the murine encephalomyelitis (MM virus), Eastern equine encephalomyelitis and rabies viruses were antigenic but no longer infectious to mice following treatment with 0.1% BPL. A greater concentration of BPL was required for inactivation of mouse brain suspensions of these viruses.

By using Newcastle disease virus and BPL the alteration of infectivity and antigenicity of the virus could be measured in the chicken, which is the natural host for the virus. The absence of infectivity with retention of high antigenicity indicated that BPL is ideal for manufacture of certain viral vaccines. This is especially true when viruses propagated in chicken eggs may become contaminated with other viral agents.

Summary. 1. A vaccine made by treating Newcastle disease virus wtih 0.025% beta-propiolactone was found not to be infectious to the chicken, its natural host. 2. The sera from chickens receiving the vaccine were found to contain protective antibodies 16 days following vaccination. 3. Paired sera collected before and after vaccination showed a marked rise in hemagglutination inhibition and neutralizing antibodies.

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Growth of *Micrococcus lysodeikticus* as Substrate for Lysozyme.*† (22241)

GERALD LITWACK AND DAVID PRAMER. (Introduced by V. Groupé)

Departments of Agricultural Biochemistry and Agricultural Microbiology, Rutgers University, State University of N. J., New Brunswick.

Need for a procedure whereby large quantities of cells of *Micrococcus lysodeikticus* can be prepared conveniently for use as substrate for lysozyme, was encountered in studies of lytic enzymes from mammalian tissues(1). Present methods for production of *M. lysodeikticus* depend mainly on surface culture on agar media(2-6). Although a number of reports describe growth on liquid media(7-14), they are concerned primarily with nutritional requirements of the organism or pertain to mass cultivation without regard to lysability.

The present paper describes a simple procedure for production of cells of *M. lysodeikticus* in submerged culture. The influence of certain nutrients on growth and lysability of the cells is reported.

Methods and materials. M. lysodeikticus, strain No. 19 of the collection of the Institute of Microbiology, Rutgers University, was used throughout. All inoculations were made with 24 to 36 hour old agar cultures suspended in sterile, distilled water. Growth studies were carried out in test tubes (19 x 150 mm) containing 10 ml of medium. The tubes were set in a rack mounted on a rotary shaker and incubated at 28°C with agitation. Growth measurements were made with a Coleman Junior Spectrophotometer at 635 mμ. When quantities of cells were required, 250 ml Erlenmeyer flasks containing 100 ml of media were inoculated and incubated at 28°C on a rotary shaker. Method of preparation of dried cells and assay of lysozyme activity have been described previously(1). Crystalline egg white lysozyme was purchased from Worthington Biochemical Corp. The peptones used were: Lactalysate, Trypticase, Gelysate,

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Thiotone—Baltimore Biological Laboratories, Md.; N-Z Amine—Sheffield Farms, Inc., New York City; and Bacto-peptone, Bacto-tryptose, Neo-peptone, Proteose-peptone and Protone—Difco Laboratories, Detroit, Mich.

Results. M. lysodeikticus was cultivated on a medium containing yeast extract, 0.1%; glucose, 0.5%; NaCl 0.5% and Bacto-peptone, 0.5 or 1.0% at pH of 7.2 and on similar media with various components withdrawn singly to determine essentiality for growth. Development in the complete nutrient medium was satisfactory; however, by doubling the peptone content and omitting yeast extract and glucose, growth was even better. Omission of yeast extract from a medium with glucose resulted in definite inhibition of growth and may reflect a need for biotin(12). The most rapid development was obtained on a broth consisting of 1% peptone and 0.5% NaCl. Dried cell preparations from each medium were tested as substrates for lysozyme using 5, 10, 20 and 100 μg of crystalline enzyme in a 5.5 ml system at 25°C. Since linearity between enzyme concentration and activity begins to fall off above 20 μg of enzyme(1,15, 16), the activity on the linear part of the curve as well as non-linear portion may be compared. The most susceptible cells in respect to linear and extra-linear concentrations of enzyme were those from the medium which contained 1% Bacto-peptone and 0.5% NaCl. This medium also gave the best growth.

