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THE B-VITAMIN REQUIREMENTS OF THE PROPIONIBACTERIA

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The nutritional requirements of the Propionibacteria have long been regarded as complex. It has been shown that amino acids, although beneficial, are not essential (Fromageot and Loroux, 1936; Tatum, Wood and Peterson, 1936a; Wood, Anderson and Werkman, 1938). They may be replaced by $(NH_4)_2SO_4$, but growth of many species is seriously impaired upon such a medium. Thiamin has been reported by Tatum, Wood and Peterson (1936b) as stimulatory to certain species. However, Silverman and Werkman (1939) found that cultures could be "trained" to grow vigorously in its absence. Riboflavin has been reported by Wood, Anderson and Werkman (1938) as stimulatory, but it can apparently be dispensed with (Krauskopf, Snell and Peterson, 1939). Pantothenic acid has been shown by Krauskopf, Snell and Peterson (1939) to be required by several species. No success in the culturing of any of these organisms on a completely synthetic medium has been reported. Perhaps the nearest approach was that of Wood, Tatum and Peterson (1937), who succeeded in growing a number of species in a synthetic $(NH_4)_2SO_4$ medium supplemented with an ether extract of an aqueous yeast extract. Growth was rather meager, however, and addition of hydrolyzed casein was necessary for sub-culturing.

It was the purpose of this investigation to ascertain which, if any, of the Propionibacteria could be grown on an essentially synthetic medium containing all of the known B-vitamins; and, if growth were obtained, to show which of the vitamins were essential for growth. The organisms investigated were the following: *Propionibacterium jensenii* (1), *P. jensenii* (29), *P. pentosaceum* (4), *P. thonii* (15), *P. rubrum* (19), *P. peterssonnii* (20), and *P. technicum* (22), all derived from Van Niel's strains (1928); and *P. arabinosum*, and *P. zeae*, derived from Hitchner's (1932) original strains.

EXPERIMENTAL

Medium

The basal medium used throughout the investigation had the following composition:

Glucose	10.0 g.
Acid hydrolyzed, charcoal treated, vitamin-free casein	5.0 g.
$NaC_2H_3O_2 \cdot 3H_2O$	8.0 g.
Cystine	0.1 g.
Tryptophane	0.025 g.
Adenine	0.01 g.
Guanine	0.01 g.

Uracil	0.01 g.
Xanthin	0.01 g.
K_2HPO_4	0.25 g.
KH_2PO_4	0.25 g.
$MgSO_4 \cdot 7H_2O$	0.10 g.
NaCl	0.005 g.
$FeSO_4$	0.005 g.
$MnSO_4 \cdot 4H_2O$	0.005 g.
Water to make	one liter
pH 6.0	

The casein hydrolysate was prepared by refluxing 100 g. of Labco "Vitamin-Free" casein with one liter of constant boiling HCl for 8–10 hours. The HCl was then removed by repeated vacuum distillation, and the pH adjusted to 3.0. 10 g. of Darco G-60 decolorizing charcoal were then added and the mixture shaken for fifteen minutes. The charcoal was then removed by filtration and the solution preserved under toluene.

The vitamins investigated and the amounts in which they were used are as follows:

Nicotinic acid	1.0 γ/culture
Pantothenic acid	1.0 γ/culture
Riboflavin	1.0 γ/culture
Thiamin	1.0 γ/culture
Pyridoxin	1.0 γ/culture
Inositol	1.0 γ/culture
p-Aminobenzoic acid	1.0 γ/culture
Biotin	0.1 γ/culture
Folic acid	1.0 mg. unit (described below)/culture

These amounts were thought to be sufficient in the light of the requirements of other organisms. All of the vitamins employed were crystalline products with the exception of folic acid, which was a concentrate of potency 3,000. The amount of folic acid added per culture was equivalent to one milligram of Liver Fraction B (Wilson).

