H. M. Sheffer: "A set of five independent postulates for Boolean algebras, with application to logical constants."

Mildred Sanderson: "Formal modular invariants with application to binary modular covariants."

THE opening (October) number of Vol. 20 of the *Bulletin of the American Mathematical Society* contains: "Note on the gamma function," by G. D. Birkhoff; "Some properties of space curves minimizing a definite integral with discontinuous integrand," by E. J. Miles; "The degree of a cartesian multiplier," by D. R. Curtiss; "On closed continuous curves," by Arnold Emch; "Let us have our calculus early" (review of Mercer's "Calculus for Beginners"), by E. B. Wilson; "Shorter Notice": Ziwet and Field's "Introduction to Analytical Mechanics," by Kurt Laves; "Notes"; and "New Publications."

THE November number of the *Bulletin* contains: Report of the twentieth summer meeting of the society, by H. E. Slaught; "Intuitionism and formalism," by L. E. J. Brouwer; "Shorter Notices": Arnoux's "Essai de Géométrie analytique modulaire à deux Dimensions," by L. E. Dickson; Padoa's "La Logique déductive dans sa dernière Phase de Développement," by J. B. Shaw; Hun and MacInnes's "Elements of Plane and Spherical Trigonometry," by Cora B. Hennel; "Notes"; and "New Publications."

THE articles in *The American Journal of Science* for November are as follows:

"Upper Devonian Delta of the Appalachian Geosyncline," by J. Barrell.

"Optical Bench for Elementary Work," by H. W. Farwell.

"Volcanic Research at Kilauea in the Summer of 1911," by F. A. Perret; with Report by A. Brun.

"Observations on the Stem Structure of *Psaronius Brasiliensis*," by O. A. Derby.

"Fauna of the Florissant (Colorado) Shales," by T. D. A. Cockerell.

"The Photoelectric Effect," by L. Page.

"Graphical Methods in Microscopical Petrography," by F. E. Wright. (With Plates II. to IX.)

"A Graphical Plot for Use in the Microscopical Determination of the Plagioclase Feldspars," by F. E. Wright.

"On the Influence of Alcohol and of Cane Sugar upon the Rate of Solution of Cadmium in Dissolved Iodine," by R. G. Van Name and D. U. Hill.

"Comparative Studies of Magnetic Phenomena. IV. Twist in Steel and Nickel Rods due to a Longitudinal Magnetic Field," by S. R. Williams.

A NOTE ON PENFOLD'S MODIFICATION OF BACILLUS COLI COMMUNIS[1]

PENFOLD'S[2] observation, that the cultivation of *Bacillus coli communis* upon monochloracetic acid media permits the selection of strains whose power to produce gas from certain sugars is permanently lost, has an important bearing not only upon mutation, but upon the mechanism of the carbohydrate metabolism of coliform organisms.

Through the work of Scruel,[3] Frankland and Frew,[4] Pakes and Jollyman,[5] Harden[6] and others, there has been gathered considerable evidence that the hydrogen and carbon dioxide, liberated in the fermentation of various sugars and allied compounds by coliform organisms, are the products of the decomposition of formic acid in accordance with the equation:

$$H_2CO_2 = H_2 + CO_2.$$

This decomposition has been attributed to the activity of a specific enzyme for which

[1] From the U. S. Department of Agriculture, Bureau of Animal Industry, Dairy Division.

[2] Penfold, W. J., *Proceedings of the Royal Society of Medicine*, Pathological Section, Vol. 4, Part 3, p. 97, 1910–11; *Journal of Hygiene*, Vol. II., p. 487, 1911.

[3] Scruel, *Arch. med. Belges*, ser. 3, t. 42, p. 362, 1892; ser. 4, t. 1, pp. 9 and 83, 1893.

[4] Frankland, Percy F., and Frew, William, *Journal of Chemical Society Transactions*, Vol. 61, p. 254, 1892, London.

[5] Pakes, Walter Charles Cross, and Jollyman, Walter Henry, *Journal of Chemical Society Transactions*, Vol. 79, Part 1, p. 386, 1901, London.

[6] Harden, Arthur, *Journal Chemical Society Transactions*, Vol. 79, Part 1, p. 610, 1901, London.

Franzen and Stuppuhn[7] have proposed the name formiase.

The important point in Harden's comparison of the products of fermentation of *Bacillus coli* and *Bacillus typhosus* lies in the fact that the products are very similar, with the exception that *typhosus* leaves considerable formic acid and no gas, while *coli* leaves little formic acid and produces considerable hydrogen and carbon dioxid. This suggests that an essential characteristic of *coli* and of similar gas-producing bacteria is their ability to elaborate the enzyme formiase. This enzyme was supposed to be active both in the gaseous fermentation of sugars and of the related alcohols.

Penfold's observation that by artificial selection a strain of *coli* may be isolated which retains its power to produce gas from certain alcohols while it has lost this power in its attack upon sugars, has therefore a profound theoretical significance.

