Summary and Conclusions: It is possible to obtain highly potent skin preparatory and reacting factors in filtrates of washings of 20-22 hour old *Bacillus typhosus* cultures on plain agar. Under these conditions there is avoided much of the extraneous material present in tryptic digest broth previously employed, as well as much of the bacterial autolysis occurring in the older method. This would indicate that the skin preparatory and reacting factors are bacterial soluble substances, the production of which is independent of massive cell destruction.

The filtrates prepared in this manner produce negligible primary effect upon the skin (erythema, swelling), notwithstanding their high skin preparatory potency. This is in agreement with the view previously expressed[1, 2, 5] that the skin preparatory potency of a given filtrate bears no relation to its primary effect on the skin.

It is of considerable interest to note that there exists a distinct reciprocal quantitative relationship between the skin and intravenous doses. This observation merits further study.

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Nicotine Tolerance in the White Rat.

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The minimal effective dose (M.E.D.) of nicotine for white rats of both sexes, from 29 to 33 days old, was 0.3 mg. per kilo body weight. This dose was sufficient to cause slight but definite weakness of the hind legs. After determining the M.E.D., one-half of each of 3 litters were injected twice daily with 2 to 3 times the M.E.D., producing symptoms ranging from instability to convulsions and prostration. At intervals of 3 and 9 weeks following the first injections, after a rest day of no injections, all rats were tested for the M.E.D. Whereas the controls, 10 in number (injected twice daily with salt solution), responded each time to 0.3-0.4 mg. nicotine per kilo body weight, the test rats, 13 in number, showed a definite decrease in susceptibility, in that at the end of 3 weeks, the M.E.D. averaged 0.65 mg. nicotine, and at the end of 9 weeks, 0.85 mg. per kilo body weight. This was considered to be evidence of an acquired nicotine tolerance.

An interesting phenomenon of "acute tolerance" was also observed. That is, the second injection of the day caused much milder symptoms than the first injection. Although not yet well worked out, it seems that the optimal interval between injections for demonstrating this phenomenon is from 2 to 4 hours.

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The Complement Fixation Test in Yellow Fever.*

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Yellow fever offers opportunity for study of the immune reactions of a virus disease under circumstances which eliminate error due to collateral antigens. According to Schultz[1] collateral antigens have been a frequent source of error in previous studies of the complement fixation reaction in virus diseases. Material quite free from collateral antigens can be obtained in yellow fever as antigen for the complement fixation test.

Aragao[2] has reported unfavorably upon the complement fixation test in yellow fever with antigens prepared from phenolated tissues. On the supposition that the antigenic substances in tissues affected by virus diseases might be within the cells, Ciuca[3] has made use of a process described as septic maceration in order to liberate the cellular contents. He reports success in differentiating between the 3 principal types of foot and mouth disease by complement fixation with antigens prepared in this way. The method of preparing the antigen for our tests is based on a procedure followed by Hindle[4] in making vaccine from yellow fever tissues. He produced rupture of the cells by causing a sudden change in the osmotic pressure of the fluid in which the tissues were suspended.

In preparing antigen, pieces of liver and spleen were taken at autopsy from monkeys which had died from experimental yellow fever. This material was ground thoroughly with sterile sand and

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[1] Schultz, E. W., *J. Immunol.*, 1928, xv, 229.

[2] Aragao, H. de B., *Compt. Rend. Soc. Biol.*, 1928, xlix, 1341.

[3] Ciuca, A., *J. Hyg.*, 1929, xxviii, 22.

[4] Hindle, E., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1929, xxii, 405.