The results of tests when 10 different peptones were substituted singly for Bacto-peptone are shown by Fig. 1. No growth was obtained on Protone or Neo-peptone. Although there was greatest development with Bacto-tryptose, growth was preceded by a lag period of approximately 25 hours. Growth on Bacto-peptone showed the shortest lag. Cells from each of the 10 peptones were tested for susceptibility to lysozyme and rate of lysis at linear and non-linear concentrations of enzyme was greatest in cells which grew optimally. With cells from media supporting poor growth (curves 5 through 8), this relationship was not apparent. Susceptibility of cells from the medium containing Proteose-peptone was lower with high concentrations of the enzyme. This may reflect a decreased availability of sodium ion(16). Although rate and extent of growth on peptones 5 through 8 differed greatly, the cells were comparable with regard to lysability. Gelysate, for example, produced cells whose lysis was comparable to that of cells grown on Lactalysate which produced 2 times as much growth. Of the 10 peptones investigated, Bacto-tryptose‡ and Bacto-peptone‡ were chosen for further study.

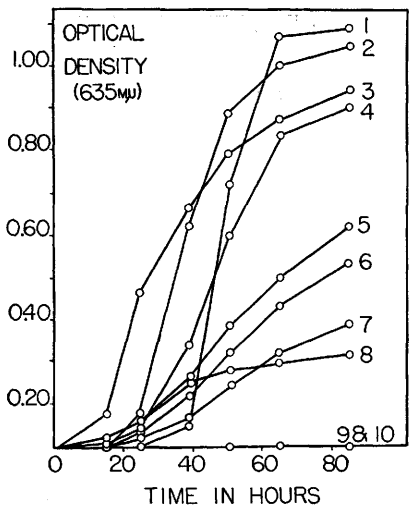

FIG. 1. Comparison of growth of *Micrococcus lysodeikticus* on 10 nitrogen sources. Curve 1—Bacto-tryptose, 2—Thiotone, 3—Bacto-peptone, 4—Proteose-peptone, 5—Lactalysate, 6—Trypticase, 7—N-Z Amine, 8—Gelysate, 9—Protone, 10—Neopeptone.

After demonstrating that the lag period for growth in Bacto-tryptose could not be eliminated by prior adaptation of the cells in this medium and that NaCl did affect the lag, cells were maintained on Bacto-tryptose agar and Bacto-peptone agar. These grew equally well when transferred to Bacto-peptone broth, however, addition of NaCl increased both extent and rate of development. Cells grown on Bacto-peptone agar and inoculated into Bacto-tryptose broth grew at a reduced rate compared to cells that had been maintained

‡ The composition of Bacto-peptone (Difco) and Bacto-tryptose (Difco) are presented in the Difco manual, Difco Laboratories, Inc. Detroit, Mich., 1953, pp. 256 and 262.

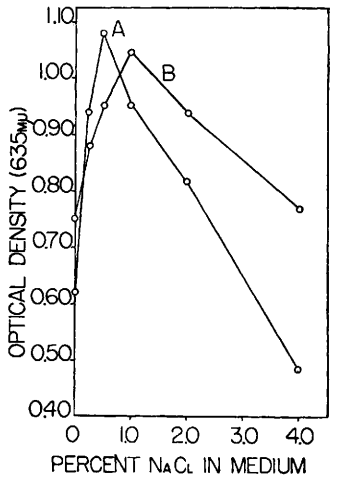

FIG. 2. Optimal concentration of NaCl for growth of *Micrococcus lysodeikticus* in Bacto-peptone and Bacto-tryptose media. Curve A, growth on Bacto-peptone; Curve B, growth on Bacto-tryptose.

on Bacto-tryptose agar. Cells maintained on Bacto-tryptose agar continued to show a prolonged lag period in Bacto-tryptose broth. This lag period was reduced markedly by addition of 0.5% NaCl to the medium. Cells that had been maintained on Bacto-peptone agar were influenced similarly by addition of NaCl.

The influence of concentrations of NaCl on growth of *M. lysodeikticus* in 64 hours is shown by Fig. 2. The optimal concentration of NaCl for growth was 0.5% with Bacto-peptone and 1% with Bacto-tryptose, even though Bacto-tryptose contains twice as much sodium ion and 10 times as much chloride ion as Bacto-peptone.‡

Tests of the activity of lysozyme on these cells indicated that susceptibility of cells grown on Bacto-peptone was greater than that of cells grown on Bacto-tryptose (Fig. 3). Bacto-peptone and Thiotone produced cells on which enzyme activity appeared to parallel growth. This was not the case for the other peptones.

