It seems certain, however, that this tissue need not be that of the infundibular region.

8319 P

Immunization of Rabbits with Inactive Vaccinia Virus.

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It is generally accepted that immunity to virus diseases can only be produced by an infection with a live, even though highly attenuated, virus. From time to time experimental evidence contrary to this view has been published, but the results have not been convincing. In this preliminary report references may be limited to experiments with vaccine virus. Gordon[2] used virus heated to 57°C. for 30 minutes, a period insufficient to kill this virus. Hunt and Falk[3] reported positive results with virus treated with a weak solution of formalin, but Olitsky and Long[6] showed that vesicular stomatitis virus thus treated still contained live virus. The most careful work has been carried out by Bland,[1] who tested his vaccine for live virus and used both heat and formalin killed virus. He reported positive results in guinea pigs and equivocal results in rabbits.

The results obtained by Bland, as well as our own observation[4, 5] on the antigenic nature of purified phage and of typhus rickettsia in cultures, suggested that the failure to induce immunity with dead virus was due to the relatively small amount of antigen contained in tissue suspensions of viruses. In the phage work it was found that an amount of suspension containing not less than 20 million plaques was necessary to produce an antiserum with moderate neutralizing power. In the case of rickettsia it was estimated that an infected guinea pig brain weighing 3 gm. would have a maximum only of 12,000,000 organisms, and this seemed sufficient reason why infected lice or cultures made an efficient vaccine, whereas, a whole guinea pig brain produced at best only a slight degree of immunity.

1 Bland, J. O. W., *J. Hyg.*, 1932, **32**, 55.

2 Gordon, M. H., *Med. Counc. Rep.*, 1925, No. 98.

3 Hunt, L. W., and Falk, I. S., *J. Immunol.*, 1927, **14**, 347.

4 Kligler, I. J., and Olitsky, L., *Brit. J. Exp. Path.*, 1931, **12**, 172.

5 Kligler, I. J. and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.

6 Olitsky, P. K., and Long, P. H., *J. Exp. Med.*, 1928, **47**, 835.

The experiments reported below were undertaken in order to test the validity of this view in regard to vaccine virus.

The vaccine used consisted of a 10% suspension of infected testicle, or an eluted virus of the same strength, either heated 2 hours at 56°C., or treated with formalin (final concentration 1.0%), and kept 24 hours in the incubator and one or more weeks in the icebox. The vaccine was tested for live virus by intracutaneous injections of 1.0 cc. of the stock material into susceptible animals.

The virulence of the virus suspension and the eluate were titrated on a rabbit skin before heating. The strength ranged between 10^{-6} and 10^{-7}. In other words 1.0 cc. of the 10% suspension contained about 1,000,000 infective doses. On the basis of the phage work about 20 cc. would be required to produce a measurable degree of immunity. The neutralizing power of the serum of the rabbits to be vaccinated was titrated before immunization. Immunity after the treatment was tested by intradermal injections of different dilutions of an active virus as well as by titration of the neutralizing power of the serum.

Animals given 1 or 2 injections of 5 cc. of the vaccine gave entirely negative results. Those, however, receiving 3 or more injections gave results which indicated that an immunity had developed. Below is given several protocols of experiments on rabbits receiving 3 or more injections:

Rabbits 446 *and* 553 received 4 injections of 5 cc. of a 10% testicular suspension—446 formolized, 533 heated. Titre of untreated virus suspension 1:10,000,000. Sera (1:5) mixed with virus dilutions 1:10,000 to 1:1,000,000 gave no neutralization. Injections given 4 cc. i.p. and 1 cc. i.c., at 3-day intervals. Blood taken 7 days after last injection.

Rabbit 533—serum 1:5 neutralized (1 hr. at room temperature and 24 hr. in icebox) 1:1,000,000 and 1:100,000 but not 1:10,000 dilution of virus. Intracutaneous test: 1:10,000±, 1:100,000—. Control: 1:10,000,000+. *Rabbit* 446—serum 1:5 neutralized 1:1,000,000 and 1:100,000 only partially, delayed reaction; intracutaneous test: all dilutions positive, reactions delayed.

Rabbits 489, 456 received 3 injections eluted virus; each 4 cc. i.p. and 1.0 cc. i.c.; 489 received formolized, 456 heated material. Titre of eluate: positive 1:1,000,000. Titre of serum before immunization: serum 1:5, virus 1:10,000 to 1:1,000,000, no neutralization. Blood taken 7 days after last injection.

Rabbit 489—skin test 1:10,000 to 1:1,000,000. Next day all points showed equally extensive, infiltrated, inflamed areas. Control still negative. Two days later infiltration more extensive, control

positive with normal progression of lesions. A definite allergic reaction. Neutralization: serum 1:5, virus 1:10,000 to 1:1,000,000; after 3 days 1:10,000+, 1:100,000 and 1:1,000,000—, controls on same rabbit positive at all points; after 6 days all points positive, reaction delayed; slight neutralization.

Rabbit 456—skin test not carried out because animal died. Neutralization test with serum same as 489.

Rabbit 301—heated eluate, 7 injections; each time 5 cc. i.p. and 0.2 cc. i.c. at intervals of 2 days. Eluate positive in dilution of 1:1,000,000. Neutralization test: whole serum 1:10 and virus 1:10,000 negative. Blood taken 6 and 10 days after last injection. Neutralization test: whole serum and 1:10 with virus 1:10,000; on second day control positive, test negative; on fifth day control +++, test only slight infiltration ±. Skin test: virus 1:10,000 negative. This animal had definite skin immunity and its serum considerable neutralizing power.

8320 C

Effect of Sodium Fluoride upon Experimental Thyroid Poisoning.

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Several authors[1-4] have reported beneficial effects from the oral and intravenous exhibition of the fluorides in the treatment of hyperthyroidism, rationalizing the procedure, in some instances at least, upon the well-known enzyme inhibiting action of this halogen. Laboratory findings offer a scientific basis for this treatment, since moderate doses of the fluorides produce *(a)* a diminished tissue respiration[5] and anaerobic glycolysis[6] in excised organs, *(b)* a decreased oxygen consumption and lactic acid production in muscle,[7] and *(c)* a sharp decrease in the oxygen consumption[2] and the carbon dioxide[8] production in the intact animal. Goldemberg[2] states that

1 Woakes, E., *Lancet*, 1881, **1**, 497.
2 Goldemberg, L., *Semana Medica*, 1932, **39**, 1659.
3 Gorlitzer, V., *Med. Klin.*, 1932, **28**, 717.
4 Reveno, W. S., *J. Michigan State Med. Soc.*, 1934, **33**, 359.
5 Phillips, P. H., and Stare, F. J., *J. Biol. Chem.*, 1934, **104**, 351.
6 Dickens, F., and Simer, F., *Biochem. J.*, 1929, **23**, 936.
7 Lipmann, F., *Biochem. Z.*, 1928, **196**, 3; 1929, **206**, 171.
8 Gorlitzer, V., *Arch. f. exp. Path. u. Pharm.*, 1932, **165**, 443.