The effects of similarly prepared extracts of omental fat and kidney were studied in 4 experiments. In no instance was a lowering of the oxygen consumption noted after injection. During March, 1940, further determinations were again made and the effects of crude extracts were studied in 1 normal (No. 11) and 2 thyroidized rats (Nos. 12, 13).

Summary. It would appear that in these experiments the intraperitoneal injection of crude peanut oil extracts of the brown fat of the woodchuck and the thirteen-lined ground squirrel produced a lowering of the heat production of the white rat either at once or after 1 or 2 days (as measured by the oxygen consumption). Such effects, with one exception, were not produced by the injection of peanut oil alone.

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Protection of Guinea Pigs Against Mexican Typhus Virus by Vaccine from Infected Rat Lungs (Castaneda).

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In recent years much experimental work has been done on the production of a vaccine effective against typhus. The principal effort has been to devise a method that yields rickettsiae in sufficient quantity to be of practical application to the prevention of this disease. Since the use of infected lice as a source of rickettsiae, as developed by Weigl in 1930, the most promising methods have utilized peritoneal washings of infected white rats previously treated with benzol or irradiated with X-rays (Zinsser and Castaneda[1]); infected tissue cultures on agar slants (Zinsser, FitzPatrick and Wei[2]); material from the infected yolk sac of the developing chick (Cox, and Cox and Bell[3]); and a combination of the yolk sac and agar tissue culture methods (Zinsser, Plotz and Enders[4]). Castaneda has recently reported the production of an experimental typhus pneumonia in white

1 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

2 Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

3 Cox, H. R., *U. S. Pub. Health Rep.*, 1938, **53**, 2241; Cox, H. R., and Bell, E. J., *U. S. Pub. Health Rep.*, 1940, **55**, 110.

4 Zinsser, H., Plotz, H., and Enders, J. F., *Science*, 1940, **91**, 51.

rats[5] that yields large numbers of rickettsiae separable from infected lung tissue by differential centrifugation and available for use as a killed vaccine.[6]

During a period of study in the typhus research laboratory of Dr. Castaneda, the writer had the opportunity to examine the effectiveness of the vaccine prepared from rat lungs in the protection of guinea pigs against typhus virus. This is a brief report of that work.

Methods. A Mexican strain ("L") of typhus was used both as the vaccine and as the test strain of live virus. The vaccine consisted of rickettsiae in pure suspension, killed by phenol, and numbering about one and a half billion per cc. It was injected subcutaneously into 10 male guinea pigs weighing from 400 to 570 g each. Two animals received 4 injections of 1 cc each; 2 were given 3 injections of 1 cc each; 2 were injected twice, 1 cc each; 2 once with 1 cc; and 2 once with 0.2 cc vaccine. The injections were made at intervals of 5 days and in such a way as to have all animals receive their last vaccine injections on the same day.

Eleven days later, all animals were given intraperitoneal inoculations of a test dose of virus, consisting of a suspension of scrapings from the tunica vaginalis of infected guinea pigs. Each vaccinated animal received in 5 cc sterile saline the equivalent of about 1/20 of the scrapings of one tunica; this is far above the minimal infecting dose. Four control guinea pigs, of approximately the same weight as those vaccinated, were injected with one-half the test dose of virus given the vaccinated guinea pigs.

The temperatures of all animals were taken daily, routinely late in the morning. The temperature of 40°C, and above, was taken arbitrarily to represent a fever. Degrees of scrotal reactions were recorded as from 1 to 4 plus. Daily observations were made for 17 days.

Results. The control (unvaccinated) guinea pigs had febrile readings scattered from the fourth to the eleventh day following inoculation. Each control animal also had a scrotal reaction, typical of the effect of an orchitic strain of Mexican typhus. These reactions appeared on the fifth and sixth days and lasted from 2 to 7 days. The degrees of reaction were graded from 1 to 4 plus.

One of the 10 vaccinated guinea pigs died of intercurrent infection (intestinal?) on the third day after inoculation with test virus. All other animals survived the test dose without fever or scrotal reaction. Three of these had temperatures of 40.0°, 40.2° and 40.3° on the

[5] Castaneda, M. R., *Am. J. Path.*, 1939, **15**, 467.

