Table 5. Change in Concentration of Carboxy-hæmoglobin in Blood after Incubation at 38° C. for 20 hr. with Increased Carbon Monoxide Tension, with and without Addition of Sodium Azide (1 : 200)

Method	Species	No. of separate exp.	Per cent carboxy-hæmoglobin before incubation	Per cent carboxy-hæmoglobin after incubation: without sod. azide	Per cent carboxy-hæmoglobin after incubation: with sod. azide
Horvath and Roughton	Man	8	12·50	11·46	13·84
Siösteen and Sjöstrand	Man	6	11·06	10·40	12·16

These observations seem to indicate that carbon monoxide may be both formed in and removed from the blood. If the blood is incubated at 38° C., an equilibrium seems to be reached between these antagonistic reactions. The rate of disposal of carbon monoxide is determined by its tension, and has its optimum value at approximately *p*H 7. In normal life the equilibrium may be affected by the expiration of carbon monoxide, if the concentration in the inspired air is lower than that of the alveolar air, as is usually the case with ordinary atmospheric air. It has also been shown that the reaction is shifted to give increased carbon monoxide formation during physical exertion and on breathing 10 per cent oxygen in nitrogen[5].

The results of these investigations appear to provide an explanation of the endogenous formation of carbon monoxide, and also of the observations made by Fenn and Cobb[6], Clark, Stannard and Fenn[7], and Clark[8], that carbon monoxide is oxidized in isolated muscle preparations and in the intact animal. They may also explain the well-known fact that the carbon monoxide capacity of blood is slightly greater than the oxygen capacity.

A more detailed account of these investigations will be published in *Acta Physiologica Scandinavica*.

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SOCIETY FOR APPLIED BACTERIOLOGY

ANNUAL GENERAL MEETING

THE annual general meeting and conference of the Society for Applied Bacteriology was held in Marischal College, Aberdeen, during July 3–6. Approximately sixty-five members and visitors attended one or more of the four paper-reading sessions. At the meeting the resignation of the secretary, D. A. McKenzie, was announced and G. Sykes, of the Microbiology Division of Messrs. Boots, Nottingham, was appointed to succeed him. During the conference visiting parties were received at the Rowett Research Institute, the Macaulay Research Institute and the Torry Fisheries Research Station. Seventeen original papers and four demonstrations were presented at the four paper-reading sessions.

Miss M. J. Masson (Rowett Research Institute) described the microscopical methods used in a study of the gut of the sheep. The need for the use of direct microscopy as a preliminary to, and check upon, pure cultural and biochemical work on microbial populations was emphasized. Qualitative and quantitative changes in the microscopical appearance of contents from nine regions of the gut of a sheep fed with hay and a concentrate mixture and from the rumen of sheep fed with different diets were detailed. The isolation of *Clostridium butyricum* and lactobacilli from the rumen of a sheep fed with large quantities of flaked maize and small quantities of hay, and a Gram-positive streptococcus which liquefied gelatin, clotted milk, and later digested the clot, grew on McConkey's medium and survived 60° C. for 30 min., from the rumen of a sheep fed up to 300 gm. daily of casein, was reported.

Two papers on "The Chemistry of the Nitrifying Bacteria" were presented by Dr. H. Lees and Dr. T. Hofmann (Biochemistry Department, Marischal College, Aberdeen). The free energy efficiency of *Nitrosomonas* is usually taken to be about 6 per cent, but Dr. Lees has found that in the early stages of growth the value is about 50 per cent (biochemically a far more reasonable figure). The value falls rapidly as nitrite accumulates until, at nitrite concentrations of 1,500 μgm. nitrogen/ml., it has reached about 6 per cent. The rapid fall in efficiency may well be due to an increased respiration-loss consequent upon the maintenance of a low intracellular nitrite concentration in a medium of increasing nitrite content. Experiments on enrichment cultures of *Nitrobacter* have shown that the organism is highly sensitive to chlorate and that chlorate inhibition is reversed by nitrate; this accords with the results of percolation studies on soil. Dr. Hofmann, using the paper chromatography method of Fowden, has found that the amino-acid content of the protein of nitrifying bacteria does not differ fundamentally from the proteins of other less-specialized organisms.

