SOCIETY OF AMERICAN BACTERIOLOGISTS

VI

Sanitary Bacteriology

Under the supervision of W. W. Ford

Bacteria in City, Country and Indoor Air: WILLIAM W. BROWNE.

The New York State Commission on Ventilation under the direction of Professor C.-E. A. Winslow undertook a systematic examination of the air in and about New York City. During the survey 353 samples of air were examined and may be roughly divided as follows:

1. City air ..... 134 samples.
2. Country air ..... 85 samples.
3. Office air ..... 87 samples.
4. Factory air ..... 47 samples.

The samples were collected and examined according to the methods proposed by the Committee on Standard Methods for the Examination of Air of the American Public Health Association. In each examination 5 cu. ft. of air were pumped through a sand filter by (1) hand pump in the field, (2) power pump in factories and offices. Samples were plated on gelatin and litmus lactose agar and plated at 20° C. and 37° C., respectively.

Summary of Results

Source	No.	Microorganisms per Cu. Ft.				Strep. per 100 Cu. Ft. of Air
		20° C.	Per Cent. Below 125 per Cu. Ft.	37° C.	Per Cent. Below 125 per Cu. Ft.	
Country.....	85	56	(94%)	30	(94%)	12
City.....	134	72	(82%)	32	(98%)	11
Offices.....	87	94	(80%)	80	(90%)	22
Factories.....	47	113	(82%)	63	(92%)	43
Schools[1].....	684	96	(78%)	—	.....	30

Conclusions

1. Microorganisms developing at 20° C. on gelatine are generally under 50 per cu. ft., rarely over 100 per cu. ft.

2. Microorganisms developing at 37° C. are rarely over 50 per cu. ft.

3. Number of *Streptococci* equals 10 per 100 cu. ft.

4. Air of occupied spaces contains more bacteria than open spaces and shows greater fluctuations.

[1] From Baskerville and Winslow school-room air examination in New York City, in which same methods were employed.

The Efficiency of Endo's Medium in Detecting Members of the Colon Group: G. C. SUPPLEE.

Fifty-three cultures were studied and identified. Twenty-nine of these were found to belong to the colon group. Eleven were closely allied forms, but could not be included within the group if strict adherence to the classification was observed. Three were coccus forms; four were of the acid peptonizing type and two failed to ferment any of the sugars and produced no change in milk. The data were not complete from four of the cultures.

Representatives of the colon types and the acid peptonizing type were plated upon Endo's medium after different degrees of attenuation. The results of these experiments showed that the intensity of the reaction was weakened as the attenuation increased. The color reaction of the *aerogenes* and *acidi lacti* types tended to fade to a white or pink. The fading took place much sooner if the colony developed on the surface or if the culture was at its greatest vigor.

Since sub-surface development gave rise to many doubtful reacting colonies, two hundred and thirteen such colonies were studied and fifty-seven per cent. were found not to belong to the colon group.

From experiments with pure and mixed cultures of the colon varieties it was found that about a plus four tenths acidity gave the maximum number of reactions.

Organisms which Do Not Belong to the Colon Group and Produce Black Fields on Æsculin-Bilesalt Media: J. VANDERLECK.

In the summer of 1913 more than 3,000 æsculin-agar plates made of milk were examined and 700 colonies selected for further study. As a result 10 organisms were found to produce black colonies on æsculin-bilesalt media at blood heat inside 48 hours and which did not have the least relation to the colon group. These organisms showed hardly any action in milk, gas production in sugars was absent in the majority and sometimes liquefaction combined with alkalinity was present. These organisms came from one milk district—Huntingdon—and could not be recovered from that source in the following spring. However, another exception appeared for a short time in large numbers. This organism was in many respects closely related to the colon group, produced first acid in milk followed by alkaline digestion and formed gas in saccharose. Altogether 135 organisms were tested in the examination.

Working at lower temperature and keeping the plates for at least 5 days, more exceptions would appear, 7 of which were carefully studied. Col-

onies appearing in water samples were also tested, but among 250 test cultures no exceptions were encountered. Our conclusions of these exhaustive tests are that the æsculin-bilesalt test is thoroughly reliable. Out of 1,200 samples analyzed only in one case a wrong impression was obtained on account of the presence of some exceptional organism. A full description of this investigation will appear in the *Centralblatt für Bakteriologie.*

Numbers and Efficiency of B. bulgaricus Organisms in Commercial Preparations Examined During the Period January–June, 1914: Ruth C. Greathouse.

The number and efficiency in acid production of *B. bulgaricus* in commercial preparations are an index of the value of the preparations.

Forty commercial preparations, the products of twenty-three firms, were examined between January 15, 1914, and June 1, 1914. These samples were collected fresh from the manufacturers and held under conditions of temperature and humidity which are practicable for commercial handling. They contained, in the case of dry cultures, from none to 250,000 living *B. bulgaricus* per gram; in the case of liquid cultures, from 2,300 to 320,000,000 per c.c.; in the case of sour milk drinks, from 800 to 790,000,000.

