INFLUENZA ANTIGEN DOSE AND ANTIBODY RESPONSE RELATIONSHIPS[1]

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Stevens (J. Immunol., **76,** 187, 1956) investigated the relation of antigen dose and antibody response with the Smith and St. Johns-Brooks equation:

$$K_1C^{1/n} = Ab$$

where K_1 and n are constants, C is the concentration of antigen and Ab is the antibody response. He found that aqueous influenza antigens, given subcutaneously, yielded values of n that varied from 2.5 to 5.3 in human beings and swine. Our study of antigen dose and antibody response, by intra- and subcutaneous inoculation of human beings, employing larger and more dilution increments, might be expected better to test these relations (Stille, Woolridge, and Gundelfinger, J. Lab. Clin. Med., **53,** 751, 1959).

The least square line, fitted to the data for average change in titer tubes (or log antibody response) and log dose (in chick-cell agglutinating units) of table 1 gives regression coefficients of 0.219 for the subcutaneous route and 0.057 for the intracutaneous route. These slopes give values of n of 4.6 and 17.4 for the Smith and St. Johns-Brooks equation. The high value for the intracutaneous route is clearly inconsistent with the other values determined by Stevens. The tendency of the "log log" plot of the dose response relation for the subcutaneous route, shown in figure 1 of our previous paper, to be sigmoid is wholly in agreement with comparable data given by Hirst, Rickard, Whitman, and Horsfall (J. Exptl. Med., **75,** 495, 1942) although they interpreted their data as showing a linear relation.

TABLE 1

Asian influenza hemagglutination-inhibition antibody responses

Tubes of HI* Titer Change (21 Days)	1 ml Subcutaneous Groups (CCA units†)				0.1 ml Intracutaneous Groups (CCA units)				Placebo (0.000)
	800	80	8	0.08	80	8	0.8	0.008	
−1	—	1	—	—	1	—	—	—	2
0	2	6	10	4	7	7	5	6	6
1	2	3	7	4	4	6	—	2	3
2	4	3	2	—	6	5	1	—	1
3	6	6	1	—	—	5	3	—	—
4	1	1	1	—	4	1	1	1	—
5	6	5	1	—	2	2	1	—	—
6	2	1	1	—	2	—	—	—	—
7	1	1	—	—	1	—	—	1	—
Avg change	3.38	2.52	1.26	0.50	2.22	1.73	1.82	1.30	0.25
Initial titers	18	28	32	38	21	25	19	24	38
Final titers	191	159	75	54	95	81	63	52	47
No. men	24	27	23	8	27	26	11	10	12

* HI = Hemagglutination-inhibition.

† CCA units = Chick-cell agglutinating units.

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Since the Smith and St. Johns-Brooks equation is predicated on a linear relation between log dose and log response, the evidence that a sigmoid relation obtains, at least subcutaneously, requires modification in the equation before further interpretations can be made of its parameters.

PROPAGATION OF DUCK HEPATITIS VIRUS IN TISSUE CULTURES PREPARED WITH COLLAGENASE[1]

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Duck hepatitis virus has been propagated in tissue cultures of chicken embryo explants (Pollard and Starr, Proc. Soc. Exptl. Biol. Med., **101,** 521, 1959). Further attempts to study the growth characteristics and properties of duck hepatitis virus have indicated that collagenase- but not trypsin-dispersed cells would support virus replication.

Tissue mince of 8-day-old chicken embryos was dispersed with 0.01 per cent collagenase (Hinz and Syverton, Proc. Soc. Exptl. Biol. Med., **101,** 19, 1959). Washed cells were diluted 1:300 with mixture no. 199 (Microbiological Associates, Bethesda, Md.) supplemented with penicillin (100 units/ml) and streptomycin (0.1 mg/ml). One ml of this cell suspension was added per tube and incubated at 37 C. Monolayers of cells were inoculated with 10-fold dilutions (0.1 ml) of stock duck hepatitis virus (10^6 chicken embryo LD_{50} per ml) and 0.9 ml of the following medium was added: 5 parts heat-inactivated calf serum, 5 parts lactalbumin hydrolyzate (5 per cent stock solution), and 90 parts mixture no. 199 with antibiotics. Inoculated cultures were examined daily for cytopathic changes. After 6 days, nutrient fluids and cells of each dilution were frozen and thawed twice, pooled, and stored at −20 C. Subsequent serial passages were made without dilution in monolayer cultures of collagenase-dispersed cells of chicken embryo tissue, using 0.1 ml inoculum from respective preceding culture pools. LD_{50} titers were determined in 7-day-old chicken embryos inoculated via the chorioallantoic cavity, after 6 days.

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Serial passage of duck hepatitis virus in monolayer cultures of trypsin-dispersed cells was attempted in parallel experiments after the pattern outlined above with collagenase-dispersed cells. A final concentration of 0.25 per cent trypsin (Difco) 1:250 was used.

TABLE 1

Comparison of duck hepatitis virus titers obtained on serial passage in tissue cultures prepared from chicken embryo by collagenase and by trypsin treatment

Tissue Preparation	Passage No. and Virus Titer*							
	1	2	3	4	5	6	7	8
Collagenase	2	4	3	3	3	4	3	3
Trypsin	3	2	0	0	0	0		

* Negative log dilution, LD_{50}.

Results of the duck hepatitis virus serial passage experiments are shown in table 1. With trypsin-prepared cell cultures, duck hepatitis virus titers declined with passage. Trypsin treatment of tissues did not appear to exert a deteriorative effect on duck hepatitis virus and the decline in demonstrable virus titer coincided with its passage on dilution (Pollard and Starr, Proc. Soc. Exptl. Biol. Med., **101,** 521, 1959). Propagation was evident in collagenase-prepared cell cultures. Cultures on first passage were infected by as little as 10 LD_{50} of virus and produced as much as 10^5 LD_{50} per ml. In subsequent passages, the cumulative virus production far exceeded the amount in the original inoculum. In addition the cumulative incubation time of duck hepatitis virus in tissue culture with sustained titer was 48 days which exceeded the decay time of the virus at 37 C. Infected cultures did not show either evidence of