salivary mucin; ovo-mucoid.

Samples of the following protein products, prepared by the best methods and of the highest purity, selected safranin from such a safranin-methyl green sol. and *stained red:* albumin (egg), chrondroalbumoid, collagen, deutero proteose, edestin, elastin, fibrin, gelatin (from bone, muscle, ligament), hemoglobin, metaproteins (acid albumin and alkali albuminate), myosin, nucleoprotein (from ligament and yeast), ossein, osseoalbumoid, peptone. Dry blood serum, egg white and gluten also stained red in the safranin-methyl green mixture. All these protein preparations stain green in a 0.25 per cent. sol. of methyl green alone.

Glucothionic acids from the various glucoproteins named above behave like mucins and mucoids in staining green in the safranin-methyl green sol. The products obtained from glucothionic acid by hydrolysis with boiling 5 per cent. hydrochloric acid sol. failed to show the selective action.

One part of tendon mucoid mixed with one hundred parts of collagen or edestin can easily be detected by this differential staining process.

The technique, practical utility, and applications of the test are now under investigation.

93. Two new histons. WALTER H. EDDY. *Tom-cod histon.* Tom-cod milt contains a histon which, like thymus histon, is precipitable in two forms: (*a*) an ammonia-precipitated product which is partially soluble in water, and (*b*) a sodium-chlorid precipitated product which is readily soluble in water. Both forms give characteristic histon tests. *Shad histon.* Shad milt contains a histon which may be precipitated in two forms: (*a*) an ammonia-precipitated product which is insoluble in water, and (*b*) a complex precipitable by saturation with sodium chlorid, which appears to be a combination of histon and a non-histon protein fraction. The products were prepared by the method already described.[9]

94. Histon nucleoprotein: A protein salt. WALTER H. EDDY. Continuing the work on histon-nucleoprotein that has already been described,[10] and proceeding with the aid of the improved method recently outlined,[11] a neutral water-sol. of thymus histon (which had been precipitated by saturation with sodium chlorid) was added to a neutral solution of sodium nucleoprotein from yeast until precipitation was complete. This precipitate was insoluble in water but soluble in 0.05 per cent. sodium carbonate sol. and could be reprecipitated, without decomposition, by 0.1 per cent. hydrochloric acid sol. The 0.05 per cent. sodium carbonate sol. was precipitable by conc. nitric acid sol., by very dil. hydrochloric acid sol., by picric acid, alcohol, mercuric chlorid and neutral lead acetate but was not precipitable by ammonia, sodium phosphotungstate, potassium ferrocyanid, potassium mercuric iodid, or albumin. It gave positive biuret, Millon, xanthoproteic and Molisch tests. In all these respects the aqueous sol. of this precipitate agreed with the water-sol. of sodium nucleoprotein. The precipitate obtained with 0.1 per cent. hydrochloric acid sol., after purification, was extracted for twenty four hours with 0.8 per cent. hydrochloric acid sol. Ammonia pre-

[9] Eddy: BIOCHEMICAL BULLETIN, 1913, ii, p. 435.
[10] Eddy: *Ibid.*, 1912, ii, p. 121.
[11] Eddy: *Ibid.*, 1913, ii, p. 435.

cipitated from the extract a product which gave additional histon tests, proving the presence of histon in the compound.

An interesting confirmation of these results was obtained by repeating the preparation with histon precipitated with sodium chlorid from a hydrochloric acid extract of tom-cod milts (see preceding abstract). This product resembled, qualitatively, the thymus-histon salt but was not precipitated from the sodium carbonate sol. by hydrochloric acid. It was precipitated readily with nitric acid and this precipitate yielded histon on extraction with 0.8 per cent. hydrochloric acid sol.

95. Further experiments on the preparation of modified collodion membranes for use in dialysis experiments. FRANK R. ELDER. In continuance of the studies, in this laboratory, of collodion membranes,[12] we have endeavored to determine the comparative permeabilities of collodion-fat combinations. The tests were conducted with aqueous solutions in small bags made by Dr. Gies' method, as recently published by Clark,[13] from mixtures of U. S. P. collodion sol. and pure olive oil. Uniform mixtures of 1 part of olive oil and 3 parts of collodion sol. did not yield bags strong enough for the use intended, but satisfactory membranes could be obtained from mixtures containing 1 part of oil and 4 of collodion sol. Bags made from mixtures of 1 part of olive oil and 9 of collodion sol. were impermeable to chlorid, sucrose and peptone in dialysis experiments of a week's duration. Bags made from mixtures of 1 part of oil and 99 of collodion sol. were occasionally impermeable to each of these substances, though such bags frequently permitted all of them to dialyze freely when associated pigment was unable to diffuse. It is our intention to determine, if possible, whether such diffusion differences depend upon inequalities in the distribution of oil in the bags, "age" of the membranes, etc.

96. Comparative studies of the permeability of collodion and collodion-fat membranes. SAMUEL GITLOW. Bags were prepared as usual (see the preceding abstract), and the tests were conducted in water. The following water-soluble dyes diffuse through **plain collodion** *membranes used immediately after removal from the*

[12] Gies: *Proc. Amer. Soc. Biol. Chem.*, 1912, ii, p. 75; *Journ. Biol. Chem.*, 1912, xi, p. xli; *Science*, 1912, xxxv, p. 396.

[13] Clark: BIOCHEMICAL BULLETIN, 1912, i, p. 198.

mold: (*a*) *Very rapidly*—auramin, bismarck brown, chrysoïdin, erythrosin, martius yellow, metanil yellow, orange G, rose bengal; (*b*) *in fifteen minutes*—eosin A, phloxin, safranin; (*c*) *in one hour*—Biebrich scarlet; (*d*) *in one day*—cape aloes, fast red A, fustic extract, rhodamin; (*e*) *indiffusible*—benzopurpurin, malachite green, methylene blue. The following dyes (those named above) diffuse through **collodion-fat** *membranes used immediately after removal from the mold:* (*a*) *Very rapidly*—chrysoïdin, eosin A, martius yellow, orange G, phloxin, safranin; (*b*) *in fifteen minutes*—erythrosin; (*c*) *in thirty minutes*—Biebrich scarlet, metanil yellow, rose bengal; (*d*) *in one day*—auramin, cape aloes, fast red A, rhodamin; (*e*) *indiffusible*—azolitmin, benzopurpurin, bismarck brown, malachite green, methylene blue.

The older the bags of each kind, that is the more completely the residual ether and alcohol solvents of the collodion solution (U. S. P.) were removed from the membranes, the less permeable the bags became. In most cases, each particular pigment diffused through the different membranes at the same general rate, but in the following instances diffusion through collodion-fat membranes was faster or slower than through plain collodion membranes: (*a*) *faster*—Biebrich scarlet, eosin A, phloxin, safranin; (*b*) *slower*—auramin, bismarck brown, erythrosin, metanil yellow, rose bengal.

Certain variations have been noted which appear to depend on irregularities in the dyes and in commercial U. S. P. collodion solutions, also on the "age" of the bags. The study is in progress.

97. Further studies of edema: On the absorption of water by white lupin seeds. TULA L. HARKEY. The swelling of weighed, normal, white lupin seeds in various media, as compared with the increase in weight of such seeds in distilled water, was determined at short intervals for a week or more. At the end of 24 hours, seeds gained more weight in the chemical solutions than in the corresponding water controls, in the instances cited on the opposite page.

Effects of other acids, and of electrolytes and non-electrolytes on the imbibition of water from acid solutions, will be reported later.

98. Further studies of edema: On the postmortem absorption of water by tissues from well nourished and fasting animals.

Data pertaining to the absorption of water by white lupin seeds

Solution		Percentage gain in 24 hours	
Nature	Concentration, per cent	Water control	Specified solution
Hydrochloric acid	0.125–0.5	141.8	148.6–173.6
Sulphuric acid	0.25 –1.0	154.4*	156.9–156.2*
Phosphoric acid	0.125–0.5	126.1	139.5–153.6
Lactic acid	1.0 –2.0	155.9*	165.6–163.5*
Oxalic acid	1.0	152.1*	159.2*
Sodium chlorid	0.25	135.0	138.3
Sucrose	2.0	136.7	137.2
Urea	1.0 –8.0	144.8	153.4–164.5

TULA L. HARKEY. We tested assumptions that the tissues of fasting dogs contain, at death, relatively less acid-yielding material than the tissues of well nourished dogs and that, on the basis of Fischer's collochemical theory of edema, the fasting tissues would therefore imbibe less water than the latter, under uniform postmortem conditions. Well nourished dogs and dogs which, after preparatory periods on the standard diet, had fasted completely for 8–11 days, were bled to death, in each case from a femoral artery, and the more important parts treated in stoppered, wide-mouth bottles with moderate excesses of water. The weights of the swollen tissues were recorded at regular intervals. Diffusion gains and losses occurred under uniform external conditions for each tissue. The results do not support the assumptions on which the work was conducted. The percentage gains in weight were irregular—fasting brains, for example, gaining more weight relatively than well nourished ones in some cases, or vice versa in others. There was also no apparent definite relation between the degree of swelling and the acidity of comparative extracts of the tissues.

The results do not conflict with current opinions on the role of osmosis in the absorption of water by protoplasmic structures, and they harmonize with the belief that enzymes may be important factors in such processes. The study is in progress.

These studies of edema were suggested by Dr. Gies, and conducted under his supervision.

99. A preliminary study of the resistance of fasting dogs to hemorrhage. PAUL E. HOWE and WILLIAM J. GIES. In further-

* Record at 48 hours.

ance of the preliminary study described at the last meeting,[14] we have noted the effects of hemorrhages in dogs under conditions of *partial* fasting, after suitable preparatory periods on our standard laboratory diet for dogs. Five animals (*first group*), weighing 4.7–6.4 kilos, received water (187–315 c.c.) but no other ingredients of the preparatory diet. The partial fast was maintained for from ten to thirteen days, with losses in body weight of 18.1–23.6 per cent. Blood amounting to 3.54–5.38 per cent. of the body weight (2.86–4.4 per cent. of the *initial* weight) was removed without causing any serious symptom. Three animals (*second group*), weighing 5.5–12.6 kilos, received the *standard diet minus the meat.* The only protein in the food was the small quantity in the cracker meal. No dietary compensations were made for losses due to exclusion of the meat. The partial fast was maintained for thirty-three days, with losses in body weight of 16.05–24.95 per cent. Blood amounting to 4.50–5.11 per cent. of the body weight (3.62–3.86 per cent. of the *initial* weight) was removed without causing special respiratory difficulty or distress.

100. **Biochemical studies of selenium.** V. E. Levine. Opposed to the current opinion that organic substances, generally, reduce alkali-selenite solutions, we find that reduction is not induced by alcohols, phenols, saturated and unsaturated organic acids (except formic acid, lactic acid, gallic acid), amino acids, purin bases, proteins, fats and other lipins such as lecithin and cholesterol. Acetylene, hydroxylamine and phenylhydrazine cause very strong reducing effects. Acetone and formaldehyde reduce acidified solutions of sodium selenite. Many carbohydrates reduce alkaline sodium selenite to colloidal or red amorphous selenium. Inorganic substances, *e. g.,* ferrous sulfate, stannous chlorid, zinc and hydrochloric acid, sulfurous acid, arsenious acid, phosphorous acid, hydrobromic acid, hydriodic acid, also exhibit reducing power. Hydrogen peroxid, free halogens, nitric acid, potassium permanganate, and aqua regia, because of their oxidizing activity, inhibit or may entirely prevent the formation of colloidal or precipitated selenium (reduction).

The possibility of using sodium selenite as a reagent for the detection of reducing substances was investigated. Arabinose,

[14] Howe and Gies: Biochemical Bulletin, 1913, ii, p. 468.

rhamnose, xylose, galactose, glucose, fructose, maltose, and lactose promptly reduce alkaline sodium selenite. A selenite reagent containing 2 per cent. sodium selenite, 10 per cent. sodium citrate and 10 per cent. sodium carbonate has thus far been as efficient as the Fehling-Benedict reagent.