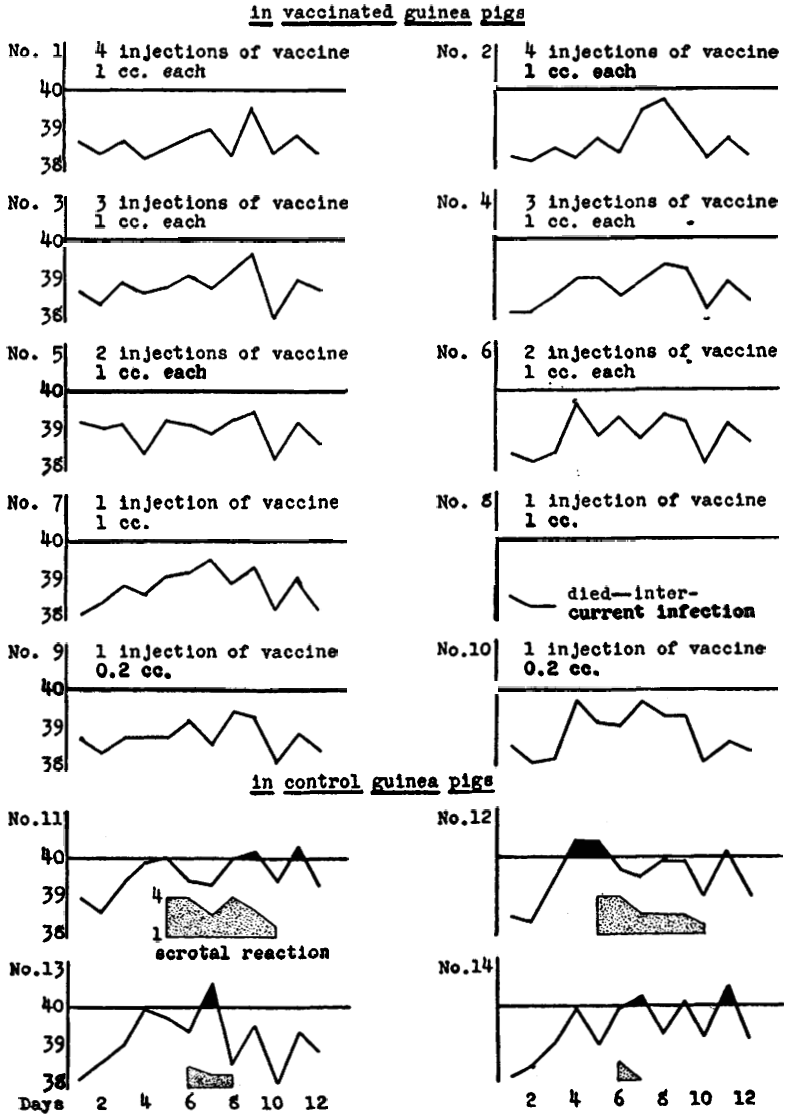
[6] Castaneda, M. R., prepared for publication.

fourteenth day after inoculation and after the febrile and acute scrotal reactions in the controls had subsided; these temperatures were not considered significant because of their late occurrence.

Observations made on the animals during the 12-day period immediately following inoculation of the test dose of virus are presented in Chart 1.

CHART I.

Temperatures and scrotal reactions following test dose of typhus virus



Summary. Typhus vaccine, consisting of killed rickettsiae from infected rat lungs prepared according to the method of Castaneda, protected guinea pigs against infective doses of virus injected intraperitoneally. Immunization was accomplished by the subcutaneous injection of the vaccine in as small a dose as 0.2 cc. The virus used in the preparation of the vaccine and for the test for immunity was the "L" strain of Mexican typhus.

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Inhibition of Tryptases by Heparin.*

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It has been demonstrated by Fischer[1] that the isoelectric point of proteins shifts towards the acid side in the presence of heparin. He concluded that the acidic groups of heparin combined with the basic groups of proteins, forming salt-like compounds. Since trypsin attacks negatively charged proteins very readily,[2] it might be expected that the addition of heparin would facilitate proteolysis by making the protein behave as a stronger anion (at constant pH). However, trypsin is also a protein, and it may be that heparin can alter its activity by combining with it. The results of this paper indicate that heparin *decreases* the proteolytic activity of serum tryptase and crystalline trypsin.

Two methods were used to study the inhibitory action of heparin: (1) the increase in the non-protein nitrogen which resulted from the digestion of casein by trypsin was followed by means of micro-Kjeldahl analyses; and (2) the rate of inactivation of thrombin by trypsin or serum tryptase ("progressive" antithrombin) was estimated by assaying the coagulant activity of the residual thrombin.[3]

A. *Action of Heparin on Casein + Trypsin.* Various amounts of a heparin preparation† were added to 5 cc portions of a 3% casein solution, buffered to pH 8.2 with M/10 phosphate. The final volumes were adjusted to 7 cc, the mixtures warmed to 38°, and 1 cc

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1 Fischer, A., *Biochem. Zeits.*, 1935, **278**, 133.

2 Northrop, J. H., *Crystalline Enzymes* (New York), 1939.

3 Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.*, 1940, **24**, 169.

† Connaught Laboratories. The preparation assayed 110 units per mg.