opinion among physicists and engineers as to the order or importance of the definitions of some of the physical quantities, particularly those having to do with electric and magnetic fields.

Those among the purest of mathematicians who fear too much contamination with the world of matter will probably shudder at the statement on page 16 to the effect that over a given range a function of x may always be represented by a curve with the slope as its derivative. However, such matters are mentioned, not as criticisms, but to clear the conscience of the reviewer. In the same category are such statements as the definition of matter, on page 26, as a physical entity which possesses mass. Personally, this reviewer is happy with the thought, even though the entity concerned is a quantum of radiation. However, some might question the definition if they were looking for trouble. Perhaps the definition coming nearest to the realm of inviting valid criticism is that of kinetic energy, which is defined as $mv^2/2$, where m is the mass and v the velocity. Even in the domain of electrical engineering, when approaching such modern appliances as are involved in cyclotrons, etc., and even in some problems of thermionics, one has to recognize the relativistic significance of kinetic energy.

Again referring to the matter of definitions, one who read the book without already having established in his mind a consistency of order in the matter of definitions might become confused when, on page 30, he reads that "an electric current through a surface is the time rate at which positive or negative electricity passes through the surface" and finds that, up to this point, he has had no definition of a quantity of electricity in the numerical sense. However, the book is intended primarily for those who have stabilized their thoughts on these matters, and for such it is an invaluable work, both as regards its scope and presentation.

The book carries a very copious index of some fifty or more pages, which adds materially to its value. Those responsible for the preparation of this work deserve the greatest commendation and the gratitude of all students of electrical science.

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SPECIAL ARTICLES

ANTIBACTERIAL PROPERTIES OF PROTAMINE AND HISTONE

IT is known that antibacterial compounds such as gramicidin and anionic detergents attack with almost complete selectivity only Gram-positive micro-organisms.[1, 2, 3] It has been demonstrated that the activity of these compounds is inhibited by phospholipids.[4] The possibility has been suggested that the inability of gramicidin and anionic detergents to inhibit most Gram-negative bacteria may be caused by the phospholipids of these organisms.[1, 3, 4] On this basis, it seemed possible that compounds such as protamine sulfate which are known to precipitate cephalin[5] might cause Gram-negative micro-organisms to become susceptible to these selective inhibitors. In testing this hypothesis we found that (a) certain selective inhibitors in the presence of protamine became active toward the Gram-negative organism *Escherichia coli,* and (b) the basic proteins protamine and histone themselves possess anti-bacterial properties.

Despite the extensive literature on protamines, their antibacterial properties appear to have been largely overlooked except for the isolated observation of McClean[6] that protamine inhibited the growth of *Eberthella typhosa.* This investigator also found that both protamine and histone inhibited the growth of vaccinia virus;[7] and Reiner, deBeer and Green recently showed that the respiration of *Trypanosoma equiperdum* was partially inhibited by these compounds.[8]

In our experiments, metabolic effects were measured in Warburg respirometers as previously described.[2] Each vessel contained from 5 to 15 billion organisms suspended in 3.0 ml of 0.038*M* phosphate buffer containing 0.02*M* glucose. Bactericidal power was determined by the F. D. A. phenol coefficient technique with the following modifications: (a) washed cells were employed, and were exposed to the test compounds for 5-, 15- and 45-minute periods, and (b) all tests were performed at 37° C. For the experiments with protamine several samples of salmine sulfate obtained from Dr. George A. Harrop, of E. R. Squibb and Sons, were used. The histone was prepared from fresh calf thymus by the method of Felix and Harteneck.[9]

SENSITIZATION OF GRAM-NEGATIVE MICRO-ORGANISMS

Neither Tergitol-7, a typical anionic detergent, nor

[1] R. J. Dubos and R. D. Hotchkiss, *Trans. and Studies Coll. Phys. Philadelphia,* 10: 11, 1942.
[2] Z. Baker, R. W. Harrison and B. F. Miller, *Jour. Exp. Med.,* 73: 249, 1941.
[3] *Ibid.,* 74: 611, 1941.
[4] *Ibid.,* 74: 621, 1941.
[5] E. Chargaff and M. Ziff, *Jour. Biol. Chem.,* 131: 25, 1939.
[6] D. McClean, *Jour. Path. and Bact.,* 34: 459, 1931.
[7] *Ibid.,* 33: 1045, 1930.
[8] L. Reiner, E. J. deBeer and M. Green, *Proc. Soc. Exp. Biol. and Med.,* 50: 70, 1942.
[9] K. Felix and A. Harteneck, *Z. physiol. Chem.,* 157: 76, 1926.

tyrothricin[1] nor the selective antibacterial compound of Hoogerheide[10] had any effect on the respiration of *E. coli.* Protamine sulfate had an inhibitory action which could be minimized by performing the experiments at or below pH 7.0. However, when protamine was mixed with either Tergitol-7, tyrothricin or Hoogerheide's compound, the mixture inhibited respiration completely within 5 minutes. A typical experiment is shown in Fig. 1. No such potentiation of action could be demonstrated with non-selective inhibitors such as merthiolate or phenol.