Procedure

Inoculum tubes containing 10 ml. of the basal medium plus 1 mg. of Difco yeast extract were inoculated from yeast-extract glucose stabs and incubated forty-eight hours at 33°C. The cells were centrifuged out, the supernatant medium decanted, sterile saline added, and the cells suspended by shaking. They were again centrifuged out and resuspended in sterile saline. It was hoped by this washing process to reduce to a minimum any carryover from the yeast-extract inoculum medium. The suspensions of the various strains were diluted to an approximately uniform, slight turbidity, and 1 ml. of this suspension added to 150 ml. of double-strength, sterile, basal medium. A 5 ml. amount of this inoculated medium was added to each of a series of tubes containing all of the B-vitamins but one, and also control tubes containing all of the vitamins,

none of the vitamins, 1 mg. yeast extract, and 10 mg. yeast extract—all of these dissolved in 5 ml. of water, so that the final culture had a volume of 10 ml. By thus inoculating the medium before distribution to the various tubes it was hoped to obtain uniform inoculation, personal experience and the work of Chaix and Fromageot (1935) having indicated that the growth of the Propionibacteria is very sensitive to the size of the inoculum. All of the above operations were carried out aseptically. The cultures were incubated for three and one-half days at 33°C. Growth was then estimated turbidimetrically with the aid of the thermoelectric turbidimeter described by Williams, McAlister and Roehm (1929).

RESULTS

The results of the preliminary experiment described above are shown in table 1. The amount of growth is indicated by the galvanometer reading, a reading of zero corresponding to pure water and a reading of 100 indicating complete opacity. It must be borne in mind that the galvanometer response is not linear. Thus the difference between a reading of 80 and 90 indicates a considerably greater increment of growth than the difference between 10 and 20.

These results would seem to indicate that *P. pentosaceum*, *P. arabinosum*, *P. zeae*, and probably *P. rubrum*, require factors other than the eight B-vitamins tested. The remaining strains grow optimally, or nearly so, in the presence of these eight B-vitamins. The individual vitamins required by each organism are also indicated. Confirmation of these indications will be considered separately for each strain studied.

P. jensenii-1: The data in table 1 indicate that this organism requires only pantothenic acid and biotin. This was confirmed by culturing the organism through seven serial transfers on the basal medium supplemented by pantothenic acid and biotin. Transfers (in this and the following cases, unless otherwise indicated) were made at three-day intervals, one drop of the old culture serving as inoculum. Vigorous growth was maintained throughout the subculturing.

P. jensenii-29: Pantothenic acid and biotin are indicated in table 1 as the required factors. The organism was carried through seven serial transfers on the basal medium supplemented by these two vitamins; however, growth was very poor in the sixth and seventh tubes. Much better growth was obtained toward the end of the subculture series by addition of *p*-aminobenzoic acid. Even with this addition, however, growth was sub-optimal.

P. pentosaceum-4: This organism grew only slightly on the basal medium plus all eight vitamins. This slight growth, however, was carried through four serial transfers (on the basal medium plus eight vitamins) and, although still weak, there was more growth in the final subculture than in the original culture. Growth on this medium was slow, six days being allowed between subcultures. Subcultures could not be maintained on the basal medium plus pantothenic acid alone.

P. thonii-15: Pantothenic acid, biotin, and possibly thiamin and/or *p*-aminobenzoic acid are indicated by table 1 as probably required factors for this or-

ganism. It was carried through seven serial transfers on the basal medium plus these four vitamins, abundant growth occurring in all subcultures. With only pantothenic acid and biotin added, however, growth failed on the fourth subculture. Addition of either thiamin or *p*-aminobenzoic acid in addition to pantothenic acid and biotin allowed growth to continue through seven transfers, although growth was somewhat less abundant than when all four vitamins were present.

P. rubrum-19: This organism in the preliminary experiments grew slightly on the completely vitamin-supplemented medium (table 1), but all succeeding attempts to secure growth on this medium were unsuccessful.

TABLE 1

	P. jensenii-1	*P. jensenii*-29	*P. pentosaceum*-4	*P. thonii*-15	*P. rubrum*-19	*P. petersonnii*-20	*P. technicum*-22	*P. arabinosum*	*P. zea*
None	7	51	15	6	4	17	6	3	4
1 mg. yeast extract	89	85	41	89	84	75	85	59	42
10 mg. yeast extract	97	92	99	94	95	91	91	99	96
All vitamins	87	79	17	84	28	65	90	4	4
All vitamins except nicotinic acid	84	82	*	84	23	73	90	*	*
All vitamins except pantothenic acid	7	20	*	7	4	15	9	*	*
All vitamins except riboflavin	87	80	*	86	29	72	89	*	*
All vitamins except thiamin	81	75	*	71	32	67	86	*	*
All vitamins except pyridoxin	87	81	*	85	4	73	91	*	*
All vitamins except inositol	87	81	*	84	49	72	87	*	*
All vitamins except *p*-aminobenzoic acid	84	71	*	77	30	74	88	*	*
All vitamins except biotin	28	49	*	17	7	37	87	*	*
All vitamins except folic acid	84	80	*	85	43	71	91	*	*

* Growth (observed visually) substantially the same as blank with no added vitamins.

