chanics, the engine continued to run after a clutch of some kind was thrown out. But what sort of a theory could we evolve to explain matters when, after the administration of nicotin, we found that the rhythmic contractions continued unchanged and the electrogram disappeared?

In a typical experiment a short segment of rabbit's intestine was excised and allowed to contract rhythmically in a layer of mineral oil floating on top of warm Ringer-Locke's solution. Simultaneous records were obtained of the mechanical and electrical changes. One-half cubic centimeter of a 1 to 100 solution of nicotin was added to the 80 cc. of Ringer's solution in the bath. Immediately there was a marked disturbance in both records. The muscle contracted down and remained that way while the electrical record showed violent erratic changes. During the next 4 minutes the electrical record consisted of a series of small regular waves, interrupted by erratic variations. Many of the cycles were miniatures of the usual ones obtained during the course of normal rhythmic contractions. After 5 minutes the regular rhythmic contractions of the muscle returned while the electrical record showed erratic changes with very small amplitude. After 14 minutes the mechanical waves were vigorous, the amplitude was larger than normal, and the rate was slowed to 6 a minute. The electrical changes had almost disappeared. After 77 minutes the mechanical contractions were regular and vigorous while the electrical changes had entirely disappeared. At the end of 3 hours the rhythmic contractions became feeble and irregular and after 5 hours they stopped. At no time was there a return of electrical changes.

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Active Immunization Against Tunisian Typhus Fever with Mexican Typhus Vaccine.*

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(Introduced by Hans Zinsser.)

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Several attempts have been made to vaccinate human beings against typhus fever, both with living virus[1] and with killed rick-

* We wish to express our thanks to Doctors Manuel Madrazo and Ruiz Castaneda for their advice and help in the elaboration of the vaccine.

[1] Nicolle, C., and Conseil, E., *Compt. Rend. Acad.*, 1910, **151**, 598.

ettsia. Immunization with living virus is always dangerous, since it is extremely difficult to make accurate titrations of the virulent material, so any method yielding a rich suspension of killed rickettsia, safe to handle and at the same time producing an effective immunity would be a great help in typhus fever epidemics.

Weigl[2] obtained encouraging active immunizations by means of an emulsion of the triturated intestinal canals of infected lice, suspended in 0.5% phenolized salt solution. His vaccine corresponds to 100 lice per cc. and is administered in 3 weekly doses. In our experience this vaccine produces in man a rather severe reaction similar to that of typhoid vaccine. Although there is no doubt as to the effectiveness of the Weigl suspension, its manufacture on a large scale is not practical.

Following the discovery of Mooser,[3] who found, in the *tunica vaginalis* of guinea pigs infected with Mexican typhus, a small intracellular organism that by its characteristics has been identified with the rickettsia of the louse, Zinsser and Castaneda have attempted[4] to produce large amounts of these organisms which, killed in a convenient manner, would give suitable material for active typhus immunization. They succeeded in this by infecting rats previously treated with benzol or exposed to X-ray radiation. These rats present a massive infection of the peritoneal cavity, smears from which show an enormous number of intra- and extracellular rickettsia, giving the appearance of a culture. The peritoneal washings made with formalinized salt solution give a fairly strong vaccine.

In the following experiments we used a vaccine prepared by means of the benzol method, though actually we now prepare the vaccine by the recent X-ray technique published by Zinsser and Castaneda[5] which results in a suspension of rickettsia bodies as free as possible from rat serum and cells.

Procedure. Adult male rats are exposed to an X-ray radiation of 170 KV constant potential; 80 cm. distance; 0.5 mm. copper filter plus 4 mm. celluloid; 8 milliamperes; the effective wave length 0.160 Angstrom units; intensity 10 "r" units per minute; 1 hour exposure (600 "r" units). Immediately after radiation, the rats are intraperitoneally inoculated with an emulsion made with tunica from a Mexican typhus-infected guinea pig. The rats appear sick

[2] Weigl, *Med. Klinik.*, 1924, 1046.

[3] Mooser, H., *J. Infect. Dis.*, 1928, **43**, 261.

[4] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649.

