121. *On the Diffusibility of Vaccine Virus.*

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(Comm. by U. SUZUKI, M.I.A., Oct. 12, 1929.)

In our recent communication a method of purification of vaccine virus was reported. Further, we found, that vaccine virus diffuses with nearly the same velocity as the fuchsin molecules; in other words, the virus seems to attain nearly the size of the latter. Of the data we obtained some are given in the present paper, to show the facts which have led us to the above consideration.

Experimental.

The virus-solution used in the experiment was prepared after the method previously communicated,¹⁾ by the addition of kaolin to the raw lymph in a certain acid reaction and liberating again the virus from the kaolin into n/25 ammonia solution, and neutralizing it to phenolphthalein with acetic acid.

To test the parchment paper used in the dialysis, we resorted to several dyes in 1 % aqueous solution. Only the specimens, which proved (1) to be impermeable to congo red or night blue, (2) slowly allowing the passage to fuchsin or neutral red, and (3) easily to pass for metylene blue, were used in the experiment.

Exp. I. About 10 cc. of purified virus was put into parchment paper, suspended in a small receptacle containing 5 cc. of distilled water. The dialysis was performed at the room temperature of 10 to 15°C. After 24 hours of dialysis, the fluid in the receptacle was removed and condensed in vacuo to 1/3 of the original volume and its 0.2 cc. was inoculated intradermally into rabbits. The results read on the fifth day were as follows:

No. of exp.	I	II	III	IV	V	Control
Rabbit (1)	⑥	11	⑥	⑥	—	9
Rabbit (2)	⑤	9	⑥	⑦	—	7

Numerals in the table indicate the diameter of papulae in mm.

○ Indicates the eruption not clearly distinguishable from its surroundings.

1) Yaoi, H. and Kasai, H: Proc. 5 (1929), 179.

Exp. II. A sheet of parchment paper was tightly fixed at the lower edge of the dialysing vessels of the sort employed by Graham, and was filled with 5 cc. of the virus suspensions. This stood in a vessel containing about 10 cc. of distilled water. After 24 hours of dialysis at a room temperature, 5 cc. of liquid in the outer vessel was taken out and injected subcutaneously into rabbit. The animal thus treated reacted in a refractive manner when reinoculated with the ordinary cow-pox virus. The dermal inoculation of the dialysate into calves resulted in scattered pustules, as shown in the next table.

Calves	Inner liquid	Outer liquid
No. 34.	discrete pustules	scattered pustules
No. 35.	discrete pustules	scattered pustules

In another series of experiment using the same apparatus, the distilled water in the outer vessel was changed several times. In this case the virus placed inside the membrane was found appreciably diminished in amount.

Exp. III. 10% gelatin, neutralized with NaOH sol. was distributed in 10 cc. into test tubes, and after solidifying a series of dye solution (night blue, fuchsin and methylene blue) and the virus-suspension, at the same time, were put on gelatin to allow them diffuse into gelatin.

The observation after 24 hours revealed the fact that the height or depth of the diffusion in the tube varies according to the dimension of each dye: Night blue (colloid)=0 mm., fuchsin (semicolloid)=6 mm., and methylene blue (crystalloid)=9 mm. At this moment, the tube with the layer of the virus suspension was taken out and after sterilizing the surface of the gelatin the tube was broken up and the gelatin stab was cut into 3 pieces at the distances of 6 mm. and 15 mm. from the upper surface. These upper two portions, 6 mm. from the top (A) and the lower half (B), were separately mixed with small amount of saline water, and two rabbits were inoculated into each testicle with 1 cc. of these suspensions. As the result, a slight edema of scrotums was noticed in both animals, but upon castration on 6th day a heavy hemorrhage was found in the one injected with A, while in another, only a slight hemorrhage was detected in the parenchyma.

The testicles, being emulsified with glycerin, were respectively injected into testicle of rabbits. In these rabbits of the 2nd generation, a remarkable edema was noted on a scrotums and on 3rd and 4th

day, a parenchymatous hypertrophy was found in the animal of A series, but in another edema was found subsiding. On extraction, after 6 days, the testicle of the former showed an intensive parenchymatous hypertrophy and hemorrhage, but the latter only a slight hyperaemia and swelling. Both testicles were again emulsified with glycerin.

These two series of testicular emulsions (4 specimens altogether) were inoculated into calves. The following table shows the results obtained.

Emulsions	A series		B series	
	I generation	II generation	I generation	II generation
Calf No. I.	confluent pustules	confluent pustules	—	scattered pustules
Calf No. II.	scattered pustules	confluent pustules	—	discrete pustules

Above findings indicate that the vaccine virus passed through gelatin over 6 mm. in 24 hours but the amount of the virus was not sufficient to cause an eruption, whereas the second generation caused a minute pustules on the skin of calves.

Exp. IV. A similar experiment was made using a Petri-dish in place of a test tube, as the receptacle of gelatin. After 48 hours of diffusion, the gelatin was taken out en mass and a portion of gelatin was scooped out from the central part, about 6 to 15 mm. below the surface, and the yield thus obtained was used as an inoculum. A portion of the gelatin was melted at a suitable temperature (40–50°C.) and quickly inoculated on the scarified skin of calves (about 3 sq. cm.). The results obtained were as follows: Calf No. 1.—7 pustules, No. 2.—pustules, No. 3.—totally negative.

Another portion of the gelatin yield was added with saline water to make a suspension, 2 cc. of which were injected into each testicle of a rabbit, and the testicles, being removed after 6 days, were emulsified and inoculated upon the scarified skin of calves, which reacted with confluent eruptions. The depth of the fuchsin diffused within the same hours, was about 16 mm.

Conclusion.

In the light of our present observations the virus of vaccinia must be regarded exceedingly minute in size and the alleged etiological agent within the limit of microscopic visibility as demonstrated by so many authors does not seem to present the true phase of the vaccine virus.