Selenium compounds are toxic to both plants and animals. Beginning with the most poisonous, the sequence of toxicity for the group selected was the following one: hydrogen selenide, selenium dioxide, selenic acid, sodium hydrogen selenite, sodium selenite, potassium selenocyanate, sodium selenate, free selenium. Intravenous injection is followed by a marked fall in blood pressure; potassium selenocyanate, however, induces a considerable rise. Pulmonary edema, accompanied by exudation of large volumes of yellowish fluid, preceded death in the case of selenium dioxide, sodium acid selenite, sodium selenite and selenic acid. Respiratory paralysis set in before the heart stopped.

Reduced selenium can be detected in neutral urine by the addition of potassium cyanide. Decomposition by means of hydrochloric or sulfuric acid of the potassium selenocyanate thus formed results in the production of a colloidal suspension of brick-red selenium. Potassium selenocyanate can be detected by the addition of ferric chlorid, followed by a drop of dilute sulfuric acid solution. The presence of potassium selenocyanate in the urine interferes with the Fehling-Benedict reduction test. The study is in progress.

101. **The influence of electrolytes on the precipitation of soluble starch.**[15] Helen I. Mattill and H. A. Mattill. The origin of this investigation was an observation by Dr. Gies that solutions of soluble starch, when dialyzed free from electrolytes, were not precipitable by alcohol, and that the addition of a drop of dilute salt solution restored precipitability. It was the object of this work to determine at what concentrations of electrolyte the precipitation by alcohol ceased, *i. e.,* how delicate a test for electrolytes it is, and how variations in the nature of the electrolyte affected the reaction. Two percent soluble starch solutions were dialyzed until they gave no precipitate upon the addition of alcohol. Two c.c. of

[15] Some of the work was done in the Physiological Laboratory at the University of Utah, where it is now in progress.

the starch solution were diluted with 10 c.c. of alcohol (95 per cent.) and one drop of salt solution was added. The time required for precipitation and sedimentation served as a measure of the activity of the various electrolytes. It was shown that the cations in chloride solutions were effective in the following order: $Ba = Sr = Ca > Mg = Ce > Na = K > H > NH_4 > Li > Hg''$ — results which are similar to ion effects upon many other emulsoids (Hofmeister's "Ionenreihen," etc.). The lower limits of precipitation were as follows: one drop of 0.1 *n* $HgCl_2$ sol. was without effect; one drop of 0.025 *n* LiCl gave a very slight precipitate; 0.01 *n* LiCl, 0.005 *n* NH_4Cl and 0.0025 *n* HCl, NaCl, and KCl were without effect; only incomplete precipitation occurred with 0.0025 *n* sol. of alkali-earth chlorides. If alcohol of varying concentrations (from 80–10 percent) is used, the precipitability of the starch by electrolytes is rapidly decreased with decreasing concentrations of alcohol, and the difference between the effects of the various cations is rendered more marked. A cursory examination of the sulfates indicated a smaller effectiveness and one or two exceptions to the above order for chlorides. In all cases the precipitation is reversible. Further work will be done on a more accurate differentiation of the cation and anion effects, and upon variations in this phenomenon, with varying degrees of dispersion of the starch.

102. **Determinations of the acidity of fruit juices.**[16] Edgar G. Miller, Jr. In line with Dr. Gies' proposal of the use of diluted vinegar and various "food-acid" media as dentifrices, I have determined the acidity of some common fruit juices as a preliminary to the selection of the most suitable ones for prophylactic application to the teeth. The appended data for acidity represent, in most cases, the averages of *triplicate* results, in c.c. of *n*/5 sodium hydroxid solution (phenolthalein) per 10 c.c. of juice: Apple, 3.5; apricot, 3.8; asparagus, 0.9; banana, 2.6; beet (red), 1.1; cantaloupe, 0.6; carrot, 0.8; cauliflower, 1.9; celery, 0.8; cherry, 7.6; cocoanut milk, 0.4; cranberry, 19.6; cucumber, 1.0; currant, 20.4; gooseberry, 16.2; grape (white), 4.5; grape fruit, 10.3; horse radish, 9.2; lemon, 53.7; orange, 6.7; parsnip, 2.1; peach, 6.4; pear, 3.2; pineapple, 7.5; plum, 4.8; radish, 0.6; rhubarb, 11.1; strawberry, 9.3; tomato, 4.2; turnip, 0.6; vinegar, 26.1; watermelon, 0.6.

[16] Miller: *Dissertation;* Columbia University, 1913 (Part III, p. 25).

103. A study of the influence of external hemorrhages on the partition of urinary nitrogen. OLIVE G. PATTERSON. Two dogs were subjected to external hemorrhages (3.5–5.8 per cent. of body weight) under *local* anesthesia (cocain), and studied by some of the nutritional methods in use in this laboratory. One dog was subjected to four successive bleedings, at intervals of 11, 5 and 7 days, respectively. The general conclusions published by Hawk and Gies,[17] on the effects of external hemorrhage in dogs under *general* anesthesia, were confirmed. Each hemorrhage caused absolute increases in the renal excretion of total nitrogen and urea. The first hemorrhage in each animal was followed by an increased output of creatinin; subsequent bleedings increased the excretion of creatinin or were without influence on it. The absolute amounts of urinary ammonia, uric acid and purins were unaffected by the hemorrhages. The results will be published in detail in the near future.

104. The action of a high frequency current on the activity of pancreatic amylase. P. W. PUNNETT. An attempt was made to discover what effect, if any, the high frequency current, such as is used in electro-therapeutics, has on the activity of pancreatic amylase. The current was furnished by a high frequency machine loaned to the department by the Van Houten and Ten Broeck Company of this city, to whom we are greatly indebted for this courtesy. The amylase of commercial "pancreatin" (Parke, Davis & Co.) was used. The method of Sherman, Kendall and Clark[18] was employed throughout for the determination of activity.

Solutions of the material were treated with the current in a beaker coated on the outside with tinfoil attached to one terminal of the machine and having a platinum electrode, connected to the other terminal, immersed in the solution, the whole being kept in an ice bath. The length of treatment varied from 5 to 30 min., and the hot-wire ammeter gave a reading of 500 to 600 milliamperes. Controls were kept at the same temperature. Because of the fact that only ordinary distilled water was available, the enzyme did not show more than 70 per cent. of its maximum activity (its power was 418, using triple-distilled water).

[17] Hawk and Gies: *Amer. Jour. of Physiol.*, 1904, xi, p. 171.

[18] Sherman, Kendall and Clark: *Jour. Amer. Chem. Soc.*, 1910, xxxii, p. 1073.

Although the results are not quantitatively exact, they strongly indicate that this type of electricity is in no degree harmful to pancreatic amylase; that the treatment of a fresh solution for from 3 to 20 min. is probably beneficial, giving an increase of from 5 to 20 per cent. in activity; that longer treatment is without effect; and that solutions which stand for about 24 hr. at room temperature Mr. Punnett's report.)

It may be noted that the *direct* current has an exactly opposite effect on enzymes, as has recently been shown by Burge.[19] Further work along these lines is desirable.

(Mr. John W. Radu, Superintendent and Chief Engineer of the Van Houten and Ten Broeck Co., made some very interesting demonstrations with the electrical apparatus at the conclusion of Mr. Punnett's report.)

105. A further study of the distribution of osseomucoid. CHRISTIAN SEIFERT and WILLIAM J. GIES. Ten years ago we published data showing that osseomucoid is a constituent of the main limb bones (the only ones investigated) in thirteen species of mammals, ten of birds, two of reptiles and one of fish.[20] At that time we concluded that osseomucoid is probably a constituent of all bones. In resumption of this study, and proceeding by the original methods, we have lately separated osseomucoid from the main limb (or skull) bones of the following additional species: *mammals*—monkey (mangabey and spider), horse, fox, raccoon, white rat; *birds*—dove, meadow hen; *fish*—sculpin. The research is in progress.

106. A further effort to prepare a colorless biuret reagent. A. W. THOMAS. In extension of the work described by Kantor and Gies,[21] I have endeavored to prepare a colorless biuret reagent. Cuprous thiocyanate was thought to be a good substitute for copper sulfate because of its white (or grayish white) color, but treatment with alkali caused decomposition of this salt, resulting in precipitation of a yellowish red modification of cuprous oxide; and the solution, which contained some of the undecomposed compounds, rapidly assumed a blue color due to oxidation. It was possible to obtain a nearly colorless reagent with cuprous iodide, but oxidation promptly brought about the inevitable result—a blue solution.

[19] Burge: *Amer. Jour. of Physiol.*, 1913, xxxi, p. 328; xxxii, p. 41.
[20] Seifert and Gies: *Amer. Jour. of Physiol.*, 1903, x, p. 148.
[21] Kantor and Gies: BIOCHEMICAL BULLETIN, 1911, i, p. 264.

The complex colorless cyanide of copper, which is readily soluble in 10 per cent. sodium hydroxid soution, was tried. The solution was colorless and it gave a strong characteristic pink to purple reaction when added to a dilute solution of Witte peptone. Unfortunately, however, the solution gradually turned blue. It was thought that this blue oxidation product might be reduced with an excess of potassium cyanide to drive the cupric ion into the colorless complex ion. Experiment proved such to be the case, but this colorless solution would not give the biuret reaction when added to peptone solution. This is an extremely interesting fact from which the inference may be drawn that the biuret reaction takes place only with the positive copper ion. But if this inference is correct, how is it that one obtains a reaction with the colorless solution made by adding $K_2Cu(CN)_3$ to 10 per cent. sodium hydroxide solution? This fact is easily understood when we consider the dissociation of this salt. The instability constant of the complex ion, $\frac{(Cu^+) \times (CN^-)^3}{Cu(CN)_3^=}$, is 0.5×10^{-27}, and the concentration of cuprous ion is approximately 3.7×10^{-8} in a tenth molar solution. But, this very small concentration of cuprous ion is sufficient to start the reaction. In the strong alkaline solution there is immediate oxidation of Cu^+ to Cu^{++}, which reacts with the protein giving some pink color; as soon as this happens more of the $K_2Cu(CN)_3$ dissociates, forming more $Cu^+ \rightarrow Cu^{++}$, which results in more of the pink product with the protein. If an excess of KCN is added, there is a driving back of the ionization of $K_2Cu(CN)_3$, positive copper ions are removed and hence no reaction occurs when protein solution is treated with the reagent.

In order to determine whether the reagent could be prepared for use during a laboratory day without oxidation, 0.2 gm. of the white double cyanide was added to 50 c.c. of sodium hydroxid solution, and observed for appearance of any blue color. From time to time the flask containing the solution was opened and portions were withdrawn for the purpose of making tests upon proteins. After five hours of such treatment the reagent began to acquire a light blue color, due to the oxidizing effect of the air, the color deepening to that of the regular reagent. If air is excluded from the bottle, the development of color proceeds more slowly.

To prepare this temporarily colorless reagent, about 0.1 gram of potassium cupro-cyanide may be dissolved in 50 c.c. of 10 percent (or *stronger*) sodium hydroxid sol. Fehling's alkaline tartrate sol. may be used. Convenient approximations of these amounts may be made in emergencies with satisfactory results.

Several of the common organic salts of copper were treated with alkali. Many of them were decomposed, yielding cupric hydroxid or cuprous oxid, and a blue solution. The citrate, salicylate and tartrate, while soluble in alkali, yield solutions that are respectively green, deep green and dark blue, thus rendering them useless for the intended purpose.

107. Biochemical studies of expired air in relation to ventilation.[22] CHARLES WEISMAN. Our preliminary results[23] have been fully confirmed. Eleven repetitions of Rosenau and Amoss' experiments have been made, with negative results in each case. Anaphylaxis failed to occur in control experiments, when expired-air condensations were used for the second injection as well as for the preliminary sensitization.

