A STUDY IN ACTIVE IMMUNIZATION AGAINST FOWL CHOLERA.*

KANKICHIRO SAKAMOTO.

(From the Veterinary Laboratory, Ministry of Agriculture and Commerce, Nishigahara, Tokyo, Prof. N. Nitta, Director.)

The purpose of this work has been to study comparatively the antigenic properties of vaccines prepared after various methods from the organism of fowl cholera and organisms of the hemorrhagic septicemia group, and to find out practicable and effectual ones against fowl cholera.

Throughout my experiments pigeons were used as experimental animals, which received single subcutaneous inoculation in the breast. Intervals between vaccination and control inoculation were about two weeks. The minimum lethal dose of the fowl cholera (agar) culture used for control inoculation was $1/10000000$ loopful.

1. Attenuated Organism of Fowl Cholera.

The organism of fowl cholera was cultivated on agar slants and incubated for ten days at $42°-43°C$. The growth was suspended in physiological salt solution in proportion of one tube : 5 c.c. and four pigeons received a subcutaneous inoculation with doses of $0.05-0.5$ c.c. The results obtained were unsatisfactory.

2. Organism of Fowl Cholera Destroyed by Shaking.

A suspension of the growth of twenty-four-hour agar cultures of the organism (three tubes : 10 c.c. physiological salt solution) was shaked for about twenty-four hours. Having ascertained the complete destruction of the organism by means of microscopic examination and cultivation four pigeons were inoculated with doses of $0.1$ to $0.5$ c.c. In the results of the control inoculation there were no remarkable differences between treated and untreated animals.

* Received for publication, August 12, 1922.
3. **Dried Organism of Fowl Cholera.**

The growth of twenty-four-hour agar cultures of the highly virulent organism was smeared on sterilized slides and placed in a desiccator for forty days in a dark room. The dried organism was rubbed off into a mortar and carefully ground with physiological salt solution (one slide: 3 c.c.). The treatment with the suspension gave no good results.

4. **Organism of Fowl Cholera Killed by Heating.**

A suspension of the organism in physiological salt solution (one tube: 5 c.c.) was heated in a water bath for one hour at 60°C. After ascertaining the complete death of the organism by means of cultivation in broth the suspension was inoculated into four pigeons in doses of 0.1–0.5 c.c. The results of this experiment were also unsatisfactory.

5. **Organism of Fowl Cholera Killed with Disinfectants.**

To a suspension prepared in the same manner as described above was added a 2.5% solution of iodine in proportion of 1:0.5; after forty-eight hours eight pigeons were treated with doses of 0.1–1.0 c.c. of the iodized suspension. The animals which received the largest quantity (0.5 c.c.) of the suspension resisted the inoculation of tenfold minimum lethal dose of the virulent organism.

The results of the experiment with a suspension of the organism treated with glycerine (1 c.c.: 6 c.c.) and incubated for ten days were nearly the same.

6. **Aggressine.**

From a rabbit dead from intraperitoneal injection of a small dose of agar culture of the organism the pleural exsudate was collected into a test tube and heated for three hours at 44°C., then passed through a Berkefeld filter and added with carbolic acid in a proportion of 0.5 per cent. After twenty-four hours the aggressine was inoculated into seven pigeons with doses of 0.1–1.0 c.c. The animals treated with 0.5 c.c. or more of the
aggressine resisted inoculation of a minimum lethal dose of the virulent organism.

7. Condensed Broth Culture of the Organism of Fowl Cholera.

A eleven-day broth culture of the organism was evaporated and condensed to one-tenth original volume in a vaccum for four hours at 45°-50°C. Four pigeons were inoculated with doses of 0.1-3 c.c. of the condensed culture. The animals treated with 1 c.c. or more safely stood a tenfold minimum lethal dose of the virulent organism.

8. Polyvalent Vaccine.

The above-mentioned vaccines were prepared from the organism of fowl cholera alone. Thus an attempt has been made to prepare a polyvalent vaccine from four kinds of the organisms of septicemia hemorrhagia group (fowl, rabbit, swine and guinea-pig). Each of four kinds of the organisms was cultivated on agar medium, from which was made a suspension in physiological salt solution (one tube to 1.5 c.c). Four different suspensions thus prepared were then mixed in equal quantities and heated in a water bath for one hour at 60°C. and inoculated in doses of 0.1-1 c.c. The animals treated with 1 c.c. resisted inoculation of a hundredfold minimum lethal dose of the virulent organism of fowl cholera.


Nearly all the vaccines mentioned above produced an abscess at the site of inoculation, when comparatively large doses used, and such a reaction is not favorable from a immunological point of view. Therefore were tried nucleoproteids prepared after Lustig-Galleotti.

The growth of fourty-eight-hour agar cultures was treated with 1% solution of caustic potash (one tube to 8 c.c.) and left for fourty-eight hours at room temperature. On neutralizing it with 1% solution of acetic acid a white precipitate formed. After repeating the centrifugal separation and washing with distilled
water five times the precipitate was solved in 1% solution of sodium carbonate in a proportion of one tube to 1.5 c.c. Four pigeons were inoculated with doses of 0.1-1 c.c. The animals which received 0.5 c.c. or more resisted a tenfold minimum lethal dose of the virulent organism of fowl cholera.

The polyvalent nucleoproteids have been prepared from four kinds of the organisms mentioned above. The results of the treatment were much better, the animals inoculated with 1 c.c. or more resisted a thousandfold minimum lethal dose of the virulent organism of fowl cholera.

Summary.

1. The drying of fowl cholera attenuated, or killed by shaking, drying or heating proved to be of no value as vaccines.

2. The organism killed with disinfectants, fowl cholera aggressine and condensed broth culture of the organism, both mono- and polyvalent, gave better results; the production of an abscess at the site of inoculation was, however, a common defect of these vaccines.

3. Nucleoproteids, both mono- and polyvalent, gave very satisfactory results, conferring a solid immunity to animals treated with no ill effects.

Finally I wish to express my best thanks to Prof. N. NITTA for his valuable suggestions in this work.
A STUDY IN ACTIVE IMMUNIZATION AGAINST FOWL CHOLERA

(1) 減毒生菌液  (2) 振蕩破壊菌液  (3) 乾燥死菌液  (4) 加熱死菌液
(5) 加薬死菌液  (6) あぐれっしん  (7) 培養濃縮液  (8) 多価死菌液
(9) ぬくれおぶろていど等9種類ノ豫防液ヲ製シテ試験シタル
結果ヲ総括スルニ右ノ中家禽ケラ菌ヲハ本菌及他ノ出血性敗血
症菌ヨリ製セルぬくれおぶろていど不良ノ注射反射ヲ呈スルコ
トナク1回0.5—1.0 c.c. ノ注射スレバ可ハり高度ノ免疫性（最低
致死量1000倍ノ注射＝耐セ）ヲ賦與シメ得ルヲトヲ證明セリ

（自抄）