

**ABSTRACTS OF PAPERS PRESENTED AT A
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OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS**

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Ilda McVeigh, President
Raymond W. Beck, Secretary

Spectral studies of Chrysogenin, a Pigment Produced by Penicillium chrysogenum. Frederick T. Wolf, Y. T. Kim and E. A. Jones, Department of Biology, Division of Molecular Biology, Vanderbilt University, Nashville, Tennessee.

The absorption of spectra of chrysogenin, the yellow to orange pigment produced by *Penicillium chrysogenum*, have been studied in the ultraviolet and visible regions. In either solution, there are two absorption maxima at 270 $m\mu$ and 365 $m\mu$. In water, the positions of the maxima are shifted to 295 $m\mu$ and 375 $m\mu$. Chrysogenin is a fluorescent compound. Its fluorescence maximum in aqueous solution is very broad, in the 460-520 $m\mu$ region. Evidence obtained from infra-red spectra indicates that chrysogenin has the structure of a methyl polyhydroxy anthraquinone.

A Simplified Method for Counting Anaerobic Rumen Bacteria. D. W. Claypool, D. R. Jacobson and R. F. Wiseman, Departments of Dairy and Microbiology, University of Kentucky, Lexington, Kentucky.

Sections of small glass tubing are employed as modified Burri tubes in overcoming the obstacles often encountered when counting anaerobic rumen bacteria by conventional plating procedures. Selected dilutions of rumen content are prepared in liquified Bryant and Burkey RCGA medium and 1 ml amounts drawn into sterile glass tubing by means of a sterile syringe, fitted with a rubber adapter. The filled tubing is removed from the syringe and both ends sealed by flame. Carbon dioxide is passed through all tubing, syringes, and media to displace air and rezaurin placed in the medium to indicate anaerobiosis throughout preparation and incubation. Incubation is carried out in a water bath at 39°C for 72 hours. Counts of colonies are made under a wide field microscope at 30X. Selected colonies are picked for further study by breaking the tubing at the site of the colony.

Studies on the Loss of Penicillin Activity in the Rumen of Cattle Fed Leguminous Diets. B. Pivo, R. F. Wiseman, D. R. Jacobson, and W. M. Miller, Departments of Microbiology and Dairy, University of Kentucky Lexington, Kentucky.

It has been reported that the administration of levels of penicillin which are effective in controlling bloat in legume-fed cattle (50-100 mg) had little effect upon the numbers of bacteria in the rumens of non-bloating cattle. The present study revealed that a level of 250 mg of penicillin per day did not alter the microbial balance in the rumens of steer fed cut alfalfa. No persistent changes in numbers of lactobacilli, streptococci, coliforms, or anaerobic bacteria were detected when steers were fed either alfalfa or clover, or during penicillin administration. Analyses revealed that isolates of lactobacilli and streptococci were sensitive to 1 μg of penicillin per ml of media, while the coliform bacteria were capable of growing in media containing 50 μg per ml. Assay of the rumen content showed that the penicillin decreased to apparently ineffective levels during the first week of treatment and the activity completely disappeared within 1-3 hours in

the third week of treatment. Penicillin activity could not be detected in the blood of treated animals. Preliminary experiments suggest the resistant coliform bacteria may be a major contributor to the inactivation of the penicillin.

Bacteriocinic Strains of Enterobacteriaceae. Guy T. Barry and Vella Abbott, University of Tennessee Memorial Research Center, Knoxville, Tennessee, and Ray Leffler and Edna Mynatt, East Tennessee Baptist Hospital, Knoxville, Tennessee.

Analysis of over two hundred strains of bacteria isolated from patients suffering from various infections has revealed that 46% are producers of bacteriocine. Of the 100 bacteriocinic strains examined, one is a *C. freundii* microorganism, 4 are *E. intermedium* and 95 are *E. coli* bacteria. The bacteriocines elaborated by the isolated and 17 known strains were separated into two groups on the basis of their sensitivity to heat; bacteriocines A, C, E, V, K, S3 and 17 of the undetermined bacteriocines retain their biological activity, whereas bacteriocines B, D, F, G, H, I, J, S1, S4, S5, ML and 83 of the undetermined bacteriocines are inactivated. The spectrum of activity of the known and unknown bacteriocines against various test strains employed for the detection and identification of bacteriocines showed that among the isolated strains which elaborate a heat stable bacteriocine, 3 produce A, 2 produce V, 5 produce a bacteriocine which appears related to V, one produces E and 6 are unidentified. Of the strains which elaborate heat labile bacteriocines, one produces D, one produces B, 62 produce a bacteriocine which appears related to G and H and 19 remain unidentified. In sum, bacteriocinic strains of the enterobacteriaceae are frequently present in infections of man; several of the bacteriocines elaborated by these strains differ from those previously described.