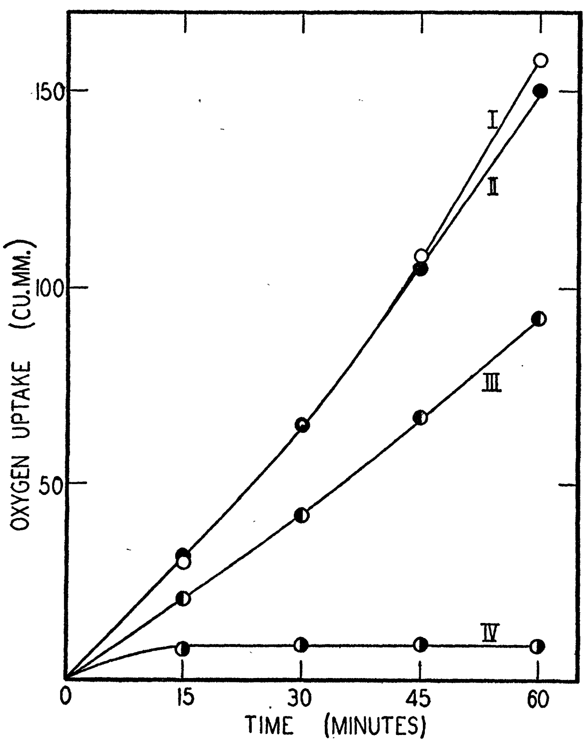

FIG. 1. Effect of Protamine and Tyrothricin on Respiration of *E. coli.* T. 38° C. Atmosphere, air. pH 5.3. 5×10^9 cells per vessel. I. Control Respiration. II. Tyrothricin (1: 15,000). III. Protamine (1: 3,000). IV. Mixture of Tyrothricin (1:15,000) and Protamine (1: 3,000).

Mixtures of protamine and tyrothricin, or protamine and Tergitol-7, were bactericidal toward *E. coli* at pH 5.3 even though considerably higher concentrations of the individual compounds were inactive.

EFFECT OF PROTAMINE OR HISTONE ALONE

Salmine sulfate exerts a powerful inhibitory action on the respiration or anaerobic metabolism of a number of species. *Gram-negative micro-organisms: E. typhosa* was completely inhibited at a 1: 38,000 dilution of the protamine, pH 8.1; and *Shigella paradysenteriae* at 1: 28,000, pH 7.0. *E. coli* was somewhat more resistant, requiring a 1: 9,000 concentration for complete inhibition at pH 8.1; *Neisseria catarrhalis* was slightly less sensitive, and *Proteus vulgaris* was completely resistant. *Anaerobic species: Clostridium perfringens (welchii), Clostridium tetani* and *Clostridium histolyticum* were extremely sensitive to protamine, *e.g.,* 1: 24,000 protamine completely inhibited anaerobic glucolysis by the Welch bacillus. *Gram-positive aerobes:* Of the members of this group tested, *Bacillus subtilis* was inhibited completely at a 1: 15,000 protamine concentration; *Staphylococcus albus,* completely at 1: 3,000; and *Staphylococcus aureus,* partially at 1: 3,000, pH 8.1.

Thymus histone also inhibited respiration of bacteria, but differed from salmine in that it had its optimum activity at an acid pH, whereas the protamine was most active at an alkaline pH, *e.g.,* 8.1. At its optimum pH, the activity of the thymus histone compared favorably with the optimal inhibitions obtained with salmine.

The bactericidal activity of protamine appears to parallel the effects on metabolism. Salmine sulfate was much more bactericidal at pH 8 to 9 than at pH 7, and was inactive at pH 5. Certain strains of *E. typhosa* were killed in 5 minutes at pH 8 by a 1: 32,000 dilution of protamine. *E. coli,* Group A streptococci and Type 1 pneumococci were also killed at relatively high dilutions of protamine, although a longer time was required. Salmine sulfate was bacteriostatic toward the Welch bacillus at a dilution of 1: 12,000, and *S. aureus* at 1: 20,000. Against *Proteus vulgaris,* the only other organism tested, protamine had neither bactericidal nor bacteriostatic power. In contrast to these results, thymus histone did not show any bactericidal or bacteriostatic activity, even at the optimum pH for its inhibition of metabolism.

It was noted that certain strains of *E. typhosa* and *E. coli* became completely resistant to the bactericidal action of the protamine.[11] None of the strains showed any variation in their resistance toward phenol.

The presence of serum, blood or broth caused a considerable reduction in the antibacterial effects of both the protamine and histone.