The maximum acidity produced in milk by the *B. bulgaricus* in these preparations varied from 1.20 per cent. to 3.41 per cent. acid calculated as lactic. The ability of the *B. bulgaricus* to produce acid was decreased in the old preparations. The amount of decreases averaged 38.5 per cent. in the case of dry cultures kept on ice for two months, and 26.4 per cent. in the case of liquid preparations kept on ice for two weeks.

The strains producing different degrees of acidity were examined for differences in morphology, staining properties and curd production in milk, which would indicate that they were separate organisms, but no such differences were found.

Agglutination Studies of Milk from Cows Affected with Contagious Abortion: L. H. Cooledge.

Milk studied was obtained from a herd in which a high percentage of animals have repeatedly given positive complement fixation and agglutination tests for contagious abortion and having a record of frequent abortions.

The milk from each quarter of 61 cows has been examined at intervals during the last 6 months. Of these the milk of 18 (30 per cent.) has given a positive agglutination test with *Bact. abortus,* in one or more quarters, at some time, or during this period. The power of the milk of one quarter to agglutinate the abortion bacterium has been observed to spread to another quarter and finally to all four; it has also been observed to gradually die out. Milk drawn at about the middle of the milking has the strongest agglutinating reaction.

An attempt to demonstrate the presence of *Bact. abortus* in milk that agglutinates the organism has resulted as follows. Out of 18 quarters the milk of which agglutinate the abortion bacterium the milk of 14 produce lesions in guinea-pigs which are like the typical lesions caused by a pure culture of *Bact. abortus.*

In the 7 cows whose milk has gradually acquired the power of agglutinating the abortion bacterium during this experiment one or both of the rear quarters have been the first to show agglutination. This suggests contamination of the rear quarters by genital discharges.

The Presence of Bacillus abortus in Milk: Alice C. Evans.

Special methods of plating milk samples which were drawn aseptically have shown that the bacillus of contagious abortion occurs commonly in certified milk in the vicinity of Washington, D. C., and Chicago, Ill. These organisms grow profusely on serum agar plates. About 30 per cent. of the samples of milk from two certified dairies near Chicago, which were plated on serum agar, showed this organism to be present in milk at the time of drawing from the udder, in numbers varying from 110 to 4,300 per cubic centimeter. In one sample taken from a herd which does not produce certified milk, 50,000 of the *Bacillus abortus* were found per cubic centimeter. This organism grows abundantly in the cream layer, with the formation of acid, but it grows sparingly in milk from which the cream has been removed. Four per cent. of lactic acid in the milk does not check the multiplication of *Bacillus abortus* in the cream layer.

The Influence of Milk and Carbohydrate Feeding on the Bacteriology of the Intestine: Leo F. Rettger and Thomas G. Hull.

The intestinal flora of white rats and of fowls is determined in a very large measure by the diet. White rats that were fed ordinary white bread and green vegetable food exhibited an intestinal flora which closely resembles that of man. Soon after the diet was changed to mixed grain a marked transformation took place. When to the diet of bread and vegetables a liberal amount of milk or of lactose was added the ordinary mixed flora quickly became simplified, and often presented the picture of only two or three types of bacteria, namely *B. bifidus* of Tissier and *B. acido-*

philus of Moro. During continued milk or lactose feeding the *acidophilus* type may give way eventually to *B. bifidus*. Similar results were obtained in the domestic fowl, the *acidophilus bacillus* being the most prominent. The feeding of other carbohydrates, dextrose, maltose, levulose, dextrin and starch did not bring about such a change.

The feeding of bacteria, even in large numbers, will in itself exert very little if any influence on the intestinal flora. *B. bulgaricus* suspensions obtained from plain agar growths could be recovered only occasionally from the feces, and then in very small numbers only. On the other hand, when sterile milk, whether sweet or sour, was fed to white rats which exhibited the usual mixed flora in which organisms of the *acidophilus* type were very few or absent, *B. acidophilus*, which in many respects is practically indistinguishable from *B. bulgaricus*, rapidly made its appearance in the intestine and for a time occurred there in relatively large numbers.

A Simple Test for B. sporogenes in Milk and Water: JOHN WEINZIRL.

The sample of milk to be tested is placed in a sterile test tube, and enough solid paraffin is added to make when melted, a layer one eighth of an inch in thickness. The tubes are then placed in the Arnold and heated at 80° C. for ten minutes. After heating, they are cooled rapidly; this causes the melted paraffin to solidify and form a cover which effectively excludes atmospheric oxygen. The cultures are then incubated at 37° C. for 24 hours. If *B. sporogenes* is present, it digests the lactose and forms gas which lifts the paraffin plug.

The test is simple, cheap and easy of application. When applied to market milk it gave the following results:

90 samples of 5 c.c. milk each gave 28 per cent. positive.

112 samples of 10 c.c. milk each gave 37.5 per cent. positive.