Effect of the Endotoxin of Salmonella typhosa on the Coronary Vascular Bed of the Dog. Jerry B. Scott, Edward D. Frohlich, and Elmo S. Dooley, U. S. Army Medical Research Laboratory, Fort Knox, Kentucky.

Despite the extensive literature on the hemodynamic alterations of endotoxin, study of its action in the coronary vascular bed has received little attention. The effects of *S. typhosa* endotoxin were studied in the entire coronary bed of the dog by shunting blood around the heart and lungs, clamping the aortic arch, and perfusing arterial blood at a constant rate of flow into the ascending aorta. After a control period, 0.6 mg/kg of endotoxin was injected into the coronary arteries. Coronary and systemic pressures were monitored for the next 40 minutes. Coronary artery pressure (CAP) fell in each of 7 experimental dogs with an average of 32% at 40 minutes. There was a concomittant fall in systemic pressure of 29%. In 6 control dogs there was no reduction in the CAP after 40 minutes following placebo. There was no change in myocardial utilization of Na and K and there was no change in the hematocrit across the coronary bed. Four of 5 dogs showed an insignificant increase in myocardial O₂ consumption. Post mortem examinations of all experimental animals confirmed endotoxin shock. It is concluded that endotoxin does act on the coronary vascular resistance either by local or remote mechanisms.

Studies on the Relationships of Bacterial Cell Walls, Antigenic Structures and Bacteriophage Adsorption Sites. Sidney D. Crouch and James C. Humphries, Department of Bacteriology, University of Kentucky, Lexington, Kentucky.

Cell walls were prepared by lysing young viable cells by treatment with 2-3-M glycine. The lysates first were freed of any unaltered cells by low speed centrifugation and the cell walls were then harvested and washed by high speed centrifugation or filtration.

Walls were prepared from four strains of *Klebsiella pneumoniae*. They were found to be almost invisible by light microscopy but were readily visible by dark field and light phase microscopy, having the general form of the original cells. By electron microscopy they appeared as flattened, empty hulls.

The presence of antigens in the walls was determined by agglutination using antisera homologous to the bacterial strains, and bacteriophage absorption sites were tested for with three phages lytic for one or more of the parent bacteria. The walls of all four bacterial strains were clumped by the corresponding antisera, indicating the presence of antigens in these structures. The results of the bacteriophage tests varied with the phage and with the bacterium, from no absorption to very good absorption showing that, although the receptor sites for other phages were absent indicating that they either are not a part of the cell wall or were destroyed by the preparation procedure.

Cellular Immunity in Tularemia. Margaret Stansberry and John M. Woodward, Department of Bacteriology, University of Tennessee. Knoxville, Tennessee.

Leucocytes harvested from peritoneal washings of rats immune to tularemia and transferred to normal rats have been shown to confer significant specific resistance against this infection. However, the protective effect is not observed unless the cells are centrifuged several times prior to transfer. Neither leucocytic extracts nor supernatants of centrifuged peritoneal washings have a beneficial effect. Investigations are in progress to determine whether a specific antibody is released by the shock of manipulative procedures to which the white cells are subjected. It is possible that such entity, in association with the leucocytes, enhanced resistance to infection with *Pasteurella tularensis*.

The Effect of 2, 4 Dinitrophenol on Rats Treated with ATP and Inorganic Phosphate. Gennaro J. Miraglia and John M. Woodward, Department of Bacteriology, University of Tennessee, Knoxville, Tennessee.

Earlier studies conducted in our laboratory have shown that the administration of phosphate in the form of ATP not only alleviated disease symptoms in tularemic animals but also reduced the mortality rate markedly. Infected animals receiving no treatment were found to have slightly elevated serum inorganic phosphate levels suggesting an uncoupling of phosphorylation. Since this may be accomplished specifically by DNP, this compound was administered to normal rats to give one LD₅₀. These were treated subsequently with ATP and inorganic phosphate which have been shown to be protective in tularemia.

Significantly higher survival rates were observed in animals treated with ATP as compared to those treated with KH₂PO₄ and K₂HPO₄. This was the same order of effectiveness by which these compounds protected against tularemic infection. A comparison of these data suggests that the symptoms in this disease may be the result of uncoupled phosphorylation.