Dr. James Shewan and his colleagues of the Torry Research Station discussed some aspects of the bacteriology of marine fish. The taxonomy of the groups present in marine fish is one of great difficulty, and a comprehensive study of these groups is at present being carried out. It has been found, for example, that it is very necessary, in order to explain different rates of spoilage in the same fish caught at the same time and handled and stored under identical conditions, to know not only the number but also the types of bacteria present. The three main families of bacteria found are Achromobacteriaceæ, Pseudomonadaceæ and Micrococcaceæ. The important species in these groups also show certain common characteristics, namely, capacity for growth in low concentrates of organic nutrients, enhanced growth on solid surfaces (presumably due to increased concentrates of organic nutrients on the solid surfaces), fairly low optimum temperatures (20°–25° C.) and marked heat sensitivity; they are actively proteolytic and usually weakly saccharolytic. Some have rather unusual biochemical characteristics, for example, liquefaction of agar, digestion of chitin and reduction of trimethylamine oxide; a few are luminescent.

A symposium on the chemistry and bacteriology of silage was presented during the conference. Dr. A. J. G. Barnett (Marischal College, Aberdeen) reviewed some recent studies on the estimation of the lactic acid content of silage and directed attention

to the fact that, while the lactic acid content of silage is directly related to *p*H, if the amount of lactic acid is re-calculated on the basis of the dry-matter content of the fresh silage, a similar curve results indicating that the lactic acid formed is proportional to the dry matter content of the initial crop. In further work, at present in progress, Dr. Barnett is studying the change of *p*H in relation to the lactic acid increase and the decline in sugar content.

Mrs. A. C. Stirling (College of Agriculture, Edinburgh) presented the results of preliminary experiments with grass silage made in laboratory containers under controlled conditions. Investigation of the bacterial content of such laboratory silage indicates that the fermentation proceeds more quickly at 30° C. than at 22° C. and that a temperature of 40° C. is selective of different types of organisms. Wilting of the grass delays bacterial action, whereas maceration gives a more rapid fermentation and a lower *p*H.

Miss R. E. A. Allen and Miss A. B. Dickinson (King's College, Newcastle upon Tyne) have investigated the variation in *p*H and total bacterial count (microscopical) in a pit of unmolassed grass silage. While they stress that it is not possible to draw final conclusions from the examination of a single pit, from their results it would seem that the variation of quality of silage in a pit, as measured by *p*H, is greater from top to bottom than from side to side. This agrees with the findings of Dudley and Townsend on the variation of crude protein and dry matter content through a cylindrical silo. Miss Allen's and Miss Dickinson's results were of interest in demonstrating the constancy of *p*H found in the middle layer of the pit.

Some preliminary results and suggestions on the role of plant cells in the ensilage problem were described by L. A. Mabbitt (Edinburgh). His paper dealt primarily with the problem of producing an apparatus which would be capable of doing all operations from sowing the seed to ensiling the fodder in the same sterile chamber. The illustration of Mr. Mabbitt's apparatus created considerable interest. Bacteriological examination of material obtained during 1950 showed that the 'silage' was sterile. Chemical analysis of the material showed that no alcohol, no volatile acid and no lactic acid were present. The amino-acid and volatile base content showed an increase. This work is also continuing.

The enumeration of lactobacilli on grass and silage has been studied by R. M. Keddie, Edinburgh. Mr. Keddie has found that addition of oleic acid and later 'Tween 80' stimulated the growth of lactobacilli. A medium containing 'Tween 80' and acetate in the form of an acetic acid/sodium acetate buffer was used at a final *p*H of 5·4. The medium gave good growth of lactobacilli apart from a group of homofermentative species. It is hoped from this preliminary work to develop a medium suitable for the enumeration of lactobacilli on grass and silage.

The methods hitherto used for the study of the anaerobic bacterial flora of silage have not been satisfactory. The development of an improved technique has been undertaken by R. F. Rosenberger (Edinburgh). Through the work of Barker in California the problem of the anaerobic fermentation of lactate has been largely solved, and, basing his work on Barker's conclusions, Mr. Rosenberger has been able to evolve a dilution method which appears to enumerate quantitatively the number of vegetative cells and spores of anaerobic lactate fermenters such as occur in normal silage. A similar method is being developed for the proteolytic anaerobes, based on the ability of these organisms to carry out an extensive protein breakdown in the absence of any other energy source, provided oxygen is excluded. With the introduction of semi-solid media and non-toxic reducing agents, thioglycollic acid and cysteine, a great advance has been made in anaerobic technique.