34 samples of 15 c.c. milk each gave 50 per cent. positive.

Utensils as a Source of Bacterial Contamination of Milk: M. J. PRUCHA, H. A. HARDING, H. M. WEETER.

This investigation attempted to measure the amount of bacterial contamination received by the milk from the utensils in which it was handled between the cow and the milk bottle.

The utensils were carefully washed in the ordinary way, being scrubbed with brush in a warm solution of Wyandotte and then rinsed out with warm water.

In the accompanying table is given the summary of the experiments.

All Utensils Sterile

	Bacteria per C.c.
1. Milk leaving the barn	2,558
2. Bottled milk	3,875

Utensils Washed—Only Bottles Sterile

3. Increase due to pails	57,077
4. Increase up to clarifier	15,353
5. Increase due to clarifier	172,763
6. Increase due to cooler	19,841
7. Increase due to bottler	247,611
8. Total in bottled milk	515,203

An Improvement in the Composition of Lactose Bile: THOMAS W. MELIA.

Lactose bile, as employed at present, has certain disadvantages.

First: The bile is not always fresh.

Second: The media, after sterilization, contains a heavy sediment in the fermentation tubes which interferes with the development of the test.

Third: Many authors have criticized the inhibiting power of the bile salts upon *B. coli.*

In regard to the first disadvantage it was found best to purchase ox gall from freshly slaughtered animals and have it delivered within a few hours to the laboratory. It is then evaporated to dryness in a vacuum dryer and stored away in one pound airtight containers. The bile will keep indefinitely.

The sediment present in fresh bile and in the sterilized media was found to contain mucin, lime salts and broken gall stones (cholesterine) probably formed by the streptococcus growth. The bile should be settled and only the clear supernatant liquid used.

The sediment in the fermentation tubes may be prevented by making a five per cent. instead of a ten per cent. solution of bile media.

The known inhibiting power of bile salts led to an investigation of the best strength of bile media to employ.

Table showing relative efficiency of the five and ten per cent. bile media on ordinary quality of drinking waters (60 in number)

	Five Per Cent. Dried Ox Gall			Ten Per Cent. Dried Ox Gall		
	C.c. 0.1	C.c. 1.0	C.c. 10	C.c. 0.1	C.c. 1.0	C.c. 10
24 hour results..	2%	22%	50%	2%	16%	31%
48 hour results..	13%	31%	75%	7%	18%	40%
72 hour results..	15%	49%	82%	9%	27%	51%

Attenuated *B. coli* is more readily shown, also less interference from overgrowths in the five-per-cent. bile media.

Infection and Immunity

Under the supervision of J. A. Kolmer

The Parasite of Oral Endamœbiasis. Endamœba gingivalis (Gros): ALLEN J. SMITH, M.D., AND M. T. BARRETT, D.D.S.

The authors present a detailed comparison of the amœbiform organisms which have been announced as discovered in the human mouth and related parts, including the amœbæ of Gros (1849), of Steinberg (1862), of Grassi (1879), of Flexner (1892), Kartulis (1893), of Prowazek (1904) and of Verdun and Bruyant (1907). They conclude that of these all save the last are really specifically identical and therefore propose as the proper nomenclature for this organism *Endamœba gingivalis* (Gros) with synonyms: *Amiba buccalis* Steinberg, 1862; *Amœba dentalis* Grassi, 1879; *Entamœba kartulisi* Döflein (*Amœba maxilaris* Kartulis, 1907); and *Entamœba buccalis* Prowazek, 1904. The organism is an amœba which ordinarily is of 30 or 35 micromillimeters in diameter in the resting stage, has a fairly differentiated ectosarcous periphery, a granular endosarc, full of nutrition vacuoles in which are found bacteria, remnants of leucocytic nuclei and red blood cells or their detritus. There is no contractile vacuole. The nucleus is small, usually central or subcentral, but occasionally excentric; is seen with difficulty if at all in the unstained specimen; is poor in chromatin and vesicular in appearance, with small central "binnenkörper" and a delicate but irregularly thickened chromatic membrane. The pseudopodia are ordinarily single or few, broadly lobose to long and digitate; cellular and pseudopodial motility active but variable; reproduction surely by binary division and by gemmation; persisting cysts formed, but no reproduction cysts as yet observed. Habitat in the pus of pyorrhea pockets, on the neighboring mucous and dental surfaces, in the tonsils, in the abscesses of the jaw, etc.

The writers urge their inability to distinguish morphologically between *Endamœba gingivalis* (Gros) and *Endamœba histolytica* Schaudinn. While stating this view of morphological indistinguishability, the writers are unwilling to declare general biological identity, although they suspect it; and acknowledge that such identity, if established, would open the door to need for important revision of our present ideas in regard to amœbic dysentery.

The Production and Detection of Specific Ferments for the Typhoid-coli Group: GEORGE H. SMITH.

The results of the application of the Abderhalden reaction to the investigation of three important problems were presented, namely:

1. To what degree does the property of specificity extend among ferments produced in the body through resistance to infective agents?