Branching in Lactobacillus bifidus. Manuel Figueroa and R. H. Weaver. Department of Microbiology, University of Kentucky, Lexington, Kentucky.

Four strains were studied by means of the microculture method and the phase contrast microscope, the electron microscope, and cell wall staining techniques. All strains retained their branching character and their requirement for anaerobic conditions throughout the study. The organisms appear to have a growing point at one end of the cell. This results in: (1) unequal cell division, simulating budding; (2) false branching, resulting from growth of a cell at the end adjacent to another cell in the chain; (3) cells that are swollen at one end; and (4) true branching from the end of the cell. The cells are less dense to the electron beam at their ends (growing points). It is postulated that the swelling of the ends of the cells and the branching is due to weakness of the cell walls at this point. This postulation is supported by the finding that: (1) swelling of the ends of cells of *L. bifidus* var *pennsylvanicus* is prevented by the addition of N-acetyl-D-glucosamine, a cell wall precursor, to the medium; (2) branching is increased by the addition of penicillin or the plant hormone 2,4,5-T, substances which appear to affect cell wall formation.

Effect of Pyridoxine Hydrochloride and Pyridoxal Phosphate on the Hematocrit Values of Chicks Infected with Salmonella pullorum. Ronald A. Le Clair and D. Frank Holtman, Department of Bacteriology, University of Tennessee, Knoxville, Tennessee.

A Babcock-Mount Hope strain of chicks exhibited a decreased hematocrit value forty-eight hours after receiving an infecting dose of *Salmonella pullorum*. The hematocrit values for twenty-four, seventy-two, and ninety-six hour testing periods are within normal limits.

The hematocrit at forty-eight hours, in infected chicks receiving pyridoxal hydrochloride, exhibited a five per cent increase over infected controls. Infected chicks receiving pyridoxal phosphate had hematocrit values two per cent above those for forty-eight hour infected controls.

In normal chicks, the administration of pyridoxine hydrochloride resulted in below normal hematocrit readings.

The increased hematocrit values after administration of the vitamin preparation may be the result of increased erythropoiesis, or to a decrease in plasma volume by a more severe endointoxication.

The Roles of Various Fractions of Pseudomonas aeruginosa in its Pathogenesis. Pinghui V. Liu, Yoshio Abe and Janice L. Bates, Department of Microbiology, University of Louisville School of Medicine, Louisville, Kentucky.

Pseudomonas aeruginosa was grown on a cellophane plate, using tryptone glucose extract agar (Difco) enriched with 1% glucose. Six fractions obtained from this growth were: I-a (pyocyanin), I-b (all dialyzable substance other than pyocyanin), II (the hemolysin), III (extracellular enzymes), IV (the slime) and V (the bacterial cells). The fractions I-a and V appeared to be the least important fractions in the pathogenesis because they were toxic neither to animals nor to plants. The fraction I-b was the only fraction capable of eliciting necrosis of plant tissues. The fraction II was lethal to mice and produced redness and induration of the skin of rabbits but it was not antigenic. The fraction III was lethal to mice and produced ulcer in the skin of rabbits. It was antigenic and species-specific. The slime was lethal to mice but did not produce lesions in the skin of rabbits. It appeared to be a good antigen and antibodies produced in rabbits with slime agglutinated bacterial cells protected mice from the infection of *P. aeruginosa*. The slime, therefore, appeared to be the most important fraction in the pathogenesis of this organism and it probably functions like the capsule of the pneumococci. The protection of mice by the antibodies to slime was aye-specific in that it was effective against the type of *P. aeruginosa* which possesses the same serological type of slime.

Preliminary Studies on the Respiration of Aerobic Actinomycetes. Thressa Redmon and Margaret Hotchkiss, University of Kentucky, Lexington, Kentucky.

Four commonly used carbohydrates (glucose, maltose, lactose and sucrose) were tested to study their effect on the fermentation and respiration of fifteen aerobic actinomycetes.

With appropriate media, all the cultures were able to produce acid from glucose, some cultures produced acid with maltose and sucrose and no culture produced acid from lactose. All the cultures grew with glucose as a sole source of carbon, and all grew with maltose, although the amount of growth was less. Eight cultures grew with sucrose and five with lactose.

Manometric methods gave more detailed information as to the effect of the four carbohydrates. In this paper the results of Q_{O_2} calculations are reported. The Q_{O_2} represents the amount of oxygen, in microliters, absorbed in one hour per mg of dried cells. An atmosphere of air was used and a pH of 6.8.

