BACTERIOLOGY

J. O. MacFarland and L. S. McClung, Chairmen

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ABSTRACTS

Intracellular inclusions in a *Caulobacter* sp. E. A. Grula and S. E. Hartsell, Purdue University.—Throughout the long history of bacterial cytology, numerous observations have been made relating to the chemical nature of bacterial inclusions. Such studies continue today with a seemingly unending array of claim and counter-claim. It should be pointed out that most of the critical studies relating to such inclusions have been performed with optical instruments.

Beginning with a standard electron microscope technique, we have modified some preparational procedures thereby permitting us direct observation of intracellular inclusions before and after specific extraction and digestion procedures, after growth on optimal and minimal synthetic media and in "specific-action" buffer solutions wherein one inclusion was synthetized "de novo" by the cells.

Where necessary, ordinary staining in conjunction with optical instruments have been employed.

Studies relating to dark and dense intracellular granules have been performed utilizing the O/R indicator dye 2, 3, 5 triphenyl tetrazolium chloride. Its intracellular reduction has been correlated with the presence or absence of the dark inclusion.

A further note will be supplied regarding fixation techniques in relation to extraction procedures.

An experimental study of the nature of bacterial pyrogens. Paul D. Ellner, University of Southern California (Now at Indiana University).—Factors influencing the production of pyrogen by two strains of *Pseudomonas aeruginosa* were studied. Experimental results indicate that 37 C approaches the optimum temperature for pyrogen production, at a neutral pH. Pyrogen is best produced on a medium in the slightly oxidized state. Media containing small amounts of sodium chloride appear to be stimulatory to pyrogen production which begins at the phase of logarithmic growth. Although the pyrogen is non-antigenic, when the bacterial cells of a pyrogenic species are suspended in specific antiserum (agglutinin), no demonstratable amounts of pyrogen are produced. Pyrogen appears to be intimately associated with the cell substance, rather than being a diffusible metabolite of the intact living cell. It is concluded that bacterial pyrogen produced by this species is associated with the cell substance, analagous to an endotoxin, and is released into the surrounding medium by cellular autolysis. Environmental conditions such as temperature, pH, O-R potential and chemical composition of the medium, which tend to stimulate cell proliferation are also conducive to the elaboration of pyrogen, since the number of autolyzed cells increases with increasing age of the cultures.

A synthetic medium for candicidin production. R. F. ACKER, Purdue University, (work done at Rutgers University).—An investigation of the nutritional requirements of *Streptomyces griseus* 3570 showed that L-asparagine and L-histidine were utilized to best advantage as sources of nitrogen for the production of the antifungal antibiotic, candicidin. Ammonium salts and nitrates were found to be poorly utilized. Among the carbon sources tested, glucose and mannose gave better antibiotic yields and growth than other sugars, sugar alcohols, or organic acids. However, certain organic acids supplied as sodium salts, e.g. sodium lactate and sodium malate were stimulatory when supplied to supplement glucose. Sodium lactate, with glucose, gave maximum candicidin yields, and sodium malate, with glucose, gave maximum mycelial growth.

The macroelements potassium magnesium, phosphate-phosphorus, and sulfate-sulfur were found essential to candicidin production, inasmuch as they were essential to growth. Such proved also to be the case with the microelements iron and zinc. It could not be demonstrated that manganese was required by culture 3570 either for growth or candicidin production.

The above findings led to the formulation of a synthetic medium for candicidin production containing L-asparagine, glucose, sodium lactate, magnesium sulfate, dibasic potassium phosphate, iron, and zinc. Potencies produced in this medium were of an order of 8 to 10 thousand dilution units per ml by streak-dilution assay, as compared with 10 to 20 thousand dilution units per ml produced in the complex production medium.

Microscope studies of red blood cell agglutination. JOSEPH CARLIN and MERWIN MOSKOWITZ, Purdue University.—Several workers have presented evidence that the addition of agglutinins to red cells brings about alterations of the red cell surface and have suggested that the resulting agglutination is due to these alterations.

