tion will deal with the details of these studies and with a report on the action of ethyl urethane on the histamine release in anaphylaxis of guinea pig tissues.

Although earlier investigations by one of us[7] on arterial muscle, and more recent ones by Farmer,[3] on the uterus, show that some narcotics inhibit the response of smooth muscle to histamine, the results shown above seem to indicate that ether prevents the fatal anaphylactic shock in guinea pigs at least partly by suppressing the release of histamine. Such views are in contrast to Farmer's,[3] who held that anesthesia acts in the secondary stages of anaphylaxis by inhibiting the action of histamine, while it does not interfere with its release.

11027 P

Effect of Varying the Volume of Injection in Calculating Number of Infectious Particles of Vaccinia.

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In the course of certain experiments with vaccinia virus it was found that the injection of a small volume of a virus dilution gave approximately the same number of positive lesions as obtained with a much larger volume. In other words it was not the number of virus particles injected but their concentration which was important. This point was thought of sufficient interest to warrant further study. This paper is a preliminary report on the subject.

Methods and Materials. The purified vaccinia virus used in these experiments was similar to that used in previous experiments.[1] Measured amounts of this virus were rapidly frozen and dehydrated in a Flosdorf-Mudd apparatus and kept sealed in a vacuum until ready for use. The number of virus particles was calculated according to the method described by Parker.[2] For example, from Experiment 1, the log of the dilution which contains 1 particle per 0.1 cc is 6.93; therefore the number of particles in the virus suspension calculated from these data would be 10 times the antilog of 6.93 which

7 Katz, G., *Arch. f. exp. Path. u. Pharm.*, 1929, **141**, 366.

1 Sprunt, D. H., and McDearman, S., in preparation.

2 Parker, R., *J. Exp. Med.*, 1938, **67**, 725.

is 8.5×10^7 infectious units per cc of the virus suspension. All the conditions of the experiment, except the volume of the injections, were kept the same as Parker's.[2] The X^2 for goodness of fit to the 1 particle curve were calculated by using both positive and negative variations as pointed out by Haldane.[3] Adult male rabbits were used for the titration of the virus. Care was taken to put the same number of injections of each size in each rabbit so as to avoid the variation in susceptibility in the rabbits. The difference in susceptibility of skin on various portions of the rabbits was also taken into consideration in a similar manner.

Results. The results are shown in Table I. It will be seen that the injection of amounts larger than 0.25 cc changes the calculated number of virus particles considerably. Amounts smaller than 0.25 cc in some instances increase the number of particles fourfold and in others the increase is only twofold.

These experiments show that the presence or absence of infection with vaccinia virus injected intradermally is not dependent entirely on the number of virus particles injected. The resulting lesions are more closely related to the concentration of the virus particles in the volume injected than to the number.

One possible explanation of this phenomenon is that in the in-

TABLE I.

Table Showing Effect of Using Different Volumes of Injection in Calculating Number of Infectious Particles.

Vol of virus injection	No. of injections of each dilution	Log of dilution containing 1 infectious unit	X^2 for fit to 1 particle curve	P	Calculated No. of infectious units per cc
		Exp. 1.			
0.50	24	6.99	3.14	.80	1.9×10^7
0.10	24	6.93	11.08	.10	8.5×10^7
		Exp. 2.			
0.50	16	7.74	6.79	.40	11.0×10^7
0.10	16	7.68	1.18	.97	47.9×10^7
0.05	16	7.60	2.75	.85	79.6×10^7
		Exp. 3.			
1.00	20	7.98	7.43	.25	9.5×10^7
0.25	24	7.83	2.31	.80	27.0×10^7
0.05	24	7.55	5.37	.50	70.9×10^7
		Exp. 4.			
2.00	18	8.27	5.38	.50	9.3×10^7
0.25	24	8.27	2.28	.90	74.4×10^7
0.05	24	7.78	6.81	.40	120.5×10^7
		Exp. 5.			
1.00	24	8.34	14.42	.75	21.9×10^7
0.25	24	8.23	4.47	.60	67.9×10^7
0.05	24	7.77	3.54	.75	117.8×10^7

[3] Haldane, J. B. S., *J. Hygiene*, 1939, **39**, 289.

jecting of a given volume only a small portion actually stays in the epidermis, the remainder going into surrounding tissues which are not susceptible to this virus. Hence, if more virus is injected than can stay in the skin it has no effect. Up to a certain point as shown in Table I the increase in the volume of injected solution does increase slightly the chance that a susceptible cell will become infected. It should be emphasized that this does not negate the theory that 1 particle causes infection. It does, however, show that an erroneous figure may be obtained in calculating the number of particles in a virus suspension if the volume of injection used is too large.

Although the above explanation at present seems the most likely, further experimentation must be done as there is the possibility that this phenomenon represents a much more fundamental character of virus infection. We are continuing the study of this subject.

Summary. Experiments are reported in which it is shown that under usual experimental conditions the concentration and not the volume of a vaccinia virus dilution is important in determining the number of infectious units contained in a given virus suspension.

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Experimental Reversal of Sex in Salamanders by the Injection of Estrone.*

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Experimental evidence derived from the work of Burns, Humphrey, Witschi and others[1] has established the fact that differentiation of sex in salamanders may be modified or even completely reversed through the action of sex-differentiating principles produced in the gonads of a parabiotic partner of opposite sex, or by a gonad of different sex type resident as a graft. This report presents briefly the results of experimental sex reversal in the salamander *Amblystoma tigrinum* (Green), during the period of sex differentiation,

* The crystalline substance used was very kindly supplied by the Schering Corporation.

[1] Allen, E. (Editor), 1939, *Sex and Internal Secretions*, 2nd Ed., Chapters III and IV. Baltimore, Williams & Wilkins Co.