P. petersonnii-20: Pantothenic acid and biotin were indicated as the essential factors for this organism. On the basal medium supplemented by these two vitamins, growth failed on the fifth subculture. Growth through seven transfers was permitted by addition of thiamin and *p*-aminobenzoic acid.

P. technicum-22: According to the data of table 1 this organism should require only pantothenic acid. This was confirmed by culturing the organism through seven serial transfers on the basal medium supplemented by pantothenic acid only. Vigorous growth was maintained on this medium.

P. arabinosum: This organism grew only very slightly on the completely vitamin-supplemented medium in the preliminary experiments (table 1). It was found, however, to be subculturable on this complete medium. It was carried through five serial transfers and, as in the case of *P. pentosaceum*, growth

became increasingly abundant with succeeding subcultures. Subcultures could not be maintained with pantothenic acid only as a supplement.

P. zeae: This organism showed no growth on the basal medium supplemented with the eight vitamins.

SUMMARY AND DISCUSSION

Five of the nine Propionibacteria studied in this investigation were found to grow satisfactorily through repeated subculture on a medium, synthetic with the exception of a charcoal-treated "vitamin-free" casein hydrolysate. All of these five required pantothenic acid. This confirms the earlier work of Krauskopf, Snell, and McCoy (1939). Four of the five required biotin. It seems probable that the ether extract of yeast extract used by Wood, Tatum and Peterson (1937) in the culture of Propionibacteria was effective due to its content of pantothenic acid and biotin. Nicotinic acid, riboflavin, pyridoxin, inositol, and folic acid were, under the conditions of this experiment, without stimulatory effect. The role of thiamin and of *p*-aminobenzoic acid is somewhat obscure. For certain of the organisms one or the other or perhaps both are apparently essential for continued subculture. Silverman and Werkman (1939) found that the thiamin requirements of *Propionibacterium pentosaceum* were widely variable depending upon the previous history of the culture. Response to *p*-aminobenzoic acid by *P. thonii* has been observed in the course of this investigation to be quite irregular. On some occasions this organism has shown practically no response (as in table 1) and at other times, under apparently identical conditions, growth would be more than doubled by addition of *p*-aminobenzoic acid. These facts, together with the apparent adaptation of *P. pentosaceum* and *P. arabinosum* to slow growth in an unfavorable medium, appear to support the conclusion reached by Wood, Anderson and Werkman (1938) in reviewing the then known nutritional requirements of the Propionibacteria, namely, that these organisms are endowed with rather remarkable adaptive capacities.

P. pentosaceum, *P. rubrum*, *P. zeae*, and *P. arabinosum* apparently require a factor or factors in addition to the eight B-vitamins considered in this study. This factor (or factors) is present in yeast extract. It should also be noted (table 1) that with the exception of *P. technicum*, all strains are stimulated by yeast extract, over and above the stimulation of the eight B-vitamins. The nature of this stimulation is being investigated.

REFERENCES

CHAIX, P., AND FROMAGEOT, C. 1935 An activator of the metabolism of propionic acid bacteria. Bull. soc. chim. biol., **17**, 874–892.

FROMAGEOT, C., AND LAROUX, P. 1936 Nitrogen nutrition of propionic acid bacteria. Bull. soc. chim. biol., **18**, 797–798.

HITCHNER, E. R. 1932 A cultural study of the propionic acid bacteria. J. Bact., **23**, 40–41.

KRAUSKOPF, E. J., SNELL, E. E., AND MCCOY, E. 1939 Growth factors for bacteria. XI. A survey of the pantothenic acid and riboflavin requirements of various groups of bacteria. Enzymologia, **7**, 327–330.

SILVERMAN, M., AND WERKMAN, C. H. 1939 Adaptation of the propionic acid bacteria to B_1 synthesis including a method of assay. J. Bact., **38**, 25–32.