Red cell agglutination was studied using both light and electron microscopy and it was observed that under the proper conditions agglutination occurred without detectable surface changes, and also that the alterations described by the other workers could be produced in the absence of agglutinins.

This study demonstrated that red cell agglutination occurs independently of alterations of the surface of the cells, giving support to the lattice theory that aggregates are formed by combination of specific groupings on the cell with specific groupings on the multivalent antibody.

Phenol extraction of the red cell altering factor of Streptococci. MERWIN MOSKOWITZ, BARBARA THOMPSON and ALEXANDER SONNENWIRTH, Purdue University.—Studies have been under way in this laboratory on the separation of the antigen(s) of *Streptococcus pyrogenes* that alter red cells in such a manner that they are agglutinated by normal human serum. A method has been found to extract the altering factor with phenol. Many difficulties were encountered in working with phenol in order to obtain consistent results. Impurities in phenol preparations, changes occurring in phenol on standing, and the phase relationships between phenol and water caused complications. The present method of extracting the altering factor and further steps in its purification will be discussed.

The effect of 2,4-diamino-5-p-chloro-phenyl-6-ethylpyrimidine in experimental toxoplasmosis. W. A. SUMMERS, Indiana University Medical School.—Low diet concentrations of Daraprim (0.01 per cent) did not prevent the death of *Toxoplasm*-infected mice nor alter the development of the parasite in the host. Higher but clearly toxic dosages of Daraprim (0.075 and 0.1 per cent) prevented the development of *Toxoplasma gondii* and protected a large percentage of infected white mice. The addition of pteryolglutamic acid to the Daraprim diet did not reverse the anti-toxoplasmal action of Daraprim and appeared to lessen the toxic action of the latter for the animals. Judging by the methods used, Daraprim did consistently eradicate Toxoplasma from the brain, spleen, liver and lungs of mice that received small to moderately heavy inocula of this parasite.

Tracer studies on threonine biosynthesis and metabolism by *Neurospora* mutants. HAROLD R. GARNER, Purdue University.—Biochemical and genetic studies of the threonine-less series of mutants of *Neurospora* have led to an accumulation of data some of which cannot be reconciled with other. No single scheme of reactions has yet been capable of explaining the biochemical needs and genetic interrelationships of these mutants. An attempt is being made to untangle this information by using C^{14} labeled compounds to trace reaction sequences.

Strain 35423 was grown on uniformly labeled glucose and unlabeled threonine. The mycelium was hydrolyzed, the individual amino acids separated by column chromatography and their specific activities determined. The results show that the unlabeled threonine is used directly and also acts as a precursor of isoleucine. Slight variations in specific activity of other amino acids can probably be explained as due to CO_2 fixation since degradation of the aspartic acid from these cells showed an average of one carboxyl group per molecule to be unlabeled. Similar experiments with two other mutants have confirmed homoserine as a precursor of threonine. However, homoserine does not appear to be a direct precursor of methionine as it appeared from previous work. To check this point further, uniformly radioactive cystathionine was biosynthesized and is being used to support the growth of a cystathionine-less mutant.

It is hoped that this work will provide a solid biochemical reaction scheme around which to explain the genetic deficiencies of the mutants.

The electron microscopy of the lysis of *M. lysodeikticus* with lysozyme. E. A. GRULA and S. E. HARTSELL, Purdue University.—Since the discovery of lysozyme in tears by Fleming, there have been several publications relating to the "modus operandi" of this mucolytic enzyme on bacterial cells. The test organism has, in most instances, been *M. lysodeikticus*—the organism originally employed by Fleming.

In the days prior to the electron microscope, several lively controversies developed regarding lysis of this organism with lysozyme. The arguments were concerned primarily with swelling—a phenomena still mentioned, but as yet unresolved, in today's literature.

With the advent of the electron microscope, the possible disruption or digestion of the cell "per se" has also been of prime concern. Unfortunately, more conflicting data has appeared concerning the lysis of this organism.