[5] Zinsser, H., and Castaneda, M. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

on the 3rd day after inoculation and on the 5th day are killed and thoroughly bled. With a specially made pipette of about 10 cc. worked with a rubber bulb, the peritoneum and intestines are carefully washed with some 100 cc. of a 0.2% formalinized salt solution. The washings are centrifuged for 30 minutes at low speed and the supernatant is decanted for further treatment. The sediment is carefully ground with pyrex glass in a mortar and successive centrifugations and washings are worked out in order to break up the cells and obtain as many free rickettsia as possible. The supernatant is added to the first material and submitted to rapid centrifugation (5000 r.p.m.) for an hour. The new supernatant is discarded and the sediment broken up with a pipette. By repeated low-speed centrifugations and washings, this sediment will give a suspension of rickettsia which appear to be free from cells. A centrifugation at high speed will give a sediment which, resuspended as before in formalinized salt solution, will give a vaccine as free as possible from rat protein. This suspension is then standardized so as to give a count of 1000 million rickettsia per cc. For use, we prepare 3 increasing doses of 500 to 1000 million, to be injected at weekly intervals.†

For our experiments we used a Tunisian strain of typhus which was kindly given us by Professor Nicolle. Our first step was to investigate the dose of guinea pig brain sufficient to infect 100% of the guinea pigs and yet not so strong as to impair the immunity produced by the vaccination. Sixteen guinea pigs were inoculated with suspensions of brain varying from 1/50 to 1/10,000 in salt solution. All animals receiving dilutions up to 1/5,000 gave typical typhus. When the dilution was as high as 1/10,000, a longer incubation time (12 days) was observed. We decided to use a dose of 1/500 for test in our vaccinated guinea pigs, an amount which would certainly infect our normal controls. Twenty guinea pigs were observed for several days and found satisfactorily healthy. Ten were injected with 0.5 cc. of the formalinized Mexican vaccine. Seven days later, the same animals received another dose of 0.5 cc.; and after another week a final dose of 1.5 cc. Nine days after the last dose of vaccine, the 20 guinea pigs were intraperitoneally inoculated with a

† In the form in which this vaccine is delivered, the suspensions are in such physical condition that it is expected that a lasting antigenic power will be obtained which seems superior to that in the raw vaccine (Kemp)—perhaps on account of the considerable amount of protein—in which the intracellular rickettsia are probably fixed in the protoplasm by the formalin, thus mechanically diminishing its antigenic activity.

1/500 suspension of the brain of a Tunisian-infected guinea pig. Temperatures were taken twice a day during the vaccination without any considerable rise being noted. This is contrary to the observation of Zinsser and Castaneda, but was probably due to the small amount of vaccine used in our experiment as compared to the doses used by them. All the controls gave typical reactions and of the 10 vaccinated animals only 5 had fever. The others proved to have been completely protected against the infection.

We find it interesting to publish these experiments, even though they are not extensive, because they prove that even with a small amount of vaccine it is possible to protect guinea pigs against the Old World strain by the Mexican vaccine of Zinsser and Castaneda.

Encouraged by the recent work of Sanchez Casco[6] we have vaccinated 2153 persons, mainly among employees of the public bathing houses for indigents and among the inmates, physicians and nurses of the Belen prison, where a focus of typhus was recently discovered. In no case did we observe any accident due to the vaccination. Dr. M. Bustamante of the Department of Transmissible Diseases has vaccinated some 6,000 persons in epidemic zones, and every day the demand and use of the vaccine is greater. We feel confident that this method of protection will prove of great assistance in the typhus problem in this country.

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On Individual Differences in Chicken Blood.

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I. The existence of numerous individual serological differences in the blood of chickens has been established by several authors by means of rabbit immune agglutinins,[1] immune isoantibodies,[2] and normal isoantibodies.[3, 4] The most interesting and exhaustive

[6] Sanchez-Casco, R., *Medicina*, 1932, **12**, 316.

[1] Landsteiner, K., and Miller, Jr., C. P., PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 100.

[2] Todd, C., *Proc. Roy. Soc.* B., 1930, **106**, 20, **107**, 197; see Hadda, S., and Rosenthal, F., *Z. Immunitätsforsch.*, 1912-1913, **16**, 524.

[3] Karshner, W. M., *J. Lab. Clin. Med.*, 1928-1929, **14**, 346.

[4] Shimidzu, T., *Tohoku J. Exp. Med.*, 1931, **18**, 97; see Bailey, C. E., *Am. J. Hyg.*, 1923, **3**, 370.