Dr. M. Ingram (Cambridge) recalled that experiments on whales have shown that the anaerobic bacteria (*Cl. welchii* type A) which occur naturally in whale flesh do not begin to develop actively until the muscles have passed through rigor mortis. Attempts to observe this effect on more normal meat (horse) have so far been unsuccessful, whether the flesh was incubated anaerobically with *Cl. welchii* as in the whale (artificial inoculation was necessary) or aerobically with species of *Pseudomonas*.

Some preliminary results on a small chlorinating plant suitable for farm water supplies were presented by Dr. L. F. L. Clegg (Wolverhampton) and W. A. Cuthbert (Leeds). The paper discussed the possibility and practicability of improving by chlorination those farm supplies used for dairy purposes which were either potentially dangerous or of such poor bacteriological quality as to be liable to affect the keeping quality of the milk produced. So as to overcome the difficulty of variation in the organic matter content, which in turn would cause a variation in the chlorine demand, super-chlorination followed by de-chlorination were employed. The principle of dosage was that of drawing in concentrated hypochlorite solution by a vacuum pump through a Venturi tube, followed by de-chlorination, on the same principle, with sodium thiosulphate. Incomplete sterilization was afforded with a dosage of 10 parts per million and a contact of 90 seconds, but satisfactory results were obtained by dosing at both 5 and 10 p.p.m. with a contact of 6½ minutes. In order to reduce contamination from the sodium thiosulphate solution itself, it was found necessary to add a by-pass from the contact tank so as to leave a residual of 0·1–0·2 p.p.m. of available chlorine. Experiments are continuing using soil suspensions as contaminating material, and it is intended to study a prototype plant under farm conditions.

Two new experiments with the methylene blue test were described by H. Barkworth (Derby). In Mr. Barkworth's results, with replicate samples, the reduction time of methylene blue in milk diminishes with storage time, but this relationship ceases, or at least is seriously modified, when the reduction time has fallen to 30 min. These results cast suspicion on the value of any routine methylene blue test with an original reading of 30 min. or less, and since in the majority of cases tubes are read only at half-hourly intervals this means that any original reading less than one hour is of doubtful value.

Improvements in the ion-exchange method of preparing silica gel media (Taylor) was the subject of a communication from W. K. Smith (Butterwick Research Laboratories, Welwyn). This type of medium has a number of advantages and has created widespread interest. Mr. Smith discussed problems in the preparation of such media and described further applications.

The need for a rapid *in vivo* screening test for compounds of possible antituberculous activity has held the attention of workers in this field for some considerable time, and because of their small size, resulting in economy of drug and cost of maintenance,

much work has been done on experimental tuberculosis in mice. Miss Betty Croshaw (Boot's, Nottingham) dealt with attempts to standardize the screening test in mice using intracerebral infection, all results being based on the extent of lung tuberculosis when the experiments were deliberately terminated four to six weeks after infection. Miss Croshaw's paper also dealt with the virulence of cultures according to the type of culture medium used and the advantage of objective interpretation of drug activity (that is, survival-time).

A note on the bacteriology of yoghurt was given by Miss E. R. Hiscox (Reading). It would appear that this fermented milk consists primarily of a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. In some types yeasts may also be present.

Miss C. M. Cousins (Milton-Deosan, London) described a preliminary screening test (bacteriological) for quaternary ammonium compounds and formulations. The test is based on a plate count method which determines the survival of a given test organism, *Bacterium coli*, in sterile homogenized whole milk, added with the bacterial suspension to the quaternary ammonium compound dilutions, for an exposure time of two minutes. The paper directed attention to the special cleaning of glassware that is necessary. The preparation and use of the quaternary ammonium compound inhibitor, lecithin in 'Lissapol N', was described, with details of the testing procedure. Examples quoted showed how this test indicates a difference in bactericidal efficiency between (*a*) two different quaternary ammonium compound solutions, and (*b*) the same quaternary ammonium compound included in two powder formulations of differing *p*H value.