2. Which method of administration—intravenous, intraperitoneal, or subcutaneous—is the most effective for immunization?

3. Is there any difference in rapidity of action between living bacteria, killed bacteria and killed sensitized bacteria when used for immunization?

As regards specificity of ferments, reference is made to previous work with *Staphyloccus, Streptococcus, Pneumococcus, Micrococcus catarrhalis* and *B. influenzæ* in which a complete specificity of ferments was demonstrated. In the present instance rabbits were immunized against *B. coli communis, B. coli communior,* paratyphoid bacillus A, paratyphoid bacillus B, and two strains of typhoid bacillus known as the Hopkins and Rawlings strains. The results of these experiments would indicate that ferments produced as a result of injections with the bacteria employed are highly specific.

The experiments with the different methods of injection, and with living, killed unsensitized and killed sensitized bacteria were conducted as follows:

The typhoid bacillus (Rawlings strain) was the organism employed. Doses of 50 million were given intravenously, intraperitoneally, and subcutaneously. The animals were bled at stated intervals and the serums tested by the Abderhalden method for specific ferments.

From this work it appears that the intravenous method of administration is most rapid in its results, and the subcutaneous gives the slowest response, and that the killed sensitized bacteria are most potent in inducing a rapid formation of ferment.

In verification of this latter conclusion, subcutaneous injections were given, simultaneously, of killed typhoid and killed paratyphoid B, of killed typhoid and killed sensitized paratyphoid B, of sensitized typhoid and killed paratyphoid B, and killed sensitized paratyphoid B. When killed un-

sensitized organisms of both types were injected, ferments for paratyphoid B appeared in 33 hours, and for typhoid in 36 hours. When killed unsensitized typhoid and killed sensitized paratyphoid B were employed specific ferments for paratyphoid B appeared after 16 hours, and for typhoid after 35 hours. When killed sensitized typhoid and killed unsensitized paratyphoid B were injected ferments for typhoid were demonstrable after 17 hours, and for paratyphoid B after 36 hours. With the final combination in which both types were sensitized the serums showed specific ferments for both kinds of bacteria after 18 hours.

The above experiments indicate that previous treatment of the bacteria with immune serum renders them more susceptible to assimilation by the body and thus enables them to bring about a more rapid formation of the specific ferments which may be detected by the Abderhalden test.

Recent Studies on Pellagra: J. F. SILER, P. E. GARRISON AND W. J. MACNEAL.

The theory that pellagra is due to the ingestion of maize or maize products, either good or spoiled, is wholly inadequate to explain the distribution of the disease actually observed in Spartanburg County, S. C.

The conception that pellagra is a specific infectious disease in some way transmissible from person to person is strongly supported by our observations. The higher incidence of pellagra in the more populous districts and its occurrence in definite foci are in accord with this view. Definite tendency to self-limitation of the attack of pellagra without specific treatment and without change in diet is very evident in many cases, and especially so in children.

The manner of origin of pellagra in its endemic foci from year to year indicates that the disease spreads from old cases as centers and that it is, as a rule, transmitted through relatively short distances, within the same house or to the house next door. The disease spreads most rapidly in communities without efficient provisions for sewage disposal and spreads hardly at all in communities with sanitary sewer systems.

We have been unable to produce a recognizable attack of pellagra in any experimental animal, nor have we as yet recognized the specific infectious agent of the disease.

The Schick Toxin Reaction for Immunity in Diphtheria: JOHN A. KOLMER AND EMILY L. MOSHAGE.

Schick has proposed a simple clinical test for immunity to diphtheria consisting in the intracutaneous injection of 1/50th the minimal lethal dose of toxin for a guinea-pig. If there is less than 1/30th of a unit of antitoxin in each cubic centimeter of the patient's serum, the injected toxin acts as an irritant and produces an inflammatory reaction. If 1/30th of a unit or more antitoxin is present the toxin is neutralized, no reaction follows and the individual is regarded as being immune to diphtheria.

The test has been advocated as a means of testing the response of active immunization with toxin-antitoxin mixtures and to detect susceptibility to diphtheria.

The objects of this study were as follows:

1. To apply the toxin skin test to a large number of apparently normal persons to determine susceptibility to diphtheria at different ages.

2. To determine quantitatively the antitoxin content of the blood serum of persons reacting positively, slightly positively, doubtfully and negatively in order to further study the toxin test under conditions where the quantity of antitoxin in the blood is known.

3. To study the degree and duration of immunity to diphtheria in normal persons following an injection of diphtheria antitoxin.

4. To study the degree and duration of immunity among persons suffering with scarlet fever and receiving an injection of diphtheria antitoxin.

5. To study immunity during and following an attack of diphtheria.

6. To study the practical value of the toxin skin test in determining which persons should be immunized with antitoxin when exposed to diphtheria.