In experiments extending the work, air was drawn through the macerated lungs from a dog and the vapors condensed into a Drechsel bottle. The guinea pigs that were "sensitized" to the condensation-liquid failed to exhibit anaphylaxis after appropriate treatment with dog-blood serum. Air was also drawn through a solution of Witte peptone, and the vapors condensed, but the liquid thus obtained did not yield the biuret, Million or xanthoproteic test.

Chemical examination of the condensation-liquids, obtained under aseptic conditions from the expired breath of several persons, gave the following results: *Absent*—sulfid, phosphate, bromid, iodid, amin, acetone (iodoform test), diacetic acid (Lipliawski test), and protein; *present*—ammonia (Nessler test), and chlorid.

The ill effects of vitiated air in poorly ventilated or overcrowded places cannot be due to a volatile protein ("sensitizing substance") from the lungs.

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[22] Weisman: *Dissertation;* Columbia University, 1913. Pp. 97.
[23] Weisman: BIOCHEMICAL BULLETIN, 1913, ii, p. 295.

BIOCHEMICAL BIBLIOGRAPHY AND INDEX

3. Second quarter, 1913 (April–June)[1]

WILLIAM J. GIES

(Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York)

Explanation of abbreviations, arrangement, notation, etc. BIBLIOGRAPHY. *Titles of papers* are freely shortened, minor words ignored, common terms conveniently abbreviated or chemical symbols substituted; *surnames* of collaborators are connected by hyphens; most *punctuation marks* are omitted—all for the sake of condensation. Heavy faced Roman numerals indicate *volumes;* heavy faced Arabic numerals designate *numbers and dates of issue* (slanting lines separate numerals for months and days). *Bibliographic items* begin with em dashes. When two or more papers by the same author occur together, they are duly numbered, and separated by semicolons, but follow the same em dash. Numerals preceding italicized names of authors indicate *sequence in the bibliography* (*index numerals*); numerals preceded by commas, at the ends of items, indicate *initial pages of the corresponding papers.*

INDEX (SUBJECTS). The numerals in the index (page 565) correspond with the numbered items in the bibliography. *Pages are not indicated.* Numerals held in groups by hyphens are plain abbreviations in accord with the indications of the first numeral of each such series (see footnote, p. 565). Abbreviations of words in the index are similar to those in the bibliography. Each *group of index references* is terminated by a semicolon; commas mark off *subdivisions of a general index subject. Names of authors are not indexed.*

JOURNALS INCLUDED: *Biochemische Zeitschrift* (**B. Z.**), *Zeitschrift für physiologische Chemie* (**Z. p. C.**), *Journal of Biological Chemistry* (**J. B. C.**), *Biochemical Journal* (**B. J.**), *Biochemical Bulletin* (**B. B.**).

PRACTICAL USE OF THE BIBLIOGRAPHY. The bibliography is helpful from several standpoints. Thus, if it is desired to ascertain whether the journals included in the bibliography contain any papers (during the given quarter) on a particular subject, *e. g., lipins,* find the key word in its alphabetical place in the index and turn to the items in the bibliographic sequence indicated by the index numerals (*i. e.,* in this case, 35, 50, 56, 415). The abbreviated items thus identified give the names of authors and suggest the nature of the corresponding papers (four papers, in the case selected for illustration), and help the reader to decide whether to examine the original publications. When the index gives a negative answer to an inquiry, a large mass of literature is removed

[1] The preceding portions of this bibliography and index were published at pages 298 and 470 of this volume (Jan. and Apr. issues).

from further consideration. During the intervals between publication of the indexes of journals, *Centralblätter* and year books, this running bibliography directs the reader to most of the main tracks through current literature on the leading biochemical subjects.

Bibliography. **B. Z.—L: 1–2; 4/11.**—1*Bokorny*Einfl versch Subst a Keim Pflanz'sam, Wachstumsförd,1; 2*Ibid.*,49; 3*Ibid.*,87.—4*Borowikow*Urs Wachstum Pflanz,119.—5*Doerr-Pick*Mechanis primär Toxiz Antiser u Eig'sch i Antigen,129.—6*Glagolew*Plasteinbild,162.—7*Landsteiner*Spezif Immunreak u ihr kol'dchem Erklärbark,176. **3–4; 4/21.**—8*Bunzel*Oxydas i Blattrollkr Zuck'rüb,185.—9*Hämäläinen*Synth β-Glucosid d Terpenalkoh,209; 10Konst Terpineol–35°–glucuronsäu, 220.—11*Kretschmer*Titr Harnsäu i Harn n vorgäng Ag-fäl,223.—12-*Arcichovskij*Wirk Giftst versch Konz a Sam,233.—13*Landsberg*Blutgerin,245.—14*Frank*Vork Kephalin u Trimyristin Leber,273.—15-*Mayer*Zuck'frei Gär b Ster'iso,283.—16*Durig*Verh Amphib i versch konz Lös,288.—17*Tögel-Brezina-Durig*Kohlenhydr'spar Wirk Alkoh, 296.—18*Walbum*Nachtrag: Verw Rotkohlausz a Indic col'im Mess H'-konz,346. **5–6; 5/7.**—19*Neumann*Fermentähn u Fermentreak d Blutser währ Gravid,347.—20*Mayer*Bestim sog "Restreduk" d Blut, 362.—21*Obermayer-Willhelm*Formoltit Unters Eiweisskör,369.—22-*Aron*Einfach Ext'app Extr fest u flüs Stof,386.—23*Lehmann*Katal Lichtwirk b Samenkeim,388.—24*Bernstein*Elek'chem Grundl bioelek Poten,393.—25*Scaffidi*Verh Musk'kreat b Ermüd,402.—26*Höber-Nast* Vitalfärb,418.—27*Bang-Stenström*Asphyx u Blutzuck,437.—28*Loeb* Milchsäu'bild a Traub'z, Glyc'aldeh u Dioxyacet Rind u Schw'blut, 451.—29*Griesbach*Milchsäu'bild a Kohl'hyd i lackf Blut,457.—30*Ishihara*Quan Best Milchsäu i Harn,468.—31*Salkowski*Wirk Antisep a Toxin,483.—32*Rosenthaler*Emulsinart Enzy,486.—33*Paladino*Veränd Stoffwech b Tier n Exstir Schildr u Parath,497.—34*Lifschütz*Erklär Schreiber-Lénárd, Cholest,508. (Pp. 510.)

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[2] This series of abbreviations, illustrating all others in the index, represents the following sequence of numerals; 17, 72, 73, 220, 253, 280. The numerals in bold-face type here are omitted from the abbreviations above.

BIOCHEMICAL NEWS, NOTES AND COMMENT

I GENERAL

Necrology. *N. H. Alcock,* prof. of physiology, McGill Univ.—*Francis Gotch,* Waynflete prof. of physiology, and fellow of Magdalen Coll., Oxford Univ.—*Max Kassowitz,* prof. of diseases of children, Vienna.—*William McMurtrie,* one of our leading industrial chemists, formerly chief chemist, U. S. Dep't of Agric., and prof. of chemistry, Univ. of Illinois.

In memoriam. At a recent meeting of the N. Y. Soc. of Anesthetists, the following memorial was drafted: "*Algernon Thomas Bristow*—His editorial and practical hospital support of the advancement of the art of general anesthesia has time and again aroused the admiration and emulation of this body of anesthetists, and so active and influential has this work among his many followers been, that this society wishes itself placed on record as considering his work monumentally constructive, and its cessation as a great loss to the surgical world."

Honors. KNIGHTHOOD. Dr. *E. A. Schäfer,* prof. of physiology, Univ. of Edinburgh, has received the honor of knighthood.

ORDER OF MERIT. Dr. *Hans Molisch,* director of the Inst. for Plant Physiology, Univ. of Vienna, has been invested with the Order of the Iron Crown.

HONORARY DEGREES. Columbia Univ.: Dr. *Alexis Carrel* (Rockefeller Inst.), Doctor of Science.—Princeton Univ.: Dr. *Simon Flexner* (Rockefeller Inst.) and Dr. *David L. Edsall* (Harvard Med. Sch.), Doctor of Science.—Univ. of Edinburgh: *James Wilson* (lately Sec'y of Agric.), Doctor of Laws.—Univ. of Michi-

gan: Dr. *Ludvig Hektoen* (Univ. of Chicago) and Dr. *Lafayette B. Mendel* (Yale Univ.), Doctor of Science.—Yale Univ.: Dr. *David F. Houston* (Sec'y of Agric.), Doctor of Science; Dr. *Harvey Cushing* (Harvard Med. Sch.), Master of Arts.

PRESIDENCY OF THE AMER. MEDICAL ASSOCIATION. Dr. *Victor C. Vaughan,* prof. of hygiene and physiol. chemistry, Univ. of Michigan, and dean of the dep't of medicine and surgery, was elected president of the Amer. Med. Assoc. at the recent annual meeting. . . . "In the selection of Dr. Vaughan, the Amer. Med. Assoc. has done deserved honor to one of the eminent members of the medical profession—one who is eminent not only as a physician, but also as a chemist, as a medical teacher, and as a scientist." (*Ed.: Jour. Amer. Med. Assoc.,* 1913, lx, p. 2053.)

RICKETTS PRIZE. Dr. *George L. Kite* and Mr. *Esmond R. Long,* graduate students in the dep't of pathology and bacteriology, Univ. of Chicago, have been jointly awarded the *Howard Taylor Ricketts prize* ($250) for original research in that dep't.

AWARDS OF MEDALS. The *Hanbury medal* of the Pharmaceutical Soc., London, has been awarded to Dr. *Frederick B. Power* (director, Wellcome Research Lab., London). The medal is awarded biennially for original research in the chemistry and natural history of drugs.—Dr. *Harry C. Jones* (Johns Hopkins Univ.) has been awarded the *Edward Longstreth medal* of the Franklin Inst., of Phila., for his work on the nature of solution.

Resignations, declination and appointments.[1] RESIGNATIONS. Dr. *L. H. Bailey,* director, N. Y. State Col. of Agric., Cornell Univ. (p. 569).—Dr. *Geo. F. Gracey,* prof. of chemistry and toxicology, Univ. of Texas.—Dr. *Paul G. Woolley,* dean, medical dep't, Univ. of Cincinnati.

DECLINATION. Dr. *Emil Abderhalden,* prof. of physiology, Univ. of Halle, has declined a call to Vienna to succeed Prof E. Ludwig as head of the Inst. for Medical Chemistry.

APPOINTMENTS. Berlin Univ.: Prof. *Ernst Friedberger,* head of the Pharmacol. Inst.

Cambridge Univ.: Dr. *F. H. A. Marshall,* univ. lecturer on animal physiology.

[1] In this summary, institutions from which *resignations* occurred are named in parenthesis. See page 574.

Columbia Univ.: Drs. *Wm. R. Williams,* assoc. prof., and *Henry S. Patterson,* assis. prof. of therapeutics (promotions).

Cornell Univ.: Prof. *W. A. Stocking,* Jr., of the dairy dep't, N. Y. State Agric. Col., acting director of the Agric. Col. vice Dr. *L. H. Bailey,* resigned.

General Mem'l Hosp. (N. Y. City): Dr. *Robert C. Lewis,* physiol. chemist.

Hamburg-Eppendorf: Prof. *Emil von Dungern* (Heidelberg), director of the newly established institute for experimental cancer research.

Harvard Univ.: Dr. *Reid Hunt* (chief of the div. of pharmacology, U. S. Public Marine Service), prof. of pharmacology, vice Dr. *Franz Pfaff,* resigned (p. 310); Dr. *W. J. V. Osterhout,* prof. of botany (promotion); Dr. *P. G. Stiles* (assis. prof. of physiology, Simmons Col.), instr. in physiology.