TATUM, E. L., WOOD, H. G., AND PETERSON, W. H. 1936 Essential growth factors for propionic acid bacteria. II. Nature of Neuberg precipitate, replacement by $(NH_4)_2SO_4$ or certain amino acids. J. Bact., **32**, 167–174.

TATUM, E. L., WOOD, H. G., AND PETERSON, W. H. 1936 Growth factors for bacteria. V. Vitamin B_1, a growth stimulant for propionic acid bacteria. Biochem. J., **30**, 1898–1904.

VAN NIEL, C. B. 1932 *The Propionic Acid Bacteria*, Harlaam.

WILLIAMS, R. J., MCALISTER, E. D., AND ROEHM, R. R. 1929 A rapid and accurate method for determining the quantity of yeast or other microorganisms in a suspension. J. Biol. Chem., **83**, 315–320.

WOOD, H. G., ANDERSON, A. A., AND WERKMAN, C. H. 1938 Nutrition of the propionibacteria. J. Bact., **36**, 201–214.

WOOD, H. G., TATUM, E. L., AND PETERSON, W. H. 1937 Growth factors for bacteria. IV. An acidic ether soluble factor essential for growth of propionic acid bacteria. J. Bact., **33**, 227–242.

NOTES

FURTHER STUDIES ON THE EIJKMAN REACTIONS OF SHIGELLA CULTURES

C. A. STUART AND ROBERT RUSTIGIAN

Biological Laboratory, Brown University, Providence, Rhode Island

Stuart *et al.* (1942) found that 15 cultures of *Shigella paradysenteriae*, with one exception, failed to grow while 17 *S. sonnei*, 4 *S. alkalescens* and one *S. dispar* (madampensis) cultures grew readily and fermented glucose at 45.5°C. Wood *et al.* (1943) found that the ability of *Shigella* species to reduce trimethylamine oxide corresponded to their Eijkman reactions. In view of this correlation it seemed advisable to extend the work on the Eijkman reactions of *Shigella*.

In the present work on 276 cultures a temperature of 45°C., ± 0.1°C., was found more satisfactory than 45.5°C. Inoculations were made from 24-hour broth cultures. Two loopfuls of group I and one loopful of group II species

TABLE 1

GROUP	SHIGELLA SPECIES	CULTURES TESTED	EIJKMAN REACTIONS			
			No growth	Growth	Slight acid	Strong acid
I	*Sh. dysenteriae*	6	6			
	Sh. paradysenteriae	61	61			
	Sh. ambigua	3	3			
	Sh. sp. (Newcastle type)	15	15			
	Sh. equirulis	1	1			
Totals		86	86	0	0	0
II	*Sh. sonnei*	17		1	2	14
	Sh. alkalescens	142		2		140
	Sh. dispar (madampensis)	22				22
	Sh. ceylonensis	9				9
Totals		190	0	3	2	185

(table 1) were inoculated into Difco Eijkman medium base with glucose. All cultures were incubated for 24 hours. Table 1 shows that none of 86 group I cultures grew at 45°C. After 24 hours at 45°C. the cultures were placed at 37°C. Seventy-nine group I cultures, after showing no visible growth for from 12 to 36 hours at 37°C., produced acid while 7 cultures failed to grow. In the previous work one carefully checked *S. paradysenteriae* gave a strong acid reaction at 45.5°C. Unfortunately this culture was not available for the trimethylamine test. All 190 group II cultures tested in the present work grew and 185 or 97.4 per cent produced strong acid from glucose at 45°C.

As pointed out by Neter (1942) *S. alkalescens* is not infrequently mistaken for *S. paradysenteriae*. To a lesser extent a similar condition holds true for *S. sonnei* cultures fermenting lactose much more slowly than the average strain. Diagnostic laboratories without adequate antiserums could use either the Eijkman or the trimethylamine test or both to good advantage with *Shigella* cultures. Considering the large number of cultures tested the single group I, Eijkman positive exception and the single group I, trimethylamine positive exception (Wood *et al.* 1943) detract but little from the practicability of these two tests.

REFERENCES

STUART, C. A., ZIMMERMAN, A., BAKER, M., AND RUSTIGIAN, R. 1942 Eijkman relationships of the coliform and related bacteria. J. Bact., **43**, 557–572.

