the body by fostering the degradation of thyroxine and/or its breakdown products is not an indispensable role under normal circumstances because the salivary glands can be removed without causing a statistically significant change in thyroxine metabolism as revealed by the BMR and thyroxine secretion rate.

Perhaps after salivariectomy, control of thyroxine level through degradation is assumed by other tissues at a slower but nonethe-less equally effective rate. The kidney and stomach have been reported to possess tyrosine iodinase in low concentrations(2). Evidence is accumulating to show that many tissues can deiodinate thyroxine with formation of triiodothyronine as for example the kidney in which the process appears to be controlled by an adaptive enzymatic mechanism(3).

On the other hand, an important point in determining the comprehensive role of the salivary glands in thyroxine metabolism still remains to be demonstrated; *i.e.*, their participation in the degradation of thyroxine itself to an extent relatively greater than that of other peripheral tissues which are metabolically stimulated by thyroxine and thus "utilize" thyroxine in their normal metabolism. If the salivary glands do not destroy thyroxine to a relatively greater extent than other tissues do, it is evident that a salivariectomy will probably not result in a recognizable change in BMR or thyroxine secretion rate.

Obviously more experimental data need to be available before an adequate appraisal of the normal role of the salivary glands in thyroxine metabolism can be made, however, in view of the present finding, it appears that this role is nonessential or adaptive.

Summary. Salivariectomy performed in young adult female rats produced no statistically significant effect on BMR or thyroxine secretion rate. It is concluded that rat salivary glands play a nonessential or adaptive role in thyroxine production and metabolism.

NOTE: After this paper was submitted for publication, two other papers(5,6) reported similar conclusions reached by different methods.

The technical assistance of Miss Gwendolyn Slover is gratefully acknowledged.

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Received October 24, 1955. P.S.E.B.M., 1956, v91.

Serological Response in Chickens to Beta-Propiolactone Treated Newcastle Disease Virus. (22240)

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In a previous study(1) it was demonstrated that Newcastle disease virus (NDV) was rendered noninfectious for chickens by treatment with 0.025% beta-propiolactone (BPL). The vaccinated birds developed no symptoms of Newcastle disease while 97.4% unvaccinated ones developed symptoms or died from the disease. Hemagglutination inhibition tests performed on paired sera from the vaccinated group showed a significant rise in titer.

The present communication presents additional information about the antibody response in the vaccinated group.

Materials and methods. White Leghorn chickens were used. All birds were from the same apparently disease-free flock. Beta-propiolactone was supplied by Dr. T. L. Gresham, B. F. Goodrich Research Center, Brecks-

TABLE I. Serological Response in Chickens to Newcastle Disease Virus Vaccine.

No. sera tested	Amt vaccine, ml	HI titer pre-vac,* avg	Post-vac, 16 day HI titer avg	No. sera tested	Pre-vac, serum index avg	Post-vac, serum index avg
13	.5	1:14	1:500	9	1.48	3.80
10	1.0	1:12	1:520	6	1.02	3.83
10	1.5	1:13	1:690	6	1.00	3.75
10	2.0	1:18	1:832	6	1.38	4.93
43	Avg	1:14	1:635	27	1.22	4.07

* vac = vaccination.

ville, Ohio. To facilitate dispensing of small volumes of BPL used, the BPL was diluted 1:10 with cold, sterile, distilled water and per cent concentration was based on undiluted BPL. In preliminary experiments, it was determined that treatment of NDV-infected allantoic fluid with 0.025% BPL for 2 hours at 37°C, destroyed infectivity of the virus for embryonated chicken eggs. It was also determined that heating NDV in allantoic fluid without added BPL for 2 hours at 37°C did not appreciably inactivate virus infectivity. Several serial embryonating egg passages were made with the virus by the allantoic route, prior to preparing a pooled allantoic fluid-virus suspension. The stock virus possessed a $10^{-9.5}$ LD_{50} titer when tested by injecting 0.1 ml of virus into embryonating eggs. All chickens in the experiment were bled from the heart to obtain pre-vaccination blood samples and stored in a frozen state. Thirty-nine birds were not vaccinated, and 43 birds were divided into 4 groups of 13, 10, 10 and 10 and received 0.5, 1.0, 1.5 and 2 ml vaccine respectively. On the 16th day following vaccination, the birds were again bled from the heart and the serum held in a frozen state until pre- and post-vaccination sera could be tested at the same time. The vaccinated and control birds were challenged by injecting 0.2 ml of virus intramuscularly. No evidence of infection occurred in the vaccinated group. Thirty-eight non-vaccinated controls died from the disease. *Standard hemagglutination inhibition* (HI) tests were performed on all paired sera. Incubation took place at room temperature and results of the tests were recorded at 15, 30 and 45 minutes. The hemagglutinating inhibition titers in 0.25 ml of undiluted serum were computed as the reciprocal of virus titer divided by the reciprocal of serum titer x dilution of the serum. *Serum neutralization* tests were performed in 8-10-day-old embryonating chicken eggs. Serial 10-fold dilutions of virus were prepared in nutrient broth. Equal parts of undiluted serum to be tested were mixed with each virus dilution. After standing 30 minutes, 0.1 ml of each virus-serum mixture was injected into the allantoic sac of 5 eggs. Viral infectivity was determined by mortality of the embryos. The *serum neutralizing titers* were the difference between the logarithms of titers of virus incubated with and without antiserum.

Results. The serological responses in chickens receiving the vaccine are shown in Table I. The HI titer rose gradually in the serum samples as the amount of vaccine given was increased. The average pre-vaccination HI titer was 1:14 which increased to average 1:635 sixteen days after vaccination. This represents a 45-fold increase in HI antibody titer.

The serum neutralizing antibodies against NDV also showed an increase in the post-vaccination serum samples. The average serum neutralizing index before vaccination was 1.22 in the 27 sera tested. The average index, taken on the 16th day after vaccination, was 4.07 or nearly a 4-fold increase over the average pre-vaccination index.

Discussion. Beta-propiolactone appears to be an ideal viricide with which to destroy the infectivity of Newcastle disease virus. Twenty-five thousandths per cent BPL was sufficient to destroy the infectivity of the virus and still leaving it capable of stimulating antibody to a high level to prevent disease. Lo Grippo and Hartman(2) have shown that

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.

Journal Article 1844, Department of Microbiology and Public Health, Michigan Agri. Exp. Station

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Received November 7, 1955. P.S.E.B.M., 1956, v91.

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

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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,

* Paper of the Journal Series, N. J. Agri. Exp. Station, Rutgers University, State University of N. J., Departments of Agricultural Biochemistry and Agricultural Microbiology, New Brunswick.

† This work was presented in part before the Theobold Smith Society of the Soc. of Am. Bact. at Princeton University, Princeton, N. J., June 2, 1955.