Johns Hopkins Univ.: Dr. *L. G. Rowntree,* assoc. prof. of exper. therapeutics (promotion).

Leipzig Univ.: Prof. *Walter Kruse* (Bonn), director of the Hygienic Inst., succeeding Prof. Franz Hofmann.

Leland Stanford, Jr., Univ.: Dr. *W. H. Manwaring* (assis. in pathology and bacteriology, Rockefeller Inst.), prof. of bacteriology and immunity; Dr. *F. W. Weymouth,* assis. prof. of physiology (promotion).

Marine Biolog. Lab. (Woods Hole, Mass.), Session 1913: Profs. *A. P. Mathews* (Univ. of Chicago), *R. S. Lillie* (Univ. of Penn.), *H. C. Bradley* (Univ. of Wis.), *W. E. Garrey* (Wash. Univ., St. Louis), dep't of physiology.

Mass. Inst. of Tech.: Dr. *E. B. Phelps,* assoc. prof. of biochem. research; *R. G. Daggett,* research assis. in sanitary chemistry; *Lester F. Hoyt,* assis. in water analysis, succeeding W. J. Daniels.

Northwestern Univ.: *Medical School*—Dr. *R. G. Hoskins,* assoc. prof. of physiology; *School of Pharmacy*—Dr. *John H. Long,* dean, to succeed the late *Oscar Oldberg* (page 476).

Rockefeller Inst.: *Promotions*—from *assis.* to *assoc.,* Dr. *F. B. La Forge,* chemistry; Dr. *J. B. Murphy,* pathology and bacteriology; Dr. *Martha Wollstein,* pathology and bacteriology. *New appointments:* Dr. *W. H. Brown,* assoc., pathology and bacteriology; Dr. *C. G. Bull,* assis., pathology and bacteriology; Dr. *F. L. Gates,* fellow, physiology and pharmacology. (See page 575.)

Rutgers Col.: Mr. *H. Clay Lint* (Kan. Agric. Col.), industrial fellowship, plant pathology.

Tulane Col. of Med. (reorganized): Dr. *J. T. Halsey,* prof. of clin. therapeutics; Dr. *C. S. Williamson,* Jr. (assoc. prof. of chemistry, Ala. Polytech. Inst.), assoc. prof. of industrial and sugar chemistry. (See page 575.)

Univ. of Minnesota: Dr. *F. J. Alway* (prof. of agric. chemistry, Univ. of Neb. and chemist of the Neb. Agric. Exper. Sta.), prof. of soil chemistry and chief of the div. of soils; Dr. *A. D. Hirschfelder* (Johns Hopkins Med. Sch.), prof. of pharmacy and director of the Sch. of Pharmacy; Dr. *E. P. Lyon* (prof. of physiology and dean of the Med. Col., St. Louis Univ.), dean of the med. dep't and director of the dep't of physiology, in succession to Dr. F. F. Wesbrook.

Univ. of Nebraska: Dr. *A. A. Johnson* (Western Reserve Univ.), instr. in clin. pathology; Dr. *Fred Upson* (Univ. of Chicago), prof. of agric. chemistry, Univ. of Neb. and chemist of the Neb. Agric. Exp. Sta., to succeed Dr. *F. J. Alway.*

Univ. of Singapore: Dr. *J. A. Campbell* (assis. to Prof. Schäfer, Edinburgh Univ.), prof. of physiology.

Univ. of Wisconsin: Dr. *P. M. Dawson,* instr. in physiology.

Univ. of Wyoming: Drs. *C. J. Oviatt* (Mich. Agric. Col.) and *A. E. Bowman* (Utah Agric. Col.), extension profs. of agriculture.

Washington State Exp. Sta.: Dr. *Ira D. Cardiff* (prof. of botany, State Col. of Wash.), director. He will remain head of the dep't of botany in the college.

Western Reserve Univ.: *Promotions*—Dr. *J. D. Pilcher,* assis. prof. of pharmacology; Dr. *Paul J. Hanzlik,* instr. in pharmacology; Dr. *Roy G. Pearce,* instr. in physiology.

Yale Univ.: Messrs. *S. Goldschmidt* and *A. J. Hogan,* lab. assis. in physiol. chemistry.

Lectures. Dr. *James W. Jobling* (Michael Reese Hosp., Chicago) delivered the annual address before the Minn. Pathological Soc., May 20, on The toxicity and antigenic properties of the cleavage products of bacterial proteins.—Dr. *Oscar Riddle* (Carnegie Inst.) lectured, May 5, under the auspices of the Cornell Chapter of Sigma Xi, on A relation between the storage metabolism of ova and the experimental control of sex.

Buildings, funds and scholarships. The committee on medical research, Amer. Med. Assoc., has awarded a grant of $250 to the dep't of bacteriology of the *Hoagland Laboratory, Brooklyn,* to defray the expenses of an investigation on the immunity reactions

of edestin.—The British Board of Agric. and Fisheries has awarded research scholarships in agric. science of the annual value of £150, tenable for three years, to the following candidates, among others: *W. Brown* (Edinburgh), plant pathology; Miss *E. C. V. Cornish* (Bristol), dairying; *E. J. Holmyard* (Cambridge), plant nutrition and soil problems; *R. C. Knight* (London and Bristol), plant physiology; *H. Raistrick* (Leeds), animal nutrition; Miss *T. Redman* (London), dairying; *G. Williams* (Wales), animal nutrition; *S. P. Wiltshire* (Bristol), plant pathology.—The N. Y. State Legislature has appropriated $450,000 for the Coll. of Agric., *Cornell Univ.,* which also receives $125,000 in the supply bill.—Dr. *William Duane,* for six years radium-research assis. in the Curie Lab., Paris, will organize, for the *Harvard Cancer Commission,* a laboratory in which cancer may be studied from the point of view of the physicist. The univ. requires $250,000 for the establishment of this laboratory. Meanwhile, experiments will be conducted in the Collis P. Huntington Building.—The Ill. State Legislature has appropriated $4,500,000 for the *Univ. of Ill.* for the next biennium. This includes $200,000 for the Coll. of Med.—The special alumni committee on the needs of the Med. Sch., *Univ. of Wis.,* has recommended the construction of a medical building to house the dep'ts of physiology, physiol. chemistry, pharmacology, toxicology and bacteriology, and the State Hygienic Lab.; also a student infirmary. —The *Vienna Society for the Investigation and Prevention of Cancer* has established a lab. for experimental work on cancer, mainly in the domain of chemistry and chem. therapeutics. It will be amalgamated with the *Spiegler Inst.,* which has been in existence nine years. Prof. *S. Fraenkel* has been appointed director.

Turck Institute, New York. Dr. *Fenton B. Turck* has removed his office and residence from Chicago to 14 E. 53 St., N. Y. City, where he devotes his morning hours to office practice and the afternoons to his Research Lab., at 428 Lafayette St. This research lab., formerly in Chicago, has been endowed by two former patients, of London, England, who have removed to New York, and reside near the laboratory. The work relates to various problems connected with the alimentary tract and is conducted by the Director, Dr. Turck, assisted by the following staff—Organic chemistry: *A.*

R. Rose (Univ. of Minn., Yale and Columbia), *Arthur Knudson* (Univ. of Missouri and Columbia), *Katherine R. Coleman* (Columbia Univ.); physiol. chemistry: *Vincent Greco* (Univ. of Palermo, Italy); bacteriology: *Otto Maurer* (K. Oberrealschule, Heilbronn, Germany, and Univ. of Wis.), *W. W. Browne* (Brown Univ.); general pathology: *P. J. Friedman* (Dept. of Health, Research Lab., City of N. Y.) and *Earle Kister* (Univ. of Toronto).

Commissions. In the report for 1912 of the Council of the Amer. Med. Assoc., the appointment of a *Commission on Electrical Shock* was announced. This commission completed its work in the fall, preparing a chart and a book of directions for resuscitation from electrical shock, both of which were printed by the *Electrical World,* and distributed free in large quantities to electric-light plants, power houses, factories and other places where electrical currents are in constant use. As a result of the work of this commission, the Council was asked by the director of the Bureau of Mines of the U. S. Dep't of Labor to appoint a similar *Commission on Resuscitation from Mine Gases.* The following were appointed such a commission: Dr. *W. B. Cannon* (Harvard Univ.), Chairman, Dr. *S. J. Meltzer* (Rockefeller Inst.), Dr. *Yandell Henderson* (Yale Univ.), Dr. *George W. Crile* (Western Reserve Univ.), Dr. *Reid Hunt* (Harvard Univ.), Dr. *Joseph Erlanger* (St. Louis Univ.).—A recent ruling of the U. S. Public Health Service has been made demanding that all interstate carriers supply certified water and ice to be used in public drinking fountains, tanks, etc. Under this ruling the various ice and water companies are compelled to obtain certification of their ice and water to be used on steamboats and trains. Accordingly, a commission, to be known as the *Chicago Ice Commission,* has been formed, composed of Drs. *Ludvig Hektoen,* director of the Mem. Inst. for Infec. Diseases; *Edwin O. Jordan,* prof. of bacteriology, Univ. of Chicago; and *John H. Long,* prof. of physiological chemistry, Northwestern Univ., which will undertake the examinations of ice, its source, transportation, delivery, etc., and certify the results when found satisfactory.—Dr. *C.-E. A. Winslow* (Col. of the City of N. Y.) has been appointed chairman of a *Commission on the Experimental Study of Ventilation Problems,* with an appropriation of $50,000 to

be expended during the next four years. The other members of the commission are: Prof. *F. S. Lee* and Prof. *E. L. Thorndike,* Columbia Univ.; Prof. *E. B. Phelps,* Mass. Inst. of Tech.; Dr. *J. A. Miller* and Mr. *D. D. Kimball,* New York. The fund is part of a gift, by Mrs. *Elizabeth Milbank Anderson,* to the Assoc. for Improving the Condition of the Poor.

Miscellaneous items. Pensions in the Rockefeller Inst. Pensions for the members and assoc. members of the Rockefeller Inst. have been provided by the generosity of Mr. John D. Rockefeller, who has, for this purpose, increased the endowment of the inst. by a special gift of about $500,000. The rules provide pensions of three-quarters of full pay for members of the inst. who retire at the age of 65, after fifteen or more years of service, and pensions of from one-half to three-quarters of full pay, according to the length of service, for members and assoc. members who retire at 60 years of age. There is also a provision for total disability after ten years of service, and for widows and orphaned children, at one-half the scale upon which members of the staff are pensioned.

American Chemical Society: Biological Division. The Sect. of Biolog. Chemistry, of the Amer. Chem. Soc., will meet in Rochester on Sep. 10 and 12, when the organization of the section into a division will be completed. Drs. *W. D. Bancroft, Edw. Kremers* and *A. W. Dox* with the officers, *Carl L. Alsberg* (chairman) and *I. K. Phelps* (secr.), are the committee in charge of this matter (p. 480).

Society of the Sigma Xi. Recent elections of officers (see page 481)—Pres. of the Leland Stanford, Jr., chapter: Prof. *R. E. Swain;* treas. of the Brown chapter: Prof. *P. H. Mitchell.*

Prizes. *Elié de Cyon prize.* The de Cyon prize ($600) is open for the third international competition until March 1, 1915. The prize will be awarded for the best printed or manuscript work (printed since Mar. 1913) on the functions of the internal ear, thyroid, hypophysis or pineal gland. The Acad. of Sci., Bologna, has charge of the administration of the prize fund.—*Emil Chr. Hansen Prize Fund* (see p. 535).

Personalia. Dr. *Martin H. Fischer,* who was seriously ill with appendicitis, has happily recovered.

Dr. *F. G. Hopkins,* reader in chem. physiology, Univ. of Cambridge, has been appointed a member of the Med. Research Commit. under the National Health Insurance Act, Eng.