WOOD, A. J., BAIRD, E. A., AND KEEPING, F. E. 1943 A primary division of *Shigella* based on the trimethylamine test. J. Bact., **46**, 106–107.

A PRIMARY DIVISION OF THE GENUS SHIGELLA BASED ON THE TRIMETHYLAMINE TEST

A. J. WOOD, ELIZABETH A. BAIRD AND FRANCES E. KEEPING

Atlantic Fisheries Experimental Station, Halifax, Canada

A recent survey (Wood and Baird 1943) of the Enterobacteriaceae has revealed that most of the species of this family are able to reduce trimethylamine oxide to trimethylamine. The only exceptions were noted in *Shigella* and *Erwinia*.

TABLE 1

GROUP	SHIGELLA SPECIES	NUMBER OF CULTURES	TRIMETHYLAMINE PRODUCTION	
			Neg.	Pos.
I	*S. dysenteriae*	16	16	
	S. paradysenteriae	87	86	1
	S. ambigua	7	7	
	S. schmitzii	1	1	
	S. sp. (Newcastle type)	5	5	
	S. equirulis	1	1	
Totals		117	116	1
II	*S. sonnei*	22		22
	S. alkalescens	98		98
	S. madampensis (dispar)	19		19
	S. ceylonensis	2		2
Totals		141	0	141

The work with *Shigella* has been extended to additional species and to a larger number of cultures of those species already examined in the hope that the findings might have some taxonomic value.

The results presented in Table I were obtained using a procedure already described (Wood and Baird 1943). They have been confirmed by repetition at three different intervals over a period of three months.

The division into two groups, one positive and the other (except for one *S. paradysenteriae* culture) negative for trimethylamine, is in striking agreement with the results of Stuart and Rustigian (1943) based on the Eijkman reactions. A primary separation of the species of this genus based on the trimethylamine test or on the Eijkman reactions appears to offer some advantage over the present one using mannitol fermentation (Bergey 1939, Glynn and Starkey 1939). As pointed out by Stuart and Rustigian (1943) the Eijkman reactions or the trimethylamine test or a combination of both may be of value to laboratories not equipped with antisera required for differential purposes.

REFERENCES

BERGEY, D. H. 1939 Manual of Determinative Bacteriology. 5th edition. Williams & Wilkins, Baltimore.

GLYNN, J. H., AND STARKEY, D. H. 1939 The cultural and antigenic properties of *Shigella sonnei*. J. Bact., **37**, 315–331.

STUART, C. A., AND RUSTIGIAN, R. 1943 Further studies on the Eijkman reactions of *Shigella* cultures. J. Bact., in press.

WOOD, A. J., AND BAIRD, E. A. 1943 The reduction of trimethylamine oxide by bacteria. I. The *Enterobacteriaceae*. J. Fisheries Research Board Can., in press.

PROCEEDINGS OF LOCAL BRANCHES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

JOINT MEETING OF THE NEW JERSEY, THE EASTERN PENNSYLVANIA, AND THE NEW YORK CITY BRANCHES

PRINCETON, N. J., MAY 15, 1943

NOTE ON ANTIBIOTIC SUBSTANCES ELABORATED BY AN ASPERGILLUS FLAVUS STRAIN AND BY AN UNCLASSIFIED MOLD. *Arthur E. O. Menzel, O. Wintersteiner and Geoffrey Rake,* The Squibb Institute for Medical Research, New Brunswick, N. J.

Aspergillic acid, the antibiotic substance elaborated by a strain of *Aspergillus flavus* (isolated and supplied by Dr. E. C. White) has been prepared by a simple extraction procedure. Preparations from plain tryptone media melted between 80° and 90°C., while preparations obtained from media containing brown sugar invariably melted above 100°.

Pure aspergillic acid melts at 93°; it is optically active ($[\alpha]_D = +14°$). Analysis and molecular weight determination agree with the formula $C_{12}H_{20}O_2N_2$. It possesses a hydroxyl group, which is responsible for its acidic character (pK 5.5); the other oxygen atom could not be derivatized. Its ultraviolet absorption spectrum shows a characteristic maximum at 325 mμ. It can be distilled with steam or *in vacuo* without loss of biological activity, and it is remarkably stable under extreme conditions of acidity and alkalinity.