I. In all 1,265 inoculations were made. Of these 447 were among persons most of whom were healthy and well; a few were suffering with various chronic diseases and were tested while in various hospitals in Philadelphia.

The reaction has demonstrated that children between the ages of one and eight years are most susceptible to diphtheria.

II. The serums of a number of persons were tested for antitoxin content with the following results:

(*a*) The serums of persons reacting negatively to the toxin test usually contained at least 1/20th of a unit of antitoxin per cubic centimeter of serum.

(*b*) The serums of persons reacting weakly positive to the toxin test usually contain from 1/40th to 1/160th of a unit of antitoxin per cubic centimeter of serum.

(*c*) The serums of persons reacting strongly positive were found to contain less than 1/30th of a unit per cubic centimeter of serum and frequently none at all could be detected.

These results corroborate those of Schick, Park and Zingher.

III. The duration of passive immunity to diphtheria was studied in 106 persons by applying the toxin skin test at varying intervals after the administration of 1,250 units of antitoxin subcutaneously.

The immunity conferred was apparently efficient for ten days; after this interval antitoxin rapidly disappeared, so that after four to six weeks the immunity may be regarded as having entirely disappeared.

IV. The toxin skin test was applied to 362 persons in the various stages of scarlet fever and at varying intervals of time following the subcutaneous injection of 2,500 units of antitoxin to study the duration of passive immunity in scarlet fever.

It was found that in scarlet fever passive immunity following an injection of diphtheria antitoxin is of shorter duration than that induced among normal children in that 10 per cent. of the former are susceptible within ten days after receiving antitoxin.

V. The toxin test was also applied to 350 persons, mostly children, suffering with diphtheria and receiving from 10,000 to 100,000 units of antitoxin by subcutaneous injection. The high percentage of positive reactions during the first ten days of the disease and after large doses of antitoxin was quite surprising. As a general rule these occurred among children with severe infections. It was also found that patients were in general just as susceptible after an attack of diphtheria as before; in other words, it would appear that the body cells produce little or no homologous antitoxin and the immune antitoxin is soon eliminated.

VI. Practical experience with a small epidemic of diphtheria has increased our confidence in the toxin test as a means of detecting persons susceptible to diphtheria. The chief practical value and application of this test is the detection of non-immune individuals and immunizing those only instead of all persons indiscriminately. The reaction has a special field of usefulness in hospitals and wards for the care of children.

While cultures were made of a large number of the persons tested, there was found no constant relation between the occurrence of diphtheria bacilli in the upper air passages and the toxin test.

The Mechanism of Abderhalden Reaction: J. Bronfenbrenner.

When the placenta and serum of a pregnant individual are placed on ice, instead of the thermostat, the Ninhydrin reaction substances do not appear in dialysate. The analysis of the ingredients, however, shows that both the serum and placenta underwent changes—namely, placenta was sensitized by fixing upon itself the specific substances from the serum, and the serum was exhausted of its specific substances. Such a serum, moreover, when separated from placenta and transferred to 37° shows gradual deterioration of its complement, and parallel with it, the increase of dialyzable Ninhydrin reacting substances as the incubation at 37° goes on. Similar tendency to apparent autodigestion can be produced also in any normal serum by placing it for a certain time in contact with sensitized placenta (but not with normal placenta). The absorption by the placenta of specific constituents of pregnant serum is not due to a mechanical absorption, but is strictly specific, at least within certain quantitative limits.

The action of this specific union between the substratum and specific constituents of the serum upon the residue of the serum is such that the normal antitrypsin of the serum is inactivated (or absorbed) and the normal proteolytic ferment is set free. The action of this non-specific ferment upon the residue of the serum is responsible for the appearance of dialyzable substances. This action of the ferment upon the serum can be arrested in the Abderhalden test by the addition of any substance acting as antitrypsin, as, for instance, the serum albumen or serum lipoids, both in the pure form and in the form of excess of whole normal serum. The products of such autodigestion of the serum are toxic to homologous animals, and their appearance can be made evident by the biological tests (anaphylaxis).[1]

Do Bacteria Produce Pyrogenic Poisons? D. H. Bergey, M.D.

It is believed that the pyrogenic substances act by (*a*) the stimulation of the heat-producing centers, or, (*b*) the inhibition of heat dissipation through conduction, radiation and evaporation, or (*c*) by the combination of these two processes.

It is well known that the injection of animals with distilled water, or with sterile bouillon leads to fever production. Hence it was thought probable that it might be possible to show the presence of fever-producing poisons in a filtered bouillon

[1] The protocols will appear in one of the following numbers of the *J. of Exper. Med.*

culture of *Bacillus typhosus*, if the filtrate produced a uniformly higher degree of febrile reaction than did the sterile bouillon, before bacteria had been grown in it.

It was also thought possible that the presence of fever-producing substances in the culture filtrate could be demonstrated by treating animals for some time with these filtrates and then using the serum of these animals to inhibit the fever production in normal animals by injecting serum and filtrate at the same time.