Dr. *Howard B. Lewis,* formerly assis. in physiol. chemistry, Sheff. Sci. Sch., Yale Univ., has been appointed one of the first incumbents of the Seessel Fellowships for the encouragement of research in biological subjects at Yale Univ.

The names of Drs. *Jacques Loeb* (Rockefeller Inst.) and *A. P. Mathews* (Univ. of Chicago) are among those constituting the latest official list of Trustees of the Marine Biolog. Lab., Woods Hole, Mass.

Prof. *Graham Lusk* is a member of the Board of Managers of the Biolog. Lab. of the Brooklyn Inst. of Arts and Sci., Cold Spring Harbor, L. I.

Dr. *W. A. Murrill,* assis. director of the N. Y. Botan. Garden, is in Europe, studying types of fungi and the effect of tar dust on the trees planted on roads where the surface binding is of tar.

Prof. *Howard S. Reed,* of the Virginia Polytech. Inst., who spent the year in Europe, was a delegate to the Tenth Intern. Congr. of Agric., in Ghent, June 8 to 12.

Dr. *H. M. Richards* (Columbia Univ.) has been elected a vice-president of the Torrey Botan. Club and reelected an editor of the *Bull. of the Torrey Bot. Club.* He is one of the three editors of *Physiological Researches* (see p. 576).

Dr. *Victor C. Vaughan* was recently reelected president of the Mich. State Board of Health.

II. COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

1. General notes

Marriage: On June 19, Miss Charlotte Cecil Marie Verlage and Dr. Marston Lovell Hamlin (Harriman Research Lab., Roosevelt Hosp., N. Y.)

Appointments.[2] Dr. *Louis E. Bisch* (N. Y. Post-Grad. Med. Sch.), lecturer in educational psychology, Teachers Col., Columbia Univ.—Dr. *J. J. Bronfen Brenner* (Rockefeller Inst.), director of

[2] See footnote, page 568.

the pathological lab'y, Western Pennsylvania Hosp., Pittsburgh.—Dr. *Allan C. Eustis,* assis. prof. of dietetics and nutrition, Tulane University (promotion).—Dr. *R. F. Hare* (prof. of chemistry), vice-director, New Mexico Col. of Agric. and Mech. Arts.—Dr. *Michael Heidelberger,* assistant in chemistry, Rockefeller Inst. (promotion).—Dr. *Burton E. Livingston* (prof. of plant physiology), prof. of plant physiology and director of the lab. of plant physiology, Johns Hopkins Univ.—Dr. *Gustave M. Meyer,* assoc. in chemistry, Rockefeller Inst. (promotion).—Dr. *Winifred J. Robinson,* assis. prof. of botany, Vassar Col. (promotion).—Dr. *Oscar M. Schloss* has succeeded Dr. Ira S. Wile as conference physician at the Riverside Kitchen of the N. Y. Diet Kitchen Assoc.—Dr. *Arthur W. Swann* has succeeded Dr. Otto von Huffman as instr. in clin. pathology, Columbia Univ.—Dr. *Edwin D. Watkins,* assoc. prof. of gynecology, and prof. of general surgery in the dental dep't, Univ. of Tennessee (Memphis).—Dr. *Wm. H. Welker,* assis. prof. of physiol. chemistry, Col. of Med., Univ. of Ill. (Chicago).

Instructors at summer sessions, 1913. Biolog. Lab. of the Brooklyn Inst. of Arts and Sci. (Cold Spring Harbor, L. I.): Prof. *David D. Whitney* (Wesleyan Univ.), compar. zoology.—Biolog. Sta. of the Univ. of Montana (Yellow Bay-on-Flathead Lake): Prof. *J. E. Kirkwood* (Univ. of Mont.), botany and forestry.—Marine Biolog. Lab. (Woods Hole, Mass): Prof. *L. L. Woodruff* (Yale Univ.), embryology. (See page 579.)

Miscellaneous items. Woods Hole Corporation. The latest official list of the "members of the corporation" of the Marine Biol Lab. (Woods Hole, Mass.) contains the following names of members of the Biochem. Assoc.: Carl L. Alsberg, *Cora J. Beckwith,* William J. Gies, A. J. Goldfarb, H. B. Goodrich, *Louise H. Gregory,* E. N. Harvey, *Mildred A. Hoge,* Jacques Loeb, Max Morse, Raymond C. Osburn, Charles Packard, A. M. Pappenheimer, Henry J. Spencer, Charles R. Stockard, *Isabel Wheeler,* David D. Whitney, and L. L. Woodruff. (*Biolog. Bull.,* 1913, xxiv, p. 454.)

Members of the association lately admitted to the Society for Experimental Biology and Medicine: Drs. *J. J.*

Bronfen Brenner (West Penn. Hosp. Pittsburgh), *Russell L. Cecil* (Presbyter. Hosp., N. Y.), *H. H. Janeway* (City Hosp., N. Y.), *Edwards A. Park* (Johns Hopkins Univ.).

PERSONALIA. Prof. *A. R. Bliss* (Birmingham Med. Coll.; Grad. Sch. of Med., Univ. of Alabama) has been elected second vice-president of the Alabama Sect. of the Amer. Chem. Soc.—Dr. *E. D. Clark* has been reelected to the editorial board of the *Bull. of the Torrey Botan. Club.*—The Board of Managers of the Vanderbilt Clinic (Columbia Univ.) have recently issued, in pamphlet form, the interesting "Report of the day camp of the Vanderbilt Clinic of the Coll. of Phys. and Surg.," by Dr. *F. Morris Class,* Attending Physician.—On July 23 an expedition for the study of marine biology, under the auspices of the Carnegie Inst., Wash., set sail from San Francisco for Thursday Island, Torres Straits, Queensland, Australia. Dr. *E. Newton Harvey* was a member of the party.—The med. col. of Phila. gave a reception, April 30, at the Bellevue-Stratford Hotel, in honor of Prof. *Philip B. Hawk,* new member of the faculty of Jefferson Med. Col.—Prof. *B. E. Livingston* is the managing editor of *Physiological Researches,* the first issue of which has just appeared. He is also a member of a special committee of Sect. G (Botany) of the Amer. Assoc. Adv. Sci., to consider affiliation with the Botanical Soc. of Amer.—The Univ. of Texas gave a "One-Week's School of Home Economics," from Feb. 10–15. Among the lectures and demonstrations were: Nutritive value of foods; the importance of the menu, by Miss *Anna Richardson* and Some problems of house building; sanitary problems of the home; the house and how to plan it, by Prof. *Mary E. Gearing.*—Dr. *Winifred J. Robinson* has been appointed an adviser for women at the summer school of the Univ. of Wisconsin. —Mr. *Carl A. Schwarze's* work (N. J. Agric. Exp. Sta.) on the relation of enzymes to "peach yellows" and "little peach" is progressing favorably. He is planning to study an enzymic disorder of the tomato plant: "filiform-leaf tomato."—Dr. *F. J. Seaver* has relinquished his editorship of the *Jour. of the N. Y. Botan. Garden* and will devote more of his time to *Mycologia.*—We are glad to note that Prof. *E. A. Spitzka* has returned from his trip abroad, with his health fully restored (p. 485).

2. Proceedings of the Association

Proceedings of the twelfth scientific meeting (page 486). *Fourth annual meeting.* The fourth annual meeting of the Assoc. was held at the Columbia Med. Sch., June 2, 1913, at 8.30 P. M. Dr. Emily C. Seaman occupied the chair. Abstracts of the communications constituting the scientific proceedings are given in this issue at page 541.

After several amendments of the constitution had been adopted (one of them eliminating the Article on Dues), the following officers were elected (1913–'14):

Honorary officers. *President,* Dr. Carl L. Alsberg; *Vice-presidents:* Dr. Hugh Auchincloss, Dr. William B. Boyd, Prof. Mary E. Gearing; Dr. James C. Greenway, Prof. Mary E. Sweeny.

Active officers. *President,* Prof. Stanley R. Benedict; *Vice-president,* Dr. Frederick T. Van Beuren; *Secretary,* Dr. Alfred P. Lothrop; *Treasurer,* Prof. William J. Gies.

At the suggestion of the Ex. Committee, Prof. *Hugo Kronecker,* Director of the Physiological Institute of the University of Bern, was unanimously elected an Honorary Member of the Assoc., and Prof. *Lafayette B. Mendel,* of Yale University, was invited to be the guest of honor at our third annual dinner, next November. The Assoc. has been highly honored by letters of acceptance from both our distinguished colleagues.

The Ex. Committee's selections of Corresponding Members were enthusiastically endorsed; the full list is given on page iv.

The secretary's official register of members, including those elected at this and all other meetings during the year, was approved as read (page iv).

By unanimous vote, and in accord with the amended constitution, the Assoc. approved the Ex. Committee's suggestions (1) that the Ed. Committee in charge of the Biochemical Bulletin be enlarged to include the members who are actively engaged in biochemical work, (2) the new Ed. Committee to take the place of the Board of Directors and the former Ed. Committee, (3) the secretary of the Assoc. to be the chairman of the Ed. Committee and (4) the treasurer of the Assoc. to be the managing editor of the Biochemical Bulletin.

The Assoc. adjourned at a late hour, after a very happy and stimulating meeting.

ALFRED P. LOTHROP, *Secretary*

3. Columbia Biochemical Department

Marriage: On June 18, Miss Harriet Beckwith Rinaker and Prof. Paul E. Howe.

Appointments from the staff. Dr. *Nellis B. Foster* (assoc.), instr. in medicine, Cornell Univ. Med. Sch., with a laboratory in the N. Y. Hosp.—Dr. *Max Kahn* (assoc.), consulting physician in dietetics, Beth Israel Hosp., N. Y. He will continue as assoc. here and as director of the chemical lab. of the Beth Israel Hosp.—Dr. *Louis E. Wise* (instr.), instr. in chemistry, Univ. of Missouri.—Dr. *Edgar G. Miller,* Jr. (assis.), instr. of physiol. chemistry, Univ. of Illinois (Med. Sch., Chicago).[3]—Mr. *Arthur Knudson* (assist.), chemist, Turck Inst., N. Y. (p. 571).—Dr. *Joseph S. Hepburn* (univ. fellow), assis. chemist, U. S. Dep't of Agric. (Food Research Lab., Phila.).

Appointments to, and promotions in, the staff. Dr. *Max Kahn* (instr.), associate.—Dr. *Frederic G. Goodridge* (assis.), instructor.—Mr. *W. A. Perlzweig,* assistant.—Mr. *Victor E. Levine,* laboratory assistant (summer session, 1913).

Laboratory at Bellevue Hospital. During the past academic year Prof. Gies, aided by Dr. Edgar G. Miller, Jr., equipped a small chemical lab. in the patholog. dep't of Bellevue Hosp., and conducted research and routine work there in collaboration with Drs. Charles Norris and Cyrus W. Field. Mr. Grover Tracy assisted in some of the work. It is planned to extend this service as the needs of the hospital may determine.

Members-elect of societies. Amer. Soc. Biol. Chem.: Dr. *Alfred P. Lothrop;* Soc. Exp. Biol. Med.: Prof. *Paul. E. Howe;* Amer. Chem. Soc.: Dr. *Walter H. Eddy;* Sigma Xi: *Robert P.*

[3] Dr. Miller had been appointed prof. of physiol. chemistry in the new med. school of the Univ. of the South, Nashville, Tenn., but Mr. Carnegie's gift of $1,000,000 to the med. school of Vanderbilt Univ., in the same city, induced the Trustees of the Univ. of the South to abandon their med. school.