The high melting entity present in brown-sugar-containing media is a closely related substance (MP 149°, $[\alpha]_D = +42°$) of the formula $C_{12}H_{20}O_3N_2$. Its biological activity is about $\frac{1}{10}$ of the activity of aspergillic acid of MP 93°.

The active substance elaborated by Glister's unclassified mold is unquestionably identical with aspergillic acid MP 93°, although the cultural characteristics of this mold are different from those of *Aspergillus flavus*.

SYNTHESIS OF PYRIDOXINE BY A "PYRIDOXINLESS" X-RAY MUTANT OF NEUROSPORA SITOPHILA. *J. L. Stokes, J. W. Foster and C. R. Woodward, Jr.*, Research Laboratory, Merck & Co., Inc., Rahway, N. J.

A pyridoxine-requiring mutant of *Neurospora sitophila* grew normally in the absence of pyridoxine (vitamin B_6) if the culture medium was buffered with sodium acetate. On adjusting the medium to different pH levels, appreciable growth of the fungus occurred only at pH 5.8 or higher. At these pH values, it is also necessary to supply the mutant strain with ammonium compounds as nitrogen sources, other forms of nitrogen being unsuitable. Under these conditions, the ability to synthesize pyridoxine is restored. Other aspects of the pH-ammonium-nitrogen relationship to pyridoxine synthesis in the mutant and also some genetic implications are discussed.

SOME EVIDENCE ON THE ETIOLOGY OF CANCEROUS PROPERTIES AS EXEMPLIFIED IN PLANT CELLS. *Philip R. White and Armin C. Braun,* Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Tumors characterized by an unrestrained and discoordinate type of growth *in situ* and *in vitro*, by capacity to produce new tumors when transplanted into fresh hosts, and in at least one case by a markedly anaerobic type of respiration, yet free of any infectious agent either bacterial or virus in nature, have now been produced in three different and unrelated hosts: sunflower, tomato and periwinkle, by three different methods, involving inoculation with crown-gall bacteria and subsequent elimination of the infecting organisms. It has been possible to demonstrate the cooperation of some one or more growth-substances of the auxine type in the initiation of the cancerous change responsible for these tumors and to establish the fact that this cancerous change goes to

completion within a few hours and perhaps minutes after establishment of contact between the tumefacient agent and the host cell undergoing change. These observations greatly narrow the field in which we must look for the basis of this change.

AN ANALYSIS OF THE ANTAGONISTIC AND THE SYNERGISTIC ACTION OF ACETONE, ETHYL ALCOHOL, BUTYL ALCOHOL, CHLOROFORM, ETHER, AND URETHANE ON SULFANILAMIDE INHIBITIONS. *Frank H. Johnson, Henry B. Eyring, and Walter Kearns*, Departments of Biology and Chemistry, Princeton University.

According to the temperature, and to the concentrations employed, the sulfanilamide inhibition of luminescence may be greatly increased, greatly decreased or unaffected by the addition of urethane. The data indicate that these two inhibitors, which are known to act directly on the luminescent system, also enter into a loose, reversible combination with each other. The equilibrium constant of the sulfanilamide-urethane mutual adsorption or combination, which can be calculated from the data on luminescence, enables a fairly accurate, quantitative prediction of the effects of a wide range of concentrations of the two inhibitors mixed in various proportions. Furthermore, the theory predicts that, at suitable temperatures and concentrations, antagonism and synergism, respectively, may be expected in the inhibition of luminescence by sulfanilamide plus ether, alcohols, chloroform, acetone, and certain other substances. Data from experiments have amply verified these predictions. Although luminescence lends itself to more direct and precise analysis than growth, experiments on the growth of several species of bacteria indicate that fundamentally the same effects are obtained. The phenomenon of loose combination of inhibitors with each other, as well as with a common protein, may also be significant in a variety of circumstances involving stimulation by low concentrations, drug antagonisms, drug fastness, *et cetera*.

THE ACTION OF AN ANTIBIOTIC SUBSTANCE (PENATIN) ON BACTERIOPHAGE. *Thomas F. Anderson*, The Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia, Pa.