In addition to this Penfold seems to have arrived at the conclusion that, if strains without the power to produce gas from sugars may be selected by artificial means, there is no certainty that they may not arise either in nature, or during ordinary laboratory cultivation, and so lessen the reliance which is to be placed upon the gas test in diagnosis. Indeed, if Penfold's conclusions are strictly interpreted, we are no longer able to attribute to an organism of the colon group, any characteristic which may be called a fundamental and immutable physiological function.

If the theory of natural selection in any of its original or modern forms is held applicable to bacteria, we must perhaps admit the probability that bacteria are subject to variation. That they do vary we will not dispute. That they may be made to undergo mutations, or that conditions may be imposed upon their growth in such a way that selection takes place in certain directions, we will not debate. We do insist, however, that before it is concluded that such mutations or selections have occurred in any specific instance, the analytical methods used to demonstrate these phenomena be methods of sufficient accuracy to establish confidence in the data.

While Penfold's conclusions may be in the main correct, there appear certain inaccuracies in his methods which detract from the confidence such important deductions should carry with them. We wish to call attention to these inaccuracies not so much as a polemic against Penfold, as a plea for greater care in the analytical procedures of bacteriological chemistry.

Penfold in his tests of gas production used the Durham tube. The Durham tube, while useful as a preliminary qualitative test for gas, is otherwise worthless. It is more inaccurate than the Smith tube, whose shortcomings were not only recognized by the originator, but more fully pointed out by Keyes.[8]

The gravest fault of each is the retention of a large proportion of CO_2 by the medium. Keyes's method of cultivating colon in vacuo, and pumping out the gas for careful analysis over mercury, seemed so promising that it was employed with certain modifications by Rogers, Clark and Davis[9] in their study of the gases produced by over 200 cultures of bacteria, among which those which we are justified in calling typical colons were abundant.

A remarkable constancy both in total amount of gas and in the ratio of the constituent gases produced by colon was demonstrated. Incident to this research, the gas production of a typical colon when grown on various media was studied. It was found that while the total amount of gas obtained after 7 days' incubation from 5 c.c. of a broth containing ½ per cent. K_2HPO_4 and 1 per cent. of sugar, was quite uniformly about 8 c.c., whether the sugar was dextrose or galactose, the total amount of gas obtainable from the same medium rose to 12 c.c. when dulcite or mannite was substituted for a hexose.

If we compare these results with the graphic representation of Penfold's determinations, on page 489 of his second article, we shall find

[7] Franzen and Stuppuhn, *Zt. f. Physiol. Chem.*, Vol. 77, p. 129, 1912.

[8] Keyes, *Journal of Medical Research*, Vol. 21, No. 1, p. 69, 1909.

[9] Rogers, L. A., Clark, Wm. Mansfield, and Davis, Brooke, paper about to be published.

some suggestive comparisons. In each set of results, the total gas produced by a *normal* colon from dextrose equals that from galactose, and the total gas from dulcite equals that from mannite. In each set, the total gas from dulcite and mannite exceeds that from galactose and dextrose. In our results, the total gas from the alcohols is one and one half times that from the sugars.

We have also found that the total gas produced by colon in a peptone water medium, such as Penfold used, is but little more than half that produced in our broth with phosphate.

With these facts in mind let us assume that we have to cultivate in peptone water a colon whose physiological powers are identical with those of a normal organism except that its activity has been greatly weakened. If it produces only enough gas from dextrose or galactose to saturate the medium, none will appear in a Durham tube, and it *might* be said that the gas-producing power was *nil.* If the same relative power to ferment alcohols that a normal organism possesses, is still preserved, the weakened organism might show some gas in a Durham tube in dulcite or mannite medium.

When grown in Durham tubes, Penfold's selected strain showed no gas in dextrose or galactose media, while it did in mannite and dulcite media. Our results show that a normal colon produces much more gas from these alcohols, and it may therefore be suspected that Penfold's strain shows gas from these alcohols and not from the sugars simply because it produces from the alcohols a sufficiently greater volume of gas to become manifest.

It is significant that Harden and Penfold[10] by applying the more exact method of Harden,[11] found that the selected organism instead of producing no gas from dextrose, as Penfold found by the Durham tube method, *does* produce both hydrogen and carbon dioxid. The ratio of these gases was not accurately determined, but the amount of hydrogen was found to be only 15 per cent. of that obtained from a normal colon. The other products, with the exception of lactic acid, were also greatly reduced.

Consequently, instead of concluding, as Penfold did, that his selected organism has had its power to produce gas from dextrose destroyed, and that its physiological characteristics have been qualitatively altered, we may just as reasonably conclude, so far as Penfold's original data are concerned, that the selected organism has merely been weakened. In addition to this it should be noted that Penfold has had difficulty in producing with *B. lactis aerogenes* modification similar to that obtained with *B. coli communis.* In view of this fact it may be illuminating to recall that Harden and Walpole[12] found that *B. lactis aerogenes* furnishes much more gas than does *B. coli* on the same medium.