Calvert and *Sidney Born;* Phi Lambda Upsilon: *Arthur M. Buswell* and *Robert P. Calvert.*

Awards of higher degrees at Columbia to students of biological chemistry. DOCTORS OF PHILOSOPHY. Of the nineteen recipients of the degree of Ph.D. under the Faculty of Pure Science, at Columbia's last commencement, nine had taken "majors or minors," or both, in the biochemical department. The names of the candidates and their major and minor subjects are given below:

Name of candidate	Major	Minor	Minor
B. G. Feinberg	chemistry	chemistry	biological chemistry
H. D. Goodale	zoology	zoology	biological chemistry
J. S. Hepburn*	biological chemistry	biological chemistry	chemistry
Benj. Horowitz	biological chemistry	biological chemistry	education
Edgar G. Miller, Jr.	biological chemistry	bacteriology	pharmacology
Anton R. Rose†	biological chemistry	bacteriology	chemistry
Clayton S. Smith	biological chemistry	bacteriology	physiology
Edward C. Stone	chemistry	chemistry	biological chemistry
Charles Weisman	biological chemistry	biological chemistry	chemistry

MASTERS OF ARTS. The A.M. degree was recently conferred upon the following advanced students in the biochemical department: *Anna M. Connelly, Helen B. Davis, Mary C. de Garmo, Ula M. Dow,* Gustave Egloff, Frank R. Elder, *Ada M. Field, Beatrix H. Gross, Clara W. Hasslock,*[4] *Grace F. Hinchliff,* Edward Plaut, David F. Renshaw,[4] *Elizabeth Rothermel, Mary E. Sweeny,*[4] Fred L. Thompson, *Helen B. Thompson, Jennie A. Walker, Isabel Wheeler.*

DOCTORS OF PHARMACY. The following students of biological chemistry at the School of Pharmacy received the degree of Phar.D.: Ainslie Buck, William G. Crockett, Albert A. Muench, Herbert C. Oehlers, *Elsa G. Pickhardt,* Hugo H. Schaefer, and Leo Stein.

Summer session. COURSES. The department is conducting six courses in nutrition and biochemical methods at the summer session now in progress at Columbia. Three of these courses are given at Teachers Coll., by Prof. Gies, Dr. E. C. Seaman and Miss Tula L. Harkey; three are given at the Col. of Phys. and Surg., by Prof. Gies and Messrs. W. A. Perlzweig and Victor E. Levine. The bio-

* University fellow in biological chemistry, 1912–'13.

† The degree was awarded in February, 1913.

[4] The degree was awarded in October, 1912.

chemical lab. at the Med. Sch. is open daily for research, and will continue so throughout the summer.

INVESTIGATORS. The workers named below have been engaged in research, in the biochemical laboratory at the medical school, at various times during the vacation: Louis Berman, A. M. Buswell, Arthur D. Dryfoos, Walter H. Eddy, *Mary L. Edward,* L. L. Falke, *Helen Gavin,* William J. Gies, Mark J. Gottlieb, *Tula L. Harkey,* Max Kahn, I. J. Kligler, W. M. Kraus, Alfred P. Lothrop, Herman O. Mosenthal, William A. Perlzweig, Nathan Rosenthal, Oscar M. Schloss, *Emily C. Seaman,* A. W. Thomas, M. K. Thornton, William Weinberger, Charles Weisman, G. H. Worthing.

Miscellaneous items. Prof. Gies is a member of the third sectional committee of the Third Internat. Cong. of Refrigeration to be held in Washington and Chicago, Sept. 15–24, 1913. He was one of the speakers at the annual banquet of the First District Dental Soc. of the State of N. Y., at the Hotel Astor, Jan. 18. He addressed the Harlem Dental Soc., April 24, on the Prevention of dental caries. On May 9 he presented, at the 45th annual meeting of the Dental Soc. of the State of N. Y., the report of the Research Committee, embodying the results of a further investigation of the origin and physiological significance of salivary sulfocyanate (under the auspices of the Research Committee), with the coöperation of Prof. C. C. Lieb, Drs. Max Kahn and Edgar G. Miller, Jr., and Mr. Arthur Knudson. At the conclusion of his report, and pursuant to a recommendation in the president's annual address, Prof. Gies was elected an honorary member of the Dental Soc. of the State of N. Y. He has been invited to continue to direct the society's study of dental caries. At his suggestion the title of *The Jour. of the Allied Societies*[5] has been changed to The Jour. of the Allied *Dental* Societies (1913, viii: Mar.).

Miss Jean Broadhurst is utilizing a year's leave of absence in special study, at Ithaca, in plant physiology and bacteriology. She has received from the Torrey Botanical Club a grant of $200 from the Esther Hermann Fund to assist her in an investigation of bacteria in milk. Miss Broadhurst was recently reëlected one of the editors of the *Bull. of the Torrey Bot. Club.*

[5] *Jour. All. Soc.,* 1912, vii, pp. 408 and 507.

The department was honored during the year by the return of Prof. John S. Adriance (Williams Col.), who reviewed the work required of first year students of medicine.

Mr. A. T. Cameron (Univ. of Manitoba) was a welcome visitor in the laboratories of the department during the months of May and June, and an auditor at some of the conferences at the close of the academic year in May.

Prof. Gies recently resigned his membership in the Board of Trustees of Irving Col. for Women (Mechanicsburg, Pa.), of which he had been a member since 1900. He was recently reëlected an alumni representative in the Board of Trustees of Gettysburg Col. for a term of six years.

Mr. Arthur M. Buswell has been awarded a university fellowship in chemistry for 1913–'14; Mr. Frank R. Elder has been appointed alternate.

Mr. Guy West Wilson, lately of the N. C. Agric. Exper. Sta., and, during the past year, research scholar at the N. Y. Botan. Garden, has recently been appointed a special agent of the U. S. Bureau of Plant Industry for the study of the relation of the chestnut blight fungus to tannin and other plant products, at Rutgers College, New Brunswick, N. J.

On May 11, Dr. Max Kahn fell headlong from a moving street car. There was no internal turmoil to account for this mishap (*official*). His prompt recovery was very gratifying to all his associates.

Prof. Gies has been requested by the N. Y. Sabbath Committee to direct, under its auspices, a study of the physiological value, if any, of the weekly day of rest, entirely apart from religious or preconceived notions, and wholly from a scientific standpoint. The work will be inaugurated in the fall.

[Makes a noise like the beginning of a reform movement in this laboratory. *Printer's devil.*]

EDITORIALS

Peroxides and nitrites in plants

We learn from a Paris correspondent that Dr. Jules Wolff, of the Pasteur Institute, is about to publish in the *Annals de l'Institut Pasteur* (July), a paper in which the author reviews current knowledge on the subject of the presence of peroxides in plants. Affirmed by some, disputed by others, the question of the occurrence of these substances in plants has outlived numerous controversies. Kastle and Loevenhart, and Chodat and Bach, have attributed to peroxides the phenomena which Aso and others attribute to nitrites. Mazé has shown that the sap of all the higher plants contains nitrites. On the other hand, Wolff has found that nitrites are decomposed by the more or less acid juices of plants, and thus can induce phenomena of oxidation comparable to those which occur with the aid of the system: peroxidase—hydrogen peroxide. Wolff doubts the importance of peroxides and peroxidases as physiologic agents *because they rarely occur together in vegetable juices.* On the other hand, the number of substances that can be attacked by nitrous acid far exceeds the number that may be oxidized by the system: peroxidase—hydrogen peroxide. However, *Wolff has found, especially in apples (unpublished data), a special peroxide which is produced only when there is a lesion of the tissue. The brown color that develops on cut surfaces of fruit is a result of the action of this peroxide on a chromogenic substance. Such phenomena of coloration, contrary to current opinion, are not due to oxidases. In Wolff's view, such pigmentation results from the combined action of a peroxide and a peroxidase.* X.

The Mathews plan for an American Biological Society

In our April issue we presented a number of typical replies to our circular note inviting expressions of opinion on the "Mathews plan for the organization of an *American Biological Society*" (p. 490). In the little space available in this concluding number of Volume II, we present a few additional selections from the comment forwarded to us on this subject. The October issue will continue this

plan of facilitating open consideration and possible removal of the difficulties in the way of more effective biological organization in this country, and will also contain a summary of the ideas expressed, in this journal, on the Mathews plan of reorganization.

JAMES P. ATKINSON, *N. Y. City Dep't of Health.* I believe the plan for the Amer. Biolog. Soc'y to be a very feasible one, especially as regards the combining of one or more of the journals. It seems to me to be especially adapted to individuals who cannot afford to subscribe to many journals, and I hope that it will be successful.

R. P. BIGELOW, *Mass. Inst. of Technology.* The plan of organization for the Amer. Biolog. Soc'y proposed by Prof. Mathews seems inadvisable for the following reasons:

1. *Details of organization.* The society as proposed would simply be a confederation of existing biological societies, which in turn would become sections of the new society. The large size, national scope, and wide range of interest in the new society would involve (*a*) difficulties in securing suitable places for meetings, (*b*) long journeys and hence less regular attendance of many members, (*c*) long programs and formality of proceedings; as contrasted with independent small societies meeting within a restricted area and limited in scope, which have for their main object the promotion of intimacy and good-fellowship, with opportunity for informal discussion among men whose interests are alike.

2. *Objects.* (a) Coöperation in the abstract seems a rather hazy basis on which to found a new society. Moreover, when a definite need of coöperation arises, organizations suitable for this purpose may be found in the A. A. A. S. and the Amer. Soc'y of Naturalists. (*b*) The starting and supporting of a *Biolog. Abstract Jour.* seems to involve competition rather than coöperation. Such a publication, to be of any practical use, should segregate in one place the results of a year's work at least in each division of the subject and should be international in scope. This is the aim of *L'Année Biologique, Ergeb. d. Anat. u. Entwickelungsges., Ergeb. d. Physiol.,* and the *Jour. of the Royal Micros. Soc'y.* Why not coöperate towards the improvement of one of these existing publications, instead of starting a rival journal. (*c*) The attempt to

induce all biologists of the country to subscribe to all biological journals published in America is foredoomed to failure. For no one can pretend to keep up with the advances in all the branches of biology, and, moreover, the cost of storing the volumes would soon become embarrassing to anyone who attempts to live on a biological salary. The suggested reduction in cost would seem to depend on the formation of a society approaching in size the Amer. Chem. Soc'y, but until biology shall have as profitable applications as chemistry, this seems rather a hope than a possibility.

G. W. CRILE, *Cleveland, Ohio.* Although I appreciate the many arguments advanced in favor of Prof. Mathews' plan, I do not see my own way clear to express an unqualified commendation of the idea, as I should want to be assured that the work of this association would cover a field which has not as yet been touched upon by any other existing society, and that could not be developed in some one of them.

ARTHUR W. DOX, *Iowa State College Agric. Exper. Station.* I fail to see any special advantage to be gained by consolidating the existing biological societies, much less by organizing a new society. The chief inducement appears to be the clubbing rate which would enable the members to secure a greater number of journals at a low cost. I am personally of the opinion, however, that the majority of the biologists, were they to increase the number of their subscriptions, would derive greater benefit by subscribing to a few *foreign* periodicals in their chosen field than by taking on a greater number of Amer. journals.

WALTER H. EDDY, *High School of Commerce, N. Y. City.* The modern biology teacher in the secondary school is largely concerned in presenting the phases of biology that are intimately related to the results of current research. At the same time he is rarely in a locality where he can keep in intimate touch with such research through libraries or research institutions, and even in so rich a community as N. Y. City the field is so broad and the interests so varied that here too he is dependent upon journals for most of his information. In view of all these facts and in view of the financial impossibility of indefinite subscription to journals, any such plan as Dr. Mathews

suggests, which aims to concentrate and assist the efforts of the secondary school biologist to keep abreast of the times, must commend itself to him most forcibly.