Growth of *M. lysodeikticus* in Bacto-peptone broth was inhibited completely at pH 4 and 5. Although growth was most rapid at pH 7.5, it was comparable at pH 6 to 9 after 87 hours. Beers(14) also observed a high pH requirement for growth of this organism.

From the foregoing studies, the following medium is suggested for production of *M. lysodeikticus*: 1% Bacto-peptone and 0.5% NaCl in water at pH 7.5. Growth on this medium was compared with that on media recommended by Smolelis and Hartsell(6) and Saz and Krampitz(11). Agar was left out of the medium of Smolelis and Hartsell. All other components were equivalent. The inoculum was obtained from a Bacto-peptone-NaCl agar. The most rapid rate of growth was obtained on the recommended medium. There was considerable lag when *M. lysodeikticus* was grown in either of the other 2 media. The amount of growth was greatest on the medium of Smolelis and Hartsell. This is understandable since it contains as much as 6.5% available organic matter, as compared to 1.8% in the medium of Saz and Krampitz,

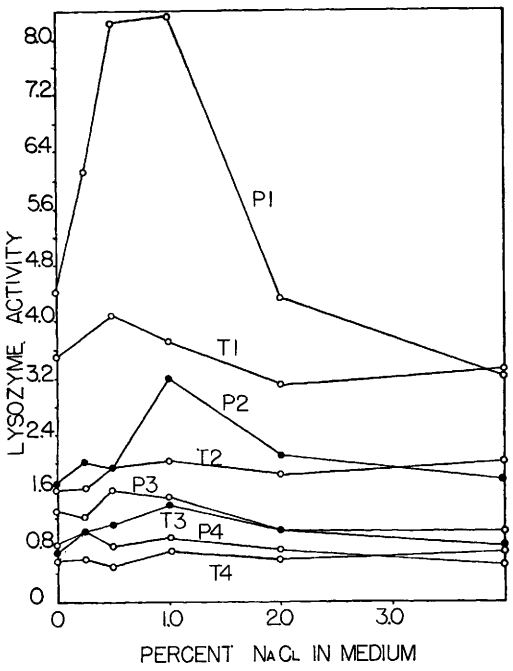

FIG. 3. Activity of crystalline lysozyme on cells grown on Bacto-peptone and Bacto-tryptose media containing various concentrations of NaCl.

Curve P1, cells from Bacto-peptone medium with 100 μg lysozyme.

T1	Bacto-tryptose	100
P2	Bacto-peptone	20
T2	Bacto-tryptose	20
P3	Bacto-peptone	10
T3	Bacto-tryptose	10
P4	Bacto-peptone	5
T4	Bacto-tryptose	5

TABLE I. Comparison of Lysozyme Activity on 3 Cell Preparations.

Medium used	Conc. of enzyme (μg)	Lysozyme activity* Δ % transmission from 30-60 sec
S and H†	5	.5
	10	.7
	20	1.5
	100	3.5
S and K‡	5	.7
	10	.7
	20	1.7
	100	5.0
Peptone-salt§	5	.8
	10	1.0
	20	2.0
	100	7.9

* Duplicate determinations.
† Smolelis and Hartsell medium without agar(6).
‡ Saz and Krampitz(11).
§ The recommended peptone-salt medium.

and only 1% in the recommended medium. It is likely that the lag in growth on the medium of Smolelis and Hartsell could be partially eliminated by adjustment of pH. Likewise, there might be more rapid development in the medium of Saz and Krampitz by addition of NaCl and adjustment of pH.

Table I shows that the lysability of cells from the recommended medium is greater than that of cells from the other 2 media.