It was soon determined that the serum even of normal animals of the same species injected into healthy animals in itself caused a febrile reaction.

It was not possible to demonstrate definitely that typhoid bacillus produces soluble toxin which is responsible for the febrile reaction in typhoid fever.

It seems more probable that the febrile reaction is due to substances liberated from the tissue cells under the influence of the organisms.

How Bacterial Vaccines Act: E. C. L. MILLER, M.D.

The protein of the dead germ bodies contained in ordinary bacterial vaccines probably produces specific immunity. The degree of this immunity is probably slight and the question is raised whether the immunity measures the entire therapeutic value of the vaccine. The fact that well-washed bacteria have much less tendency to cause a reaction is taken as evidence that besides the dead germs there are reaction-producing or pyrogenetic substances in the vaccines. The fact that bacterial vaccines must usually be administered in doses that produce some reaction is taken as indicating that the pyrogenetic substances have some part in the improvement. One way in which they may aid is in making the immunity effective and this may be either by focal or general reactions. It is suggested that the existence of these two constituents in bacterial vaccines should be more generally recognized so that they may be used separately or together, as may be indicated.

A Contribution to the Pathogenesis of the Avian Tubercle Bacterium: L. R. HIMMELBERGER AND L. A. MOSHER.

The communication deals with the pathogenic effects of living cultures of avian tubercle bacteria on rabbits. The problem was undertaken to study the type of tuberculosis, whether generalized or localized, produced by intravenous injection of living bacteria of the avian type.

The work presented in the communication involves the results obtained by intravenous injection of thirty animals. With but one exception all developed a generalized tuberculosis, usually terminating fatally in from twenty to one hundred days. In classifying the type of disease produced, the nature and distribution of the lesions and the course of the disease in the animal were the criteria used.

In view of the results obtained the authors desire to suggest that the use of avian cultures in cattle immunization is unsafe both from an economic and public-health point of view. From the economic standpoint great danger of infecting by vaccination exists since the senior author[2] has previously shown that calves can be infected by tubercular material from avian sources. From the public health point of view it is reasonable to suppose that the avian type of organism would prove equally as pathogenic for humans as the bovine type.

Reciprocal Relations of Virulent and Avirulent Cultures in Active Immunization: PHILIP B. HADLEY.

This paper presented data to show that among seventeen non-virulent strains of the fowl cholera organism only one (Culture 52) possessed an immunizing value, but that the immunizing value of this one was perfect, in so far as inoculation with rabbits with 0.000,000,01 c.c. produced permanent active immunity against the most virulent strain obtainable. When tested against other virulent strains, Culture 52 protected in many cases, but the point was especially emphasized that in all cases in which Culture 52 alone failed to protect, perfect immunity was developed through inoculation with Culture 52, followed, after an appropriate time, by inoculation with Culture 48. By the use of one or the other method, rabbits may now for the first time be permanently protected against any virulent strain of the fowl cholera bacterium yet obtained.

These experimental results were used as the basis for more general observations.

1. On the possibility of more efficient active immunization in many communicable diseases by discovery of what may be termed "Immunizing strains."

2. On the varied physiological characteristics which may be possessed by microorganisms manifesting identical morphological, cultural and biochemical features.

3. On the heretofore unconfirmed experiments of Pasteur regarding the possibility of immunization against fowl cholera by means of non-virulent cultural material.

[2] *Cent. f. Bakt. Erst. Ab.*, Bd. 73.

Symposium with Section K, A. A. A. S.
Ventilation

Under the supervision of C.-E. A. Winslow

Ventilation in Its Relation to Air-borne Diseases: Dr. A. C. Abbott.

In several hospitals for the care of contagious diseases in England, France, and in one in particular in this country, it has been conclusively demonstrated that certain of the so-called "air-borne" diseases of different natures may be treated side by side in the same ward without fear of greater transmission than commonly occurs when they are treated in separate wards. Obviously such observations justify grave doubts of the aerial conveyance of disease.

Though we do not know the causative agents of the majority of the so-called "air-borne" diseases, yet presumably they are particulate and never gaseous in nature. Therefore, they behave in the air, when they get there, just as do other suspended particles.

From information obtained through the study of another phase of the subject we know that a number of diseases may be conveyed through the air, but here it is always through the agency of insects acting as vectors or as hosts for the infective parasites. This, obviously, has more to do with wire screens than with ventilation.

In the light of the foregoing, I do not believe that ventilation has anything whatever to do with either the transmission of the so-called "air-borne" diseases, or the lessening of their transmission, and I am further of the opinion that transmission by way of the air, strictly speaking, is of infinitely less importance than transmission by animate and inanimate carriers that have been in intimate contact with the patient.

Some Fundamental Physical Factors in the Problem of the Control of Atmospheric Environment: E. B. Phelps.

The physical problem of heat dissipation from the body is conditioned externally by four prime factors; temperature, humidity, velocity of air movement and radiation. Experimental determination of the mutual relationship and individual influence of the first three of these is reported.