C. Stuart Gager, *Brooklyn Botanic Garden.* While I think that an organization such as is proposed by Prof. Mathews has admirable features, especially if it were an initial step in the organization of the biological interests of the country, nevertheless, I do not feel that it could be successful, or that another society is now desirable. I also think that the inducement of receiving periodicals over such a wide range of interests as experimental psychology to bacteriology would not be very attractive to most workers, whose interests are too narrow to respond to such an inducement. It seems to me that the Mathews plan would result in an organization which in actual fact would not prove to be much more coherent than does the present Amer. Assoc. for the Adv. of Science, with its various sections, some of which at present exist chiefly on the printed program.

Paul J. Hanzlik, *Western Reserve Univ.* So far as the unification of the various biological societies is concerned, there is already the "Federation." This should suffice. The *Biolog. Abstract Jour.* would be a supernumerary because there are already several abstract journals which cover adequately the field of the biological sciences. Among the most widely circulated are probably the *Zent. f. Bioch. u. Biophy.* and *Chem. Abstracts.* It would be an additional financial burden for the Society. This is objectionable. I understand that *Chem. Abstracts* is a very expensive enterprise and, in spite of the very large membership (running into the thousands) of the Amer. Chem. Soc'y, its financial support is not adequate for its needs. It would be much more difficult for the *Amer. Biolog. Soc'y* with its comparatively small membership to support journals to the extent that the Amer. Chem. Soc'y does. Therefore, I disagree with the idea of organizing an "*Amer. Biolog. Soc'y*" and the establishment of a *Biolog. Abstract Jour.,* as proposed in the Mathews plan.

Chas. W. Hargitt, *Syracuse Univ.* One feature of the proposition appeals to me, namely, that of the *Abstract Journal.* I have

long felt that there was a real place for such a journal among us, and have several times urged such both orally, and in written and printed communications. At present our biological journals are not fully meeting the necessities for either general or prompt publication, and we get next to nothing in the way of abstracts of literature or reviews of current publications. I should like to see something after the plan proposed by Mathews put into operation so far as a journal of this sort is concerned.

Concerning the proposed "Society" I have serious doubts. Already we have more societies than we can support decently. And to add another to the list, unless it were of such a character as to meet a real need, could hardly commend general or enthusiastic support. If one or two which long ago passed into practical desuetude could be allowed to find a niche in some mortuary hall of fame it could well be possible to fill the place by a society something after the Mathews plan. I should incline to hope very sincerely that its name might be unencumbered by that overworked adjective "American"! Pray let us have at least one society which can be trusted to stand on its own characteristic merits, without expletives, apologies, or explanations.

E. M. HOUGHTON, *Detroit, Mich.* After careful consideration of the subject, I most heartily commend the proposed plan as one that will be most desirable for those of us who are specially interested in biological subjects. I hope that the movement may gather sufficient force to put the matter into practical operation.

J. S. KINGSLEY, *Tufts Col.* I have been greatly interested in Dr. Mathews' plan for the organization of a general society to cover all sides of biology and wish that it could be consummated, but I am afraid that he has overestimated the membership and income of such an organization and has underestimated the expenses of the journals to such an extent that it vitiates the whole scheme. Thus I believe that a journal of abstracts, well done, would be of great value, but I do not see in the plan any adequate provision for the payment for the abstracts, and I know from experience that there are few who can be depended upon to do the work as a labor of love. The *Jour. of the Royal Micros. Soc'y* publishes such abstracts in a limited field, and one of the editors told me that he was paid a salary of £120 a year for about half of the zoological ab-

stracts. Then Mathews has failed to take into account the many overhead expenses in such a series of journals—postage, clerical work, corrections, bad accounts and a thousand other items which are inevitable.

F. C. Koch, *Univ. of Chicago.* The Mathews plan for the organization of the Amer. Biol. Soc'y appeals to me as practicable without doubt, and as very desirable and necessary.

Edwin Linton, *Washington and Jefferson Col.* A large number of working biologists are to be found among teachers in high schools and the smaller colleges. Many of them are obliged to conduct elementary courses in as diverse subjects as anatomy, bacteriology, botany, physiology and zoology. It is out of the question for the majority of such teachers, or of the institutions which they represent, to subscribe for the list of journals enumerated by Dr. Mathews on page 264 of the Biochemical Bulletin for January, 1913. To such persons, access to this entire list of journals would add greatly to their efficiency as teachers and as workers in biology. Therefore, any plan whereby a larger number of the various biological publications will be made available than is now the case to the majority of biologists should command the sympathetic consideration of all biologists.

F. E. Lloyd, *McGill Univ.* I am very much impressed with the Biolog. Soc'y proposition, set forth in Mathews' paper. For a long time it has seemed to me that we have a lot to learn from the chemists, and I quite believe that some such effort as outlined by Mathews would go a long way toward unifying and stimulating effort. I shall be glad to coöperate in any way that I can. I feel that the details should be gone into pretty thoroughly, so as to get the costs down as much as possible, but an abstract journal analogous to the *Chem. Abstracts* would be of immense value.

Hugh McGuigan, *Northwestern Univ. Med. Sch.* The Mathews plan suggests improvements that seem feasible. To make it more definite, the estimated costs should be worked out by a committee of publishers and scientists and an authoritative statement of the cost presented. The Federation of Amer. Socs. for Exp. Biol. forms an excellent nucleus to commence with and is a step toward the consummation of the plan.

JACOB REIGHARD, *Univ. of Mich.* I take it that each American biologist is now paying for the journals to which he subscribes as much as he can afford. Most of my friends have as many journals as they would like. Their libraries contrast unfavorably in this respect with those of their European colleagues. I do not doubt that any plan that would give them more for the same money or for less money would be welcome. On the other hand I do not know of many men who would consider the *whole list* of thirteen journals of use to them. If there were opportunity for them to get the journals they now get at less price than they now pay, they would tend to take advantage of it,—and the income of the Society would be reduced by so much.

There is one argument not touched upon by Prof. Mathews which might have great weight in starting the movement. Every biologist pays out a good deal annually for reprints. If nearly every biologist received the journals in his field, there would be little use in distributing reprints. Those furnished him by the journals without cost might meet his needs for exchange with foreign correspondents. He might thus save a great deal more than he now pays out for subscriptions.

I am not convinced of the wisdom of central control for all biological journals, and should prefer to see journals left under their present control. I fear that here, as elsewhere, central control is likely to inhibit individual initiative. The interests of the chemists seem to me less diverse than those of the biologists and therefore more likely to be adequately served by a central organization.

I am, then, in favor of Prof. Mathews' plan, with the elimination of the feature of central control. I believe that the advantages of the plan to the individual journals are such as to insure their permanency without central control.

Change of action and interest are the secrets of recreation, as the spirit of service is the spirit of happiness.—*Creelman.*

Crystals

The medical facts that have been elicited and elucidated in laboratories during the past fifty years have done more to revolutionize medical practise than the bedside observations of the past two thousand years.—*Janeway.*

INDEX

In two divisions:

(I) Author index (pp. 589–590);
(II) Subject index (pp. 591–604):

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(*A*) Impersonal *subjects (p. 591);*
(*B*) Personal *subjects (p. 599).*

References, in *roman* numerals, to parts of the *summary of contents* (pp. x–xiii) and the *list of illustrations* (p. xv), facilitate use of the volume.

I. AUTHOR INDEX

The names of the authors of the leading papers, and the general subjects treated by each, may be obtained promptly from the summary of contents (pp. x–xiii). This division of the index (I) includes not only the names of the main contributors, but also the names of accredited authors of abstracts, editorials, quotations, etc.

II. SUBJECT INDEX. A. IMPERSONAL SUBJECTS

The names of authors are given on pp. 589–590.

Personal subjects are indexed on pp. 599–604.

General subjects may be seen at a glance on pp. x–xiii. This subject index is aimed at details that the titles of papers do not include, altho it also makes due reference to the titles. A *recurrent subject* in any paper or formal section of the volume is indicated but *once,* as a rule, by the numeral on the first page of its occurrence in, or on the opening or concluding page of, the section containing it. Numerous *cross references* facilitate prompt access to details. The index ignores impersonal matters that are secondary to, or of no special interest apart from, the personal references to which they pertain (*e. g.,* items of "biochemical news, notes, and comment"). Routine matters (*e. g.,* common tests, ordinary reagents) are not indicated unless they appear in special settings.

This comprehensive index is intended to guide the reader to the main path through any and every subject, or group of subjects, in the volume.

II. SUBJECT INDEX (CONTINUED). B. PERSONAL SUBJECTS

Impersonal subjects are indexed on pp. 591–599.

The names of authors are given on pp. 589–590.

This portion of the index relates primarily to *directly personal items*, but does not include personal references in incidental historical or similar statements. A *recurrent* name in any personal item or formal section of related references is indicated by the numeral on the first of the group of pages presenting the name.

OFFICERS OF THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY, 1912–1913*

OFFICIAL REGISTER, MAY 31, 1913

WILLIAM J. GIES: *Professor and Chairman of the Staff;* Consulting chemist, New York Botanical Garden; Pathological chemist, First Division, Bellevue Hospital; Member of the Faculties of N. Y. Teachers College and N. Y. College of Pharmacy. [B.S., Gettysburg College, 1893 and M.S., 1896; Ph.B., Yale University, 1894 and Ph.D., 1897. Instructor, 1898–'02; adjunct professor, 1902–'05; professor, 1905–.]

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COURSES OFFERED BY THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY, 1912–13

(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. ELEMENTARY ORGANIC CHEMISTRY. (*First half year. Medical School.*) Introductory to course 102 (52). (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Drs. Wise and Goodridge, and Messrs. Miller and Knudson.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101(2)—**Grad.** GENERAL BIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Prof. Gies, Dr. Lothrop and Messrs. Miller and Knudson.

101(2)—**B. T.** GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* (*All year. Medical School.*) L, 1 hr. Lw, 4 hr. Dr. Eddy.

101:102—**T. C.** GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Each half year. Teachers College, School of Practical Arts.*) L, 2hr. R, 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Dr. Seaman and Misses Wickwire and Harkey. (This course is designated "Chemistry 51" and "Household Arts Education 125" in the Teachers College Announcement.)

This course is designated "Chemistry s 51" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies, Dr. Seaman and Miss Shaffer.

102 (52)—**Med.** GENERAL PHYSIOLOGICAL CHEMISTRY. (*Second half year. Medical School.*) *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Dr. Wise, and Messrs. Miller and Knudson.

This course is designated "*S*—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Dr. Smith.

104. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (*Second half year. Teachers College, School of Practical Arts.*) L, 1 hr. Prof. Gies. (This course is designated "Chemistry 52" in the Teachers College Announcement.)

209–210. CHEMISTRY OF NUTRITION. (*All year. School of Pharmacy. Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

213–214. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (*All year. Teachers College, School of Practical Arts.*) L, 1 hr. Lw, 5 hr. Prof. Howe, Dr. Seaman and Mr. Horowitz. (This course is designated "Household Arts Education 127" in the Teachers College Announcement.)

217–218. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS AND URINARY ANALYSIS IN GENERAL. (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Profs. Gies and Howe, Dr. Lothrop, and Messrs. Miller and Hepburn.

219–220. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* (*All year. Medical School.*) L, 2 hr. Lw, 14 hr. Profs. Gies and Howe, and Dr. Lothrop.

221–222. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* (*All year. Medical School.*) L, 2 hr. Lw, 14 hr. Prof. Gies.

223–224. NUTRITION IN DISEASE. (*All year. Medical School.*) L, 1 hr. Profs. Gies and Howe, and Drs. Foster, Mosenthal, Kahn and Goodridge.

225–226. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. (*All year. Medical School.*) Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Howe, and Dr. Lothrop.

TOXICOLOGY

231–232. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. (*All year. Medical School.*) Lw, 6 hr. Prof. Gies and Mr. Miller.

BOTANY

235–236. CHEMICAL PHYSIOLOGY OF PLANTS. (*All year. New York Botanical Garden.*) L, 1 hr. Lw, 5 hr. Prof. Gies.