Since Wilcox and Daniel(16) have indicated that a decrease from linearity in enzyme activity at high concentrations of lysozyme may be partially overcome by addition of sodium ions to either buffer or enzyme solutions, the influence of NaCl added to suspensions of cells which had been grown in media containing from 0 to 4% NaCl, was tested. The results are shown in Fig. 4. Doubling and quadrupling concentrations of NaCl in the suspension of cells grown in Bacto-peptone containing 0 to 1% NaCl caused a progressive inhibition of enzyme activity, but did not influence the lysability of cells which were grown in broth containing 2 to 4% NaCl. If, at a concentration of 100 μg lysozyme/0.5 ml, sodium ion is required to increase enzyme activity(16) then increasing the concentration of NaCl in the suspension should influence lysability of cells grown in the absence of NaCl. This was not the

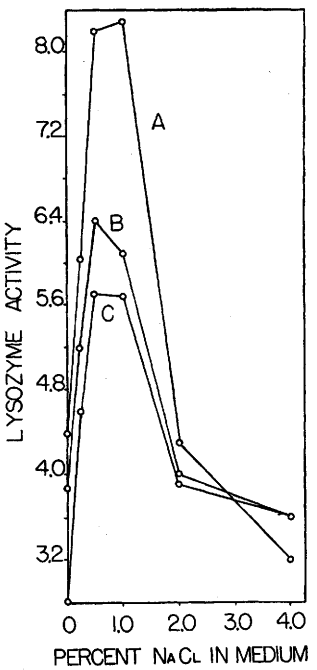

FIG. 4. Effect of adding NaCl to assay system on activity of crystalline lysozyme (100 μg). Cells grown in media containing varying amounts of NaCl. Curve A, .0171 M NaCl in assay system; Curve B, .0342 M; Curve C, .0684 M.

case. It is possible, therefore, that NaCl plays a structural role either in adsorption of the enzyme to the cell wall or in production of cellular substrate, or both; and consequently NaCl is more efficiently utilized if it is present during growth of the organisms.

Summary. (1) A broth consisting of 1% Bacto-peptone and 0.5% NaCl at pH 7.5 is recommended for growth of *M. lysodeikticus* as substrate for lysozyme. (2) Cells grown and prepared by the recommended procedure were more susceptible to lysozyme than cells produced by other methods. (3) The rate of lysis of cells of *M. lysodeikticus* was affected by the concentration of NaCl used in the culture medium.

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Effect of Ergotamine on Milk-Ejection in Lactating Rat.* (22242)

CLARK E. GROSVENOR. (Introduced by C. K. Weichert.)
Zoological Laboratory, University of Cincinnati.†

It has been generally accepted(1-5) that the oxytocic principle from the posterior pituitary gland is the humoral agent responsible for "let-down" of milk from mammary gland. It has further been demonstrated(6) that adrenalin, fright, and other stimuli, interfere with this effect, whereas acetylcholine, when injected, acts similarly to "let-down" hormone(7-8). Evidence is presented in this paper that ergotamine also interferes with normal "let-down" of milk in lactating rats.

Materials and methods. Docile lactating rats, each with its first litter and weighing 230-260 g. were housed in individual cages and given Purina Dog Chow and water *ad libitum*. Seasonal greens were fed twice weekly. Each litter was reduced to 6 young shortly after birth and, whenever possible, these were equally distributed as to sex. Rats with less than 6 young were discarded. Ten control and 10 experimental animals each with litter of 6 were used. Ergotamine tartrate‡ was injected subcutaneously into lactating mothers 3 times a day at 6-hour intervals, from ninth through twelfth days postpartum, at dosage of 3 mg/kg/day. Half of the controls received comparable injections of physiological saline to test whether or not frequency of injection and/or amount of fluid injected would affect the pattern of maternal behavior. The remaining controls were uninjected. One and one-half hours following the second of 3 injections of the day, each litter was weighed, then isolated from the mother for 6 hours during each day of injection period. The young were kept warm to prevent any undesirable effects from exposure. In some preliminary experiments, stomachs of several litters, killed at end of 6-hour isolation period, contained no milk. One and one-half hours after third injection of the day, each litter was again weighed to determine weight loss during the 6-hour interval. The young were then placed back with the mother and allowed to suckle for exactly 20 minutes. Length of time before each mother began nursing was recorded. The litter was then removed and weighed to determine amount of milk obtained during the 20-minute interval. (Table

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† Present address: Biology Department, University of Tennessee, Martin.

‡ Kindly supplied by Sandoz Chemicals, N. Y.