A simple air conditioning apparatus furnished the experimental air at temperatures of 8° to 40° C., relative humidities 30 per cent. to 90 per cent. saturation and velocities up to 250 cm. per second. The heat loss was determined from a continuously moist skin surface exposed to these various air conditions. The surface formed the only exposed portion of a calorimeter in which accurate thermo-control was provided, the actual heat lost being compensated electrically and determined by noting the volume of gas produced electrolytically by the passage of the same heating current through dilute sulphuric acid. The results between 20° and 40° are expressed by the f llowing equation:

$$c, \sqrt[3]{v}[.0072(46.7 - p) + .00294(37 - t)].$$

c, is the heat loss in calories per minute per sq. centimeter.

v, the velocity of movement in centimeters per second.

p, the absolute humidity in milligrams per liter, and

t, the temperature Centigrade.

Below 20° a complicating humidity relation was developed and at lower temperatures this relation reversed the one found above, so that increasing humidity brought about increasing heat loss. This latter relation has not yet been formulated.

Standards of Ventilation in the Light of Recent Research: C.-E. A. Winslow.

The investigations of the New York State Commission on Ventilation have indicated that even quite extreme conditions of heat and humidity (86° with 80 per cent. relative humidity) have no measurable effect upon the rate of respiration; dead space in the lungs; acidosis of the blood; respiratory quotient; rate of digestion and rate of heat production (both measured by oxygen consumption); protein metabolism (measured by determination of creatinine in the urine) or skin sensitivity.

On the other hand, the working of the circulatory and heat-regulating machinery of the body was markedly influenced by even a slight increase in room temperature, as, for example, from 68° to 75° with 50 per cent. relative humidity in both cases. In a hot room (86°—80 per cent. relative humidity) the rectal body temperature usually rose during the period of observation; in a warm room (75°—50 per cent. relative humidity) it remained on the whole about constant; in a cool room (68°—50 per cent. relative humidity) it fell. The average body temperatures attained under these three-room conditions were 37.41°, 36.99° and 37.73°, respectively. The increase of heart rate on passing from a reclining to a standing position became greater by an average of 7 beats during a sojourn in the hot room, while it became less by an average of 3 beats in the warm room and by an average of 7 beats in the cool room.

Elaborate psychological tests failed entirely to

show any effect of even the severe 86°—80 per cent. relative humidity conditions upon the power to do mental work under the pressure of a maximum efficiency test.

The results with physical work (lifting dumb bells and riding a stationary bicycle) were much more definite. Again maximum effort tests showed no appreciable influence of room temperature but when the subjects had a choice they accomplished 15 per cent. less work at 75° and 37 per cent. less at 86° than at 68°.

As to the effect of stagnant air contaminated by a group of subjects so as to contain an average of from 20 to 60 parts of carbon dioxide per 10,000 the observations of the commission are entirely negative so far as the physiological and psychological and efficiency tests above mentioned are concerned. In certain experiments the appetite of the subjects as measured by the amount of food consumed when a standard luncheon is served to them seemed to be reduced in the stagnant air.

Recent research has, on the whole, strengthened rather than weakened the arguments for ventilation. It has shown, however, that the physical quality of the air as well as the amount should be considered. Temperature standards must come into more general use, and a rise above 70° must be recognized as a sign that discomfort is being produced and efficiency decreased and vitality lowered.

Symposium with Sections C and K, A. A. A. S. The Lower Organisms in Relation to Man's Welfare

Under the supervision of John Johnson.

Theories of Fermentation: C. L. ALSBERG.

There are two types of theories of fermentation. One deals with the mechanism by which the substance fermented is converted into the end products of fermentation. The other deals with the physiological rôle which fermentation plays in the life of the fermentation organism. The latter only was considered in the paper.

The great question which has always confronted the investigator in judging the physiological significance of fermentation is the difficulty of explaining why enormous quantities of material are attacked by a relatively small mass of fermentation organisms. Until recently most investigators have looked at this question only from the point of view of matter, and not from the point of view of energy.

In the present paper it is suggested that fermentation is nothing other than the expression of the metabolism of energy of a microorganism. In the case of microorganisms in which the surface as compared with the mass is very great, the energy requirements must of necessity be enormous. Moreover, microorganisms live in a liquid medium which is an excellent conductor of heat. Therefore, the radiation losses of microorganisms must be excessive. Taking these factors into consideration it is easy to understand why a small mass of organisms converts a relatively large mass of material in its effort to satisfy the energy requirements of its protoplasm.

The Bacteria of the Intestinal Tract of Man: A. I. KENDALL.

It has been stated that the average healthy adult on a normal mixed diet excretes daily in the feces a number of bacteria, which have been variously estimated from 128 billion to 33 trillion. It is very certain that this number of bacteria is not taken in the food, and, furthermore, the fecal organisms are not necessarily the same as those found in the food. Hence the conclusion is reached that there must be a very great daily proliferation of bacteria in the intestinal tract.