In studies performed in these laboratories, we have attempted to obtain a correlated and accurate picture of the lytic phenomenon using both the light and electron microscopes. Also currently improved techniques relating to electron microscopy, that is the "freeze-dry" method has been employed.

As an adjunct to these techniques, live cells of *M. lysodeikticus* have been subjected to disruption with glass beads in order to follow further the effect of lysozyme on cellular structures.

The genus *Clostridium*—advances in the last decade. L. S. McCLUNG, Indiana University.—Recent contributions were reviewed concerning the study of the anaerobic organisms including summary of information on purification of the toxins produced by organisms causing botulinum, lock jaw, and gas gangrene. The success of toxoid immunization in tetanus and the availability of toxoids for gas gangrene and botulism was reported. The problem of classification of the clostridia was discussed and mention was made of the many new species reported recently for the genus *Clostridium.* Additional topics in which recent investigations have proved fruitful include: cellulose fermentations including microflora of the rumen, and mechanism of metabolism of anaerobic reactions.

Recent advances in the preparation of antirabies vaccines containing inactivated virus. H. M. POWELL and C. G. CULBERTSON, Lilly Research Laboratories, Indianapolis, U. S. A.—The common methods for inactivating fixed rabies virus in the preparation of vaccine have included (a) natural or spontaneous aging, (b) common antiseptics such as phenol in the presence of variable amounts of heat, (c) ultraviolet irradiation, and (d) certain nitrogen mustard or mustard-like drugs.

An early but very brief report on the use of a mustard compound in fixed rabies virus was by C. Tenbroeck and R. M. Herriot, *Viruses inactivated by mustard (Bis (B-chloroethyl) sulfide)* as vaccines. Proc. Soc. Exp. Biol. and Med. 1946, 62, 271-272. It appeared that these investigators secured better antirabic immunity in guinea pigs injected with such vaccine than with vaccine treated with phenol or chloroform. A further report by the same authors was *Mustard inactivated rabies vaccine.* Proc. Soc. Exp. Biol. and Med. 1950, 75, 523-528.

We have had occasion to try out twenty-five nitrogen mustard or mustard-like drugs for inactivating fixed rabies virus in the preparation of anti-rabies vaccine. Some of these drugs expend themselves very rapidly in dilute aqueous solutions while others last for several days or weeks. A reasonable time for inactivation of fixed rabies virus in the ice box was arbitrarily set at five to seven days or less, although some of the drugs accomplish inactivation at some time during the second or early in the third week. Slow inactivation apparently may be due to innate inertness on the one hand, or to very rapid disappearance of the drug on the other hand, necessitating a complicated repeated drug treatment of virus.

Five drugs of considerable interest in this group were as follows:

(1) 00915 B B' dichloroethylamine
(2) 01749 1 morpholino, 2,3, epoxypropane
(3) 01995 butadiene monoxide

(4) 01401 benzyl B-Chloroethylamine
(5) 01621 N(2-hydroxyethyl) ethylene imine

Rabies vaccines made by addition of from 1 to 3 mgm. of drug per ml of virus and let stand in the ice box for complete inactivation have given the following results on immunization of mice by present standard NIH methods:

(1)	vaccine mice LD_{50}	$10^{-3.1}$
(2)	" " "	$10^{-3.3}$
(3)	" " "	$10^{-4.5}$
(4)	" " "	$10^{-2.0}$
(5)	" " "	$10^{-2.0}$
Commercial vaccine 433668		$10^{-2.0}$
Control mice (no vaccine)		$10^{-6.0}$

Drug number 2 has been used to inactivate fixed rabies virus propagated in embryonated duck eggs (Powell, H. M., and Culbertson, C. G. *Cultivation of fixed rabies virus in embryonated duck eggs.* Public Health Reports 1950, **65**, 400-401) and vaccine made in this has given protection to mice against about 26,000 LD_{50} of active virus.