The endogenous Q_{02} , determined without added carbohydrate, separated the cultures into three groups. Seven cultures had a low endogenous uptake of 4 to 10 microliters, five cultures an endogenous uptake of 11 to 17 microliters and three cultures gave 22, 23, and 25 microliters respectively.

Glucose increased the Q_{02} of all the cultures, maltose of seven cultures, and sucrose had a definite effect on three cultures. Lactose increased the Q_{02} in four cultures.

The study of these cultures is continuing in order to discover basic methods which will aid in their classification.

Cellular Interactions During Bacterial Senescence and Decline. Arthur P. Harrison, Jr., Division of Molecular Biology, Bacteriology Section, Vanderbilt University, Nashville, Tennessee.

Cells of *Aerobacter aerogenes* differing in colonial morphology but otherwise of the same genotype have been utilized in the present research to determine the effect of physiological state upon survival in dearth of food. The cells were cultivated in synthetic broth at 40°C, were washed well with sodium phosphate buffer, were then suspended at various cell concentrations in the buffer at 40°C, and their response measured by means of periodic plate counts.

Cells harvested from a maximum stationary phase culture manifest better survival than cells from a logarithmic phase culture. Further, when maximum stationary phase cells, in minority, are mixed with log phase cells, in majority, the viable count of the former increases at the expense of the latter. This suppression of log phase cells by maximum stationary phase cells is not due to some occult property unique to maximum stationary phase physiology, but appears to be associated with the concentration of nutriment to which the cells were exposed at the time of harvest. Thus, when cells from a slow-growing log phase culture are mixed with cells from a log phase culture growing at the customary rate the result is the same, the cells from the former culture surviving at the expense of the latter. An interpretative model to unify these and other observations has been developed.

Evaluation of Techniques for the Demonstration of Trichomonas Vaginalis. Barbara H. Sherrard and Emil Kotcher, University of Louisville, School of Medicine, Louisville, Kentucky.

Wet preparations of vaginal secretions and cultivation of such secretions have been used in detecting *Trichomonas vaginalis*. In the former technique it is difficult to detect the non-motile, spherical forms and in the latter technique the procedure is time consuming. Identification of *T. vaginalis* in cervical smears by Papanicolaou staining is not satisfactory because of the absence of morphological details. This study evaluates the efficiency of these techniques for detecting *T. vaginalis* with fluorescein-tagged antibody procedures. *T. vaginalis* antiserum was prepared in the rabbit and the antiserum was conjugated with fluorescein isothiocyanate. Vaginal and cervical smears of 181 women were examined by (1) wet preparations, (2) culture in horse serum sodium thioglycollate, (3) Papanicolaou smear, and (4) direct fluorescein-tagged antibody procedures (FTA). Wet preparations revealed 58 positives (32.0%); culture, 69 positives (38.4%); Papanicolaou, 16 positives (9%); and FTA, 65 positives (35.8%).

Another series of 55 women were examined by wet preparations of vaginal secretions and by direct and indirect FTA procedures. In this group, wet preparations revealed 21 positives (38.1%); direct FTA, 24 positives (43.6%); and indirect FTA, 17 positives (30.9%). In this series, 14 women were positive by direct FTA and wet preparations (25.4%); 6 were positive by wet preparations but negative by direct FTA (10.9%); and 11 were positive by direct FTA but negative by wet preparations (20.0%).

The results indicate that culture and fluorescent antibody procedures are

superior to wet preparations and Papanicolaou stain in detecting *T. vaginalis*.

Studies of Hemophilus Influenzae in Acute Upper Respiratory Infection. Sarah H. Wood Sell, Kate Welch and Robert S. Sanders, Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee.

The etiological role of *H. influenzae* in acute upper respiratory infections of young children is poorly understood. A study was made to determine whether or not patients whose nasopharyngeal mucous gave heavy growth of this bacterium during the acute phase of illness later developed specific agglutinating antibodies in their serum. Seventeen infants and children were selected during the Spring of 1960. All had a clinical diagnosis of "acute upper respiratory infection" (14 with "otitis media"). All had a heavy growth of *H. influenzae* on Levinthal's agar plates streaked with nasopharyngeal mucous. Each child's acute and convalescent serum was tested against his own strain of bacteria for agglutinating antibodies with the following results:

13 developed antibodies in the convalescent serum only

11 against their own strains; 2 against stock antigens (1 vs. type b and 1 vs. type e)

4 had no difference between acute and convalescent sera.