DISCUSSION

These results suggest certain new possibilities in the development of antibacterial compounds. (1) It is possible to "sensitize" Gram-negative micro-organisms by means of a protamine to compounds which ordinarily act only on Gram-positive species. Further

[10] J. C. Hoogerheide, *Jour. Franklin Inst.,* 229: 677, 1940.

[11] In the case of *E. coli* the increase in resistance to protamine seemed to be coincident with a change from smooth to rough forms. No such correlation could be made with *E. typhosa.*

extensions of this approach may be possible since our preliminary experiments with basic dyes such as methylene blue and acriflavin indicate that these dyestuffs potentiate the action of tyrothricin and the anionic detergent, Tergitol-7, in the same way as does the protamine. (2) The similarity in chemical structure of protamine, histone, tyrothricin and the germicidal protein from wheat[12] strengthens the suggestion made by Dubos and Hotchkiss[1] that certain relatively simple polypeptide configurations may serve as the basis for a large group of antibacterial compounds. Since protamines from different species of fish vary considerably in chemical composition, it should be desirable to investigate the antibacterial effects of a number of protamines. The antibacterial properties of partial hydrolysis products of the protamines and histones, as well as of similar synthetic polypeptides, merit further study.

Chemotherapeutic applications of protamine or histone are probably greatly limited by the relatively high toxicity of these compounds when administered intravenously or intraperitoneally.[8, 13] Our preliminary tests confirm the results in the literature, but indicate that these compounds have no apparent toxicity for such a tissue as the rabbit eye.

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THE EFFECT OF VITAMIN E ON THE BLOOD PLASMA LIPIDS OF THE CHICK[1]

IN a previous communication[2] Dam and Glavind have drawn attention to the fact that the two lipotropic substances, lipocaic and inositol, can to a considerable degree protect against the exudative diathesis in vitamin E deficient chicks, whereas addition of cholesterol to the vitamin E deficient diet accelerates and aggravates the symptom.

We have now made a study of the fasting level of the lipids in the blood plasma of chicks living on vitamin E deficient diets with or without the addition of lipocaic or vitamin E. This study has shown that vitamin E exerts an effect on the plasma lipids similar to that of lipocaic and that the ingestion of cholesterol acts in the opposite direction.

The observed effect of adding vitamin E or lipocaic to the vitamin E deficient diet consists in an increase of the average ratio of the phospholipids to the other lipid fractions (total lipids, cholesterol or fatty acids) of about 20 to 40 per cent., whereas addition of cholesterol to the diet lowers this ratio without increasing the absolute cholesterol content of the plasma. The values for the individual chicks within a group of 5 chicks receiving the same diet show considerable variation so that it is not possible to predict from a simple determination of the plasma lipids of one single chick whether the animal belongs to the protected group or not. This is, however, not astonishing when attention is paid to the great individual variation of the lipid values in humans which renders it impossible, for instance, to diagnose pregnancy from a plasma cholesterol determination even if there is a definite hypercholesterolemia during pregnancy.

Since any effect on the blood plasma lipids must be a consequence of changes in the metabolism of the lipids in tissue, our observations suggest that vitamin E has a lipotropic effect similar to that of lipocaic. Further investigation of this problem must determine whether direct evidence for such an effect of vitamin E on tissue lipids can be found and whether a particular fraction of the phospholipids is involved.

Whereas a sufficient dose of vitamin E gives complete protection against exudates, lipocaic does not seem to give absolute protection but merely brings down the incidence of the symptom from 80 to 100 per cent. in the group receiving the basal diet to 10 to 20 per cent. in the lipocaic group. This seems to indicate that the effect of vitamin E is of a more fundamental nature than that of lipocaic and is not confined to the lipotropic effect alone—or that lipocaic probably remedies only one of the consequences of the lack of vitamin E.

Since vitamin E and lipocaic[3] apparently can bring about the same change of the blood plasma lipids, it is likely that the vitamin E deficient chick is lacking in the active principle of lipocaic, which would mean that the formation of this substance in the body of the chick depends upon the presence of vitamin E in the diet. This question should be elucidated by further experiments.

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VITAMIN C CONTENT OF PERSIMMON LEAVES AND FRUITS

PERSIMMON leaves have been found to give excep-

[12] A. K. Balls, W. S. Hale and T. H. Harris, *Cereal Chem.*, 19: 279, 1942.

[13] W. B. Shelley, M. P. Hodgkins and M. B. Visscher, *Proc. Soc. Exp. Biol. and Med.*, 50: 300, 1942.

[1] Aided by a grant from the Josiah Macy, Jr., Foundation.

[2] H. Dam and J. Glavind, SCIENCE, 96: 235, 1942.

[3] We are indebted to Hoffman LaRoche, Inc., Nutley, New Jersey, for supply of synthetic vitamin E (Ephynal acetate) and to Dr. L. R. Dragstedt, University of Chicago, and the Lilly Research Laboratories, Indianapolis, Indiana, for lipocaic.