BACTERIOLOGY

241–242. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Prof. Gies.

SANITATION

105. SANITARY CHEMISTRY. (*Second half year. Teachers College, School of Practical Arts*). L, 1 hr. Lw, 3 hr. Dr. Seaman and Miss Harkey. (This course is designated "Chemistry 57" and "Household Arts Education 129" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301–302. BIOCHEMICAL SEMINAR. (*All year. Medical School.*) 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance, in any of the departmental laboratories.

LABORATORIES FOR ADVANCED WORK IN BIOCHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College, New York Botanical Garden and Bellevue Hospital. Each laboratory is well equipped for research in nutrition and all other phases of biological chemistry.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all past and present workers in the Department.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds scientific meetings regularly on the first Fridays in December, February and April, and on the first Monday in June. These meetings are open to all who may be interested in them.

SUMMER SCHOOL COURSES

See page 579.

BOOKS RECEIVED

The BIOCHEMICAL BULLETIN promptly acknowledges here the receipt of publications presented to it. Reviews are matter-of-fact statements of the nature and contents of the publications referred to, and are intended *solely to guide possible purchasers;* the wishes or expectations of publishers or donors of volumes will be disregarded, if they are incompatible with our convictions regarding the interests of our colleagues. *The sizes of the printed pages are indicated, in inches, in the appended notices.*

An introduction to the chemistry of plant products. By Paul Haas (lecturer on chemistry, Royal Gardens, Kew) and T. G. Hill (reader in vegetable physiology, Univ. of London). Pp. 401—4 × 7; $2.25 net. Longmans, Green and Co., 1913.

Excellent discussion of the chemistry and biological significance of many of the most important plant constituents. Besides extended treatment of carbohydrates, lipins and proteins, chapters are devoted respectively to glucosides, tannins, pigments, nitrogenous bases (alkaloids, ptomaines, purins), colloids and enzymes. Methods of preparation, detection and quantitative determination are numerous and well described. Good *subject* index. The most valuable recent contribution of its kind to phyto-chemistry. Strongly recommended to biological chemists generally—to botanists in particular. Gies.

Practical physiological chemistry. By Sidney W. Cole, demonstrator of physiology, Trinity College, Cambridge. Third edition. Pp. 230—4 × 6½; 7s. 6d. net. W. Heffer & Sons, Ltd., Cambridge, Eng., 1913.

Very useful laboratory manual. Subject treated chiefly from static point of view. Practical throughout. Methods well selected. Quantitative procedures given satisfactory attention. Special emphasis laid upon Folin's microchemical methods of urinary analysis. Good index. See review by Walter Jones, *Jour. Amer. Chem. Soc.*, 1913, xxxv, p. 1064. Gies.

Physiological Researches. (Appears at irregular intervals.) Edited by Burton E. Livingston (Manager), Johns Hopkins Univ.: Daniel T. MacDougal, Carnegie Inst. of Wash.; and Herbert M. Richards, Columbia Univ. **Vol. I: No. 1**—*The relation of environmental conditions to the phenomenon of permanent wilting in plants, by Joseph S. Caldwell.* Pp. 1–56—4⅛ × 7½; July, 1913; $0.75 ($5.00 per vol.). Physiological Researches, Station N, Baltimore, Md.

Physiological Researches, unlike the conventional journal, appears at irregular intervals in the form of individual physiological papers, paged sequentially; each succession of about 450 pages will be a volume unit. The papers will be numbered sequentially, in each volume and in the whole series. An editorial feature will be the publication of an author's abstract in advance of the appearance of each paper, and also as a preliminary part of each paper in its final form in the series. Although the three editors are eminent *botanists,* it is their intention to make *Physiological Researches,* as its name implies, an archive for physiology in its broadest and deepest sense. This new publication begins its career auspiciously and promises not only to rival the *Amer. Jour. Physiol.* in interest and value, but also to share with that journal the high credit of stimulating the advancement of physiological research. The initial paper, by Prof. Caldwell, is a masterly treatment of an interesting and perplexing subject, and establishes a standard of merit which will doubtless characterize each issue of *Physiological Researches.* Gies.

Researches in biochemistry conducted in the Johnston Laboratory, Univ. of Liverpool. Edited by Benjamin Moore, Johnston prof. of biochem.,

and Owen T. Williams, demonstrator of biochem. Vol. II; 1908–1911. (27 reprints.)

Studies from the departments of pathology, bacteriology, experimental pathology, experimental therapeutics, Cornell Univ. Med. Coll. Vol. XII; 1912. (10 reprints.)

Collected papers: Lister Inst. of Preventive Med. No. 8; 1911–1912. Part I. Bacteriological, pathological and epidemiological papers (29 reprints); Part II. Physiological, zoological and biochemical papers (33 reprints).

Studies from the Rockefeller Institute for Medical Research. Vol. XVII; 1913. 56 reprints; *repaged, with index.*)

Collected papers: Institute of Physiology, University College, London. Edited by Ernest H. Starling, Jodrell professor of physiology. Volume XVII; 1912–13. (32 reprints.)

Collected papers: Physiological Laboratory, Kings' College, University of London. Edited by W. D. Halliburton, professor of physiology. Volume XII; 1913. (12 reprints.)

Glycosuria and allied conditions. By P. J. Cammidge. Pp. 467—4×6¾; $4.50 net. Longmans, Green & Co., New York; Edward Arnold, London, 1913.

The chemical constitution of the proteins: Part II, Synthesis, etc. 2d ed. (One of the *Monographs on Biochemistry.*) By R. H. A. Plimmer, Univ. reader and ass't prof. of physiological chem., University Coll., London. Pp. 107—4⅜ × 7½; $1.20 net. Longmans, Green & Co., 1913.

Diabetes: Its pathological physiology. (One of the *International Medical Monographs.*) By John J. R. Macleod, professor of physiology, Western Reserve University, Cleveland, O. Pp. 224—4 × 7; $3.00 net. Edward Arnold, London; Longmans, Green & Co., New York, 1913.

Collected papers: Laboratory of physiological chemistry, Sheffield Scientific School, Yale University. 1911–1912. (35 reprints.)

Practical physiological chemistry. *A book designed for use in courses in practical physiological chemistry in schools of medicine and of science.* By Philip B. Hawk, professor of physiological chemistry and toxicology in the Jefferson Medical College of Philadelphia. Fourth edition, revised and enlarged. Pp. 475—4½ × 8; $2.50 net. P. Blakiston's Sons & Co., Phila., 1912.

The protein element in nutrition. (One of the *International Medical Monographs.*) By Major D. McCay, professor of physiology, Medical College, Calcutta. Pp. 216—4 × 7, with 8 full page portraits of human subjects; $2.00 net. Longmans, Green and Co., New York; Edward Arnold, London, 1912.

Oxidations and reductions in the animal body. (One of the *Monographs on Biochemistry.*) By H. D. Dakin, The Herter Laboratory, New York. Pp. 135—4½ × 8; $1.40 net. Longmans, Green and Co., 1912.

Researches on cellulose. III (1905–1910). By C. F. Cross and E. J. Bevan. Pp. 173—3½ × 6; $2.50 net. Longmans, Green and Co., 1912.

Sigma Xi Quarterly. Vol. 1, No. 1 (March, 1913). Pp. 30. Editorial committee: J. McK. Cattell, D. C. Miller, H. B. Ward, S. W. Williston. Published by the Society of the Sigma Xi, H. B. Ward, corresponding secretary, Champaign, Ill.

Bulletin of the American Home Economics Association. Series 1. No. 1 (Nov., 1912). Published quarterly by the American Home Economics Association, Benjamin R. Andrews, secretary, 525 W. 120th St., New York City.

Abstract-bulletin of the Physical Laboratory of the National Electric Lamp Assoc. Vol. I: No. 1, pp. 1–128; Jan., 1913. Cleveland, O.

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(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. ELEMENTARY ORGANIC CHEMISTRY. (*First half year. Medical School.*) Introductory to course 102 (52). (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Drs. Wise and Goodridge, and Messrs. Miller and Knudson.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101(2)—Grad. GENERAL BIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Prof. Gies, Dr. Lothrop and Messrs. Miller and Knudson.

101(2)—B. T. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* (*All year. Medical School.*) L, 1 hr. Lw, 4 hr. Dr. Eddy.

101: 102—T. C. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Each half year. Teachers College, School of Practical Arts.*) L, 2hr. R, 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Dr. Seaman and Misses Wickwire and Harkey. (This course is designated "Chemistry 51" and "Household Arts Education 125" in the Teachers College Announcement.)

This course is designated "Chemistry s 51" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies, Dr. Seaman and Miss Shaffer.

102 (52)—Med. GENERAL PHYSIOLOGICAL CHEMISTRY. (*Second half year. Medical School.*) *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Howe, Dr. Wise, and Messrs. Miller and Knudson.

This course is designated "*S*—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Dr. Smith.

104. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (*Second half year. Teachers College, School of Practical Arts.*) L, 1 hr. Prof. Gies. (This course is designated "Chemistry 52" in the Teachers College Announcement.)

209–210. CHEMISTRY OF NUTRITION. (*All year. School of Pharmacy. Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

213–214. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (*All year. Teachers College, School of Practical Arts.*) L, 1 hr. Lw, 5 hr. Prof. Howe, Dr. Seaman and Mr. Horowitz. (This course is designated "Household Arts Education 127" in the Teachers College Announcement.)

217–218. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS AND URINARY ANALYSIS IN GENERAL. (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Profs. Gies and Howe, Dr. Lothrop, and Messrs. Miller and Hepburn.

219–220. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* (*All year. Medical School.*) L, 2 hr. Lw, 14 hr. Profs. Gies and Howe, and Dr. Lothrop.

Courses in Nutrition (continued)

221–222. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* (*All year. Medical School.*) L, 2 hr. Lw, 14 hr. Prof. Gies.

223–224. NUTRITION IN DISEASE. (*All year. Medical School.*) L, 1 hr. Profs. Gies and Howe, and Drs. Foster, Mosenthal, Kahn and Goodridge.

225–226. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. (*All year. Medical School.*) Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Howe, and Dr. Lothrop.

TOXICOLOGY

231–232. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. (*All year. Medical School.*) Lw, 6 hr. Prof. Gies and Mr. Miller.

BOTANY

235–236. CHEMICAL PHYSIOLOGY OF PLANTS. (*All year. New York Botanical Garden.*) L, 1 hr. Lw, 5 hr. Prof. Gies.

BACTERIOLOGY

241–242. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* (*All year. Medical School.*) L, 1 hr. Lw, 7 hr. Prof. Gies.

SANITATION

105. SANITARY CHEMISTRY. (*Second half year. Teachers College, School of Practical Arts*). L, 1 hr. Lw, 3 hr. Dr. Seaman and Miss Harkey. (This course is designated "Chemistry 57" and "Household Arts Education 129" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301–302. BIOCHEMICAL SEMINAR. (*All year. Medical School.*) 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance, in any of the departmental laboratories.

LABORATORIES FOR ADVANCED WORK IN BIOCHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College, New York Botanical Garden and Bellevue Hospital. Each laboratory is well equipped for research in nutrition and all other phases of biological chemistry.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all past and present workers in the Department.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds scientific meetings regularly on the first Fridays in December, February and April, and on the first Monday in June. These meetings are open to all who may be interested in them.

SUMMER SCHOOL COURSES

See page 570.

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The BIOCHEMICAL BULLETIN is a quarterly biochemical review. It publishes results of original investigations in biological chemistry, presents miscellaneous items of personal and professional interest to chemical biologists, and solicits original contributions to research, preliminary reports of investigations, abstracts of papers, addresses, lectures, criticism, reviews, descriptions of new methods and apparatus, practical suggestions, biographical notes, historical summaries, bibliographies, quotations, news items, proceedings of societies, personalia, views on current events in chemical biology, etc.

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