(In 3 there was no agglutination while in 1 there was agglutination in in both)

It is concluded that *H. influenzae* was probably of etiological importance in this small series of patients.

Bacteriology of Bacteroides Infections. James Burns and Mary Vivian Shoemaker, University of Tennessee Medical Units, Memphis, and The Baroness Erlanger Hospital, Chattanooga, Tennessee.

Gram negative non-sporeforming anaerobic bacilli ("Bacteroides") are common in clinical specimens, but their presence is frequently overlooked. The purpose of this report is to summarize our experiences with cultures of these organisms isolated in a general hospital over a two year period.

The importance of these organisms can be appreciated only when they are studied in pure culture. They should be suspected in any deep infection. They are easily recognized by their morphology and by their strictly anaerobic character. Because they frequently occur in association with other anaerobic, microaerophilic, and aerobic bacteria, it has often been difficult to study their characteristics in pure culture.

We have employed a simple two-step procedure for this latter purpose. The method consists of primary isolation in a selective medium containing high concentrations of the broad spectrum antibiotic kanamycin and subculturing to anaerobic plates prepared by the method of Carquist.

Studies on the Mechanism of Induced Radioresistance in Escherichia coli. M. S. Engel and H. I. Alder, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

It had been shown previously that cells of *Escherichia coli* grown in peptone gave typically exponential X-ray survival curves. However, when these cells were grown in the same medium supplemented with glucose they were much more resistant, yielding highly sigmoidal survival curves. This effect has now been found in six strains of *E. coli* and is not restricted to resistance to X-rays but extends to head inactivation, ultra-violet killing, and survival to hydrogen peroxide.

The mechanism of the induced resistance has been studied and it is apparent that the resistance is related to the final pH of the growth medium. The resistance is not dependent on glucose as other sugars can substitute for this substrate. There are, however, a number of other ways in which the

resistance can be varied. In the presence of some substrates the low pH is produced but the cells are not resistant and even with glucose as carbon source, certain growth factors are required for the production of resistance. The case for metabolic control of resistance is further strengthened by studies with a porphyrin deficient mutant of *E. coli* which shows the effect under investigation only when grown in the presence of hemin.

NEWS OF TENNESSEE SCIENCE

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Mr. Singer received his undergraduate work and two years of graduate training at the University of Wisconsin and has had research experience in the leather industry.

A short term In-Service Institute for High School Science teachers, under a grant from the National Science Foundation, is being conducted at Tennessee Polytechnic Institute. The first session was held on October 15 with Dr. Hollis Rogers, Professor of Botany at the University of North Carolina, as essayist for the General Meeting. Sectional meetings (Biology, Mathematics and Physical Sciences) were led by members of the faculty at TPI. At the second session, Dr. Oscar Touster, Professor of Biochemistry at Vanderbilt School of Medicine, was the speaker. The third and last session will be held December 10, with Dr. C. P. Keim of the Oak Ridge National Laboratory as speaker. Dr. G. B. Pennebaker, Director of the School of Arts and Sciences at TPI is Director of the Institute.

The University of Tennessee's new Frank H. McClung Museum will be a place where the visitor can see Tennessee's wealth of history, arts and sciences "come to life" in natural settings — a relatively new method of displaying museum pieces. The man who is preparing to give the museum exhibits this "living" quality is Joseph W. Hopkins, who came to U T this year as exhibit specialist from the Atwater-Kent Museum in Philadelphia. Although the building, now under construction on the Knoxville campus, will be open for limited use by next Fall Quarter, the visitor will have to wait for some time to see the majority of planned exhibits. Work on the many exhibits can begin only after the building is finished, and its spacious workshops and laboratories are equipped with power tools and artist's materials. Mr. Hopkins, a native Virginian and graduate of the Wilmington Academy of Art, Wilmington, Delaware, has spent much of his time thus far designing for the Green Memorial Room, named in honor of the late Judge and Mrs. John W. Green of Knoxville, who left funds for construction of the Museum. In explaining the new concept being incorporated into the McClung Museum, Mr. Hopkins says, "Museums are going through a revolution. The old way was to take a big case and display dozens of bones, pots, or what have you. This, of course, was a treasure trove for the collector — but pretty uninteresting for the average person. Now, museums are trying to interpret the object being exhibited — to put it in a setting which shows its relationship to man."

Mr. Narinder Chopra, a graduate student in the Botany Department of the University of Tennessee and employed by the Agricultural Experiment Station, suffered a fatal heart attack December 31, 1960. Mr. Chopra had received a Master's degree from Lucknow, India.