GENES AND MUTATIONS

THE Symposia on Quantitative Biology held annually in the Biological Laboratory at Cold Spring Harbor, Long Island, have become a major event in biology. Their purpose is to foster a closer relationship between biology and the other basic sciences, and they set out to achieve this not only by attempting a synthesis of existing experimental data concerning a particular subject, but also by applying new knowledge gained in other fields.

This year, the sixteenth of the series, the topic of discussion, selected by Dr. M. Demerec (Carnegie Institution), was "The Genes and Mutations". The same subject was discussed ten years ago, and the present meeting was intended to show the advances made in genetical research during the past decade. In 1941 the main subjects of discussion were the giant chromosomes of the salivary glands of *Drosophila*, the mechanism of chromosome coiling and spiralization, and the quantitative aspect of spontaneous and radiation-induced mutations. Only one contribution was concerned with the genetical usefulness of micro-organisms, viruses and bacteria. During the present Symposium the latter occupied a prominent position, and it was clearly demonstrated that the knowledge gained from studies of the genetical behaviour of bacteria, viruses and bacteriophage has deepened our understanding of the basic fundamentals of genetics themselves, and explained phenomena which baffled geneticists of ten years ago—as was so dramatically illustrated by T. M. Sonneborn (Indiana University) in the closing session.

A new theory of the gene, including its action in development and its relation to other genes, was brought to light by the work of B. McClintock (Carnegie Institution). She described very remarkable and complicated results on mutable genes in maize, which can be interpreted as due to transposition of minute pieces of chromatin from one position to another in the chromosome. The frequency of such transposition is itself under genic control, which findings emphasize the close integration or interrelationship of the gene complex. According to R. Goldschmidt (Stanford University, Cal.) the gene in the chromosome represents a "field of action" closely integrated into one system, in which gene mutation is a consequence of rearrangements on a submicroscopic scale. L. Stadler (University of Missouri) presented results on spontaneous gene mutation in maize, and E. R. Lewis (California Institute of Technology) on pseudoallelism in *Drosophila*. Both contributions have shown how it is possible by exact and extremely detailed genetical analysis to obtain insight into the complexity of genic activity. The same point was emphasized by S. G. Stephens (University of North Carolina) in describing the diversification of individual genetic loci in the diploid and amphidiploid forms of *Gossypium*.

Gene action and gene-enzyme relationship was discussed by D. M. Bonner (Yale University) and N. H. Horowitz (California Institute of Technology) in *Neurospora*. It was argued that there are genes which control only one step in the synthesis of enzymes, but an example in the synthesis of nicotinic acid was also presented to show that the blockage can be incomplete.

The genetical impact of chemical agents on genes and chromosomes was but little known in 1941. However, the present conference showed that the wealth of results obtained meantime in diverse organisms by the study of a great array of substances is not only impressive but also has widened our concept of mutagenesis itself and brought to light phenomena not observed previously. C. Auerbach (University of Edinburgh) pointed out the differences between chemically and X-ray induced mutations, by studying the mosaics in *Drosophila*, and emphasized the possibility of a delayed effect on the gene. Dr. Demerec described tests of various highly mutagenic compounds on the streptomycin-dependent mutant of *Escherichia coli*, which is a particularly suitable object for studies of the influence of environmental factors on gene-stability. On this aspect more evidence was presented by A. Hollaender (Oak Ridge National Laboratory, Tenn.) and collaborators, showing that lack of oxygen is one of the factors which greatly reduces the mutagenic effect of chemical agents and ionizing radiations. M. Westergaard (Institute of Genetics, Copenhagen) described his 'back-mutation' test in *Neurospora*, which will prove to be a very useful and rapid method for the detection of chemical mutagens, and made an attempt to interpret the chemical basis of induced mutagenesis. The aspect presented by Westergaard linked up closely with the contribution of N. H. Giles (Yale University), who described the various mechanisms which may be responsible for the spontaneous reversions of biochemical mutants in *Neurospora*.

The visible effects of various chemical agents on the chromosomes of *Allium* and *Vicia* were described by A. Levan (Institute of Genetics, Lund), who demonstrated that different chromosome regions may exhibit a different degree of reactivity to a drug,