In the presence of 0.1% glucose, 0.1 mg. of penatin per ml. reduces the concentration of plaque-forming particles of anti-coli phage to $\frac{1}{10^{6.5}}$ of its original value in 18 hours at room temperature while 0.001 mg. of penatin per ml. reduces the concentration to $\frac{1}{10}$ the original value in the same period. The penatin-inactivated phage particles retain their original morphology as seen in the electron microscope as well as their ability to adhere to susceptible bacteria. The penatin-inactivated virus suspension is also capable of inhibiting colony-formation of bacteria which are susceptible to lysis by the normal virus. This inhibitor of colony-formation is absorbed by the bacteria and the reaction may well be analogous to that observed recently by Luria and Delbrück in which phage suspensions partially inactivated by ultra-violet light were shown to have a similar effect. The significance of these findings in relation to the nature of viruses is discussed.

INCREASED INCIDENCE OF VIRUS INCLUSION BODIES IN HUMAN THROATS. *Jean Broadhurst, Estelle MacLean and Inez Taylor*, Teachers College, Columbia University, New York City.

Examination of another series of throat specimens for the inclusion bodies reported earlier by our laboratory was suggested by the prevalence of respiratory affections in our area this winter.

Throat smears of 224 unselected students in attendance in college and nursing classes between January 17 and March 15, 1943, were positive for these inclusion bodies in 35 to 87 per cent of the four groups examined or in 65 per cent of the whole number. This is a marked increase over the 8 per cent reported for 250-odd students in 1936. The positive 1943 specimens showed also a greater number of affected cells—18 per cent of the specimens being heavily affected in contrast to less than 1 per cent in 1936.

Incidentally, the Bond-Mann stain used for identifying the Negri bodies of the rabies virus gives the same differential (red) stain for these throat inclusion bodies; and in the

larger bodies, especially in specimens from long-period carriers, the stippled or composite character of these bodies is clearly observable.

PREPAREDNESS FOR DEFENSE AGAINST INFLUENZA. *Ward J. MacNeal and Ernestine R. Parker*, Department of Bacteriology, New York Post-Graduate Medical School and Hospital (Columbia University).

Influenza appears to be caused by a readily transmissible virus which develops in the superficial respiratory mucous membranes, predisposing them to penetrating bacterial invasion. Fatigue, intoxication, inhalation of dust and exposure to inclement weather increase susceptibility. Crowded quarters encourage mass infection sufficient to overcome natural resistance. In ferrets and mice, air-borne transmission is related to quantity of virus.

Brief immunity follows recovery in ferrets and the mucous membrane again becomes susceptible while the blood still contains neutralizing antibodies. Vaccination promises only relative protection. Where facilities for air-conditioning are available the virus may be destroyed by ultraviolet radiation or by minute quantities of glycols.

We have found that the virus, dried in mucin on a glazed surface, may remain potent for 45 days and, dried on the soap-free skin of the hand, for at least forty minutes. It is quickly inactivated by soap and by lysol. Commonly used mild antiseptics, as for example liquor antisepticus, inactivate the influenza virus in saliva in 30 seconds. Hence the use of these agents in toilet of the hands, face, mouth, nose and throat is recommended.

TEST OF ANTI-DYSENTERY AGENTS IN EMBRYONATED EGGS. *Ward J. MacNeal, Anne Blevins and Marcello Pacis*, Department of Bacteriology, New York Post-Graduate Medical School and Hospital (Columbia University).

Embryonated eggs inoculated with decimal dilutions of dysentery cultures were treated by the simultaneous or sometimes subsequent injection of therapeutic agents, including bacteriophage, sulamyd, sulfathiazole, sulfaguanidine and control broth. The eggs were candled daily and the dead ones examined and cultured promptly. The chicks which hatched were sacrificed and cultured. Many hundreds of eggs have been used. Only a general preliminary statement can now be given.

In the earlier experiments with Sonne strains the total survivals four days after inoculation were as follows: bacteriophage 60 per cent, sulamyd 55 per cent, sulfathiazole 49 per cent, sulfaguanidine 38 per cent and control broth 19 per cent. Survivals to maturity within the shell twelve days after inoculation were in percentages, respectively, as follows: 48, 46, 44, 19, 10. Live chicks hatched were, respectively, 28, 24, 35 (sulfathiazole), 11 and 2 per cent.

As a rule cultures failed to recover bacteria but did recover phage from the phage-treated specimens and cultures of those treated with the sulfonamides frequently recovered the bacteria even when the chicks actually hatched. Further studies with other dysentery cultures have given somewhat similar results.