If Penfold's culture is in this essential identical with that of Harden and Walpole, or with one of those organisms which Rogers, Clark and Davis have described as producing both more gas and a higher gas ratio than *B. coli,* then it may be that Penfold could not "suppress" the evolution of gas from his *lactis aerogenes* cultures, simply because he could not *weaken* it enough to prevent the formation of sufficient gas to more than saturate the medium; and not because it refused to undergo that fundamental "variation" which Penfold ascribes to *coli.*

It is of course impossible to make any accurate comparisons between our own exact determinations and those of Penfold, for the purpose of estimating the extent of his error. The unreliability and general inconstancy of gas determinations made with the Durham or Smith tubes is, or should be, universally recognized. Of special significance is the more recent work of Keyes and Gillespie[13] in demonstrating that in contrast to anaerobic growths there is a marked variation in the gas ratio of

[10] Harden, Arthur, and Penford, W. J., *Proceedings Royal Society,* B. 85, p. 415, 1912.

[11] Harden, Arthur, *London Journ. Chem. Soc.,* 1901, p. 610.

[12] Harden, Arthur, and Walpole, *Proceedings Royal Society,* B. 77, p. 399, 1906.

[13] Keyes and Gillepsie, *Journal Biological Chemistry,* Vol. 13, No. 3, p. 305, 1912.

aerobic cultures of *Bacillus coli*. This throws additional doubt upon the reliability of gas determinations made by the methods in common use.

Based largely upon his results with the Durham tube, Penfold at one time or another has come to the following conclusions:

> It may be suggested, therefore, that . . . the selective process has caused the removal of the formic-acid-forming ferment, but apparently has not interfered with the formic-acid splitting ferment.[14]
>
> The power of gas formation from sugars (always excepting isodulcite) may be lost when gas formation from alcohols is retained. It is probable, therefore, that two different ferments are engaged in the respective processes.[15]
>
> The research raises the question as to the weight to be attached to the power of fermenting glucose and lactose with gas formation in recognizing *B. coli* in routine examinations of pathological material, water, foods, etc. Hitherto, in all authoritative catalogues of the necessary properties of this organism, this has been included, but it probably ought not to be regarded as absolutely essential.[16]

Perhaps more exact work will demonstrate the essential truths there may be in these statements. If so, it will in no wise alter the contention of this article, which is that conclusions of such profound importance are worthy of being established by methods of reasonable accuracy.

It is gratifying to learn that Harden and Penfold have set out to do so. Pending the fuller publication of their results this article would not have been written but for the fact that Penfold since the publication of the preliminary report of Harden and Penfold, has published another paper,[17] in which he seems to have missed the *significance* of the discrepancy between his earlier statement that the variant colon produces no gas from dextrose, and Harden and Penfold's later statement that it does.

[14] Penfold, W. T., *Proceedings Royal Society of Medicine*, Pathological Section, Vol. 4, Part 3, p. 106.

[15] Penfold, W. T., *Journal of Hygiene*, Vol. II., p. 502.

[16] Penfold, W. T., *ibid.*

[17] *Journal of Hygiene*, April, 1913.

Unfortunately Penfold is not alone in the false confidence he has placed in the reliability of the Smith and Durham tube methods of bacterial gas determination. These instruments, which are useful only in the routine laboratory, are still being widely used in elaborate researches; and the time, therefore, seems ripe to emphasize the errors to which their use may lead, and to plead for greater accuracy in this important test of bacteriological chemistry.

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SPECIAL ARTICLES

A NEW MEANS OF TRANSMITTING THE FOWL NEMATODE, HETERAKIS PERSPICILLUM

It has been found that *Heterakis perspicillum* may be transmitted to young chicks by a dung earthworm found in horse manure. The earthworm in question is probably *Helodrilus parvus* (Eisen).[1] The experiment demonstrating this relationship was performed during the past summer at the Kansas State Agricultural College. Eleven chicks, as soon as hatched, were placed in a fly-proof field cage and kept there until the close of the experiment. The cage was so constructed that the chicks could not reach chance insects that happened to light upon the outside screen. It had two fly-proof doors enclosing an entry way and the outer door was kept locked. When entering the cage the outer door was closed and the entry inspected for chance flies before opening the inner door. On leaving the pen the same care was taken. All chicks were thrifty and were fed upon the same ration of dry food to which was added twice per day some green alfalfa. It is needless to say that the alfalfa was always examined to prevent any insects from entering the pen. The earthworms were fed to three of the chicks. To the first chick a total of 78 worms was fed in lots of six to twelve each day between July 17 and July 26, inclusive. To the second chick 64 were fed, July 18 to July 29. The

[1] The earthworm mentioned has been referred to Professor Frank Smith, University of Illinois, for identification, and the nematode has been verified by Dr. Albert Hassell, Division of Zoology, B.A.I., Washington, D. C.