The question naturally presents itself, why is there such a tremendous growth of bacteria daily, and why is it that the bacteria taken in with the food are not those which appear in the fecal contents? A rapid survey of the life history of the intestinal bacteria will explain at least some of the facts. At birth the intestinal content, the meconium, is sterile. Very shortly after birth bacteria make their appearance in the mouth of the new born, and organisms appear in the meconium from four to twenty hours post partum, depending upon environmental conditions. This is a period of mixed infection, and the number of organisms in the meconium increases rapidly after the first food enters the intestinal tract. After two to three days post partum, when the intestinal tract has become thoroughly permeated with milk, the organisms observed in the feces—for the meconium has largely disappeared by this time—begin to assume a monotony of form and a regularity of type, which contrasts sharply with the preceding period of mixed infection.

The types of bacteria which constitute the normal fecal flora of the nursling are few in number and definite in their chemical characters. The most prominent of these, *B. bifidus*, so-called because of its developmental peculiarities in artificial media, is a strict anaerobe.

B. bifidus is an organism which does not thrive in artificial media in the absence of sugars, and it

is not surprising to find, therefore, that as the breast-fed infant becomes older and its dietary demands more varied, *B. bifidus* tends to disappear from the fecal mass. In the case of bottle-fed babies, this disappearance practically coincides with the substitution of cow's milk for human milk. The decrease in the typical nursling organisms is accompanied by an increase in the numbers of *B. coli* which then dominate the intestinal tract and form about 80 per cent., roughly, of the total living fecal organisms of adolescence, and which persist in this proportion in normal individuals until death.

B. coli differs from *B. bifidus* in one noteworthy respect. *B. coli* can grow equally well in media containing protein and utilizable carbohydrate, or in media from which utilizable carbohydrates are excluded. It can accommodate its metabolism to the varying foods presented to it in the intestinal contents. This plasticity of the colon bacillus and its ability to develop in the average intestinal contents, explains in a satisfactory manner the dominance of this organism throughout life.

Turning now to the distribution of bacteria in the intestinal tract of the normal adult, it is found that the stomach contents are practically sterile under normal conditions. When the hydrochloric acid acidity of the stomach contents becomes diminished through disease, it is found that the numbers of bacteria in the stomach contents may increase greatly. The duodenum also during those periods when it is empty is practically sterile. The bacteria population increases as duodenal digestion increases, and diminishes as the duodenal contents are passed on to the lower levels.

The greatest number of living bacteria is found in the region of the ileocecal valve and the ascending branch of the colon. Here the contents stagnate, as it were, and they eventually become so desiccated through the withdrawal of water that bacterial life is retarded.

The significance of the intestinal flora has been variously interpreted. Nuttall and Thierfelder delivered guinea-pigs by Cæsarean section and attempted to raise them in a sterile environment on sterile food. For two weeks these sterile guinea-pigs increased in weight and appeared to be reasonably healthy. These observers drew the conclusion that the intestinal bacteria were not necessary for the well-being of these guinea-pigs at least. These experiments were not accepted by Schottelius as being conclusive. He claimed that the experiments were not carried on long enough. Schottelius experimented with chicks hatched from sterile eggs divided into three groups. These were incubated under sterile conditions, and the chicks developing from one group were kept in an absolutely sterile environment and fed on sterile food; a second group were kept under the same conditions for ten days and then fed with infected food; the third group were controls and were kept under ordinary conditions. The first group, the sterile chicks, did well for ten days, but after that time their development was seriously retarded. The second group also did well for ten days, and then, as the first group began to exhibit signs of abnormalities, they were placed on infected food: they gained rapidly. The third group, kept under ordinary conditions, did well from the start. Schottelius believed that his experiments showed that the intestinal bacteria were necessary for the development and well-being of chicks.

Madame Metchnikoff made similar observations on tadpoles, and Moro performed the same experiments with turtles. These observers agree with Schottelius that the intestinal flora appear to be necessary for the well-being of the animals they experimented on.

A line of evidence which is somewhat different from this was brought forward by Levin. He examined the fecal contents of many Arctic mammals in the Arctic regions, and he found few or no bacteria in them.

Attempts have been made to sterilize the intestinal contents of man, either by administering sterile food or by the use of antiseptics, but this line of experimentation has not been successful.

From the individual point of view the intestinal flora under ordinary conditions are innocuous, and perhaps even to a moderate degree protective. But under abnormal conditions, when progressively pathogenic bacteria gain a foothold in the intestinal tract, the intestinal flora may become a menace to health and may become a matter of real concern to the health of communities.

The General Mechanism of the Action of Ferments—Enzyme Action: C. S. Hudson.

A discussion of the chemical changes involved in the action of enzymes.

Use of Bacteria in the Treatment of Textile Fibers: F. P. Gorham.

Microorganisms in Their Application to Agriculture: C. E. Marshall.

Professor Marshall's complete paper has been published in Science.

A. Parker Hitchens,
Secretary