We wish to report our confirmation of the observations of Webster and Clow, since by the procedure which they describe, we have been able to carry the virus through 16 subcultures. Material from the final culture has proved infectious for Swiss mice in dilution of 10^{-2}. In addition a series of passages carried out on the same medium, but with an equivalent amount of normal rabbit serum in place of normal monkey serum yielded results which were similar in all respects to those obtained with normal monkey serum.

Attempts to propagate the virus on media containing a larger ratio of serum to Tyrode and considerably smaller amounts of tissue proved unsuccessful. The fact that Kanazawa propagated the virus in a medium containing rabbit embryo brain, without the addition of serum, suggests that possibly the concentration of serum may have been a factor and that a low concentration of serum or no serum at all, may be more favorable to growth.

Twelve separate attempts were made to propagate the virus on the chorio-allantoic membranes of developing chicks. All yielded entirely negative results. This is in accord with the experience of Waldhecker.[3]

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Differences in Spread of Dye in Skin of Normal and Tuberculous Guinea Pigs.

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In a study of the skin of guinea pigs, it has been found that the tuberculous animal reacts differently from the normal in regard to the spread of dye which has been injected intradermally.

We have made tests to see if differences could be detected in the spread of dye in the skin around the zone of primary inoculation as contrasted with other areas. The work was started in the course of completing a study of the Koch phenomenon which had been begun by the late Henry Sewall. He had obtained evidence that there are differences in sensitivity in different areas of the skin of the tuberculous guinea pig.

The dye used was pontamine sky blue which we obtained through

[3] Waldhecker, M., *Centralbl. f. Bakt., O.*, 1935, **135**, 259.

the courtesy of Dr. P. D. McMaster, and in general we have followed his technique.[1] We have used the dye as a 2.5%, approximately isotonic solution. The amount of solution injected was 0.025 cc., given with a Dewitt and Herz syringe which has an automatic guard on the plunger, assuring exact dosage. The injections were made under a binocular dissecting microscope under brilliant light, the animal having been anesthetized with sodium amytal.

It was found that there are some differences in the rate of spread of dye, according to varying thickness of the dermis. In the guinea pig the thickness of the dermis in dorsal, lateral, and ventral zones is in the proportion of 2:1.5:1, that is to say, the skin on the back is twice as thick as that on the abdomen. Hence, to be comparable, injections must be restricted to one of these 3 zones. Usually 4 injections, 2 on each side, were made in each animal but in the case of some of the dorsal injections, only 2 were made, each in the mid-line. When the injections were thus limited, the spread in a given animal was practically identical, provided that in the tuberculous animal the indurated border of the primary ulcer was avoided.

In these experiments statistically significant differences have been noted in the spread of the dye between the tuberculous and the normal guinea pig. The dye spreads more slowly in the tuberculous animal. Measurements of the area through which the dye had spread were not significantly different in 1 hour. Every intradermal injection, as Hudack and McMaster[2] have shown, involves a direct injection of dermal lymphatics which the dye enters under pressure and from which it spreads immediately into the subcutaneous plexus. After this phenomenon has been equalized by diffusion, definite differences in spread are observed; thus, after 4 hours, the spread of the dye was nearly 50% greater in the normal than in the tuberculous animals.

In Table I are given the results of one experiment in which the injections were made in the dorsal region in 9 tuberculous and 6 normal guinea pigs. The figures showing significant differences are in bold-faced type. It will be noted in the table that the difference was more accentuated at 24 hours than at 4. This phenomenon becomes still more striking when the injections are made in the ventral zone, because in 24 hours the dye had spread throughout the normal animal but was still restricted to a measurable area in the tuberculous guinea pigs. These experiments have involved 80 injections in 13 tuberculous and 46 in 7 normal guinea pigs with consistent results.

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1 McMaster, P. D., *J. Exp. Med.*, 1937, **65**, 347.

2 Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1933, **57**, 751.

TABLE I.
Measurement of Mean Area of Spread of Dye Injected into the Dorsal Region in Guinea Pigs.

	1 hr.		4 hr.		24 hr.	
	Mean sq. mm.	Standard deviation	Mean sq. mm.	Standard deviation	Mean sq. mm.	Standard deviation
Normal 18 injections 6 animals	121 ± 5.6	35.6	**237** ± 11.4	71.7	**678** ± 40.4	253.9
Tuberculous 18 injections 9 animals	103 ± 4.7	29.4	**166** ± 10.1	63.6	**411** ± 21.5	135.5

No significant differences were found in the spread of the dye in the tuberculous guinea pigs in respect to the degree of the tuberculin reaction, for the spread was about the same in animals which reacted from + to ++++. However, tuberculous guinea pigs in the terminal stages of the disease, when they are losing weight and have become negative to the tuberculin test, have lost the power of restricting the spread of the dye, which then diffuses as rapidly as in the normal guinea pig.

An increased power to restrict diffusion of dye does not seem to characterize the tuberculous state in the rabbit, for in a group of 3 normal and 3 tuberculous rabbits, intradermal injections of the dye spread at the same rate.

These observations show that there is a normal difference in the rate of diffusion of dye in proportion to the thickness of the dermis. This rate of diffusion, as indicated by injections of dye, becomes altered, that is, delayed, in the tuberculous guinea pig. It may be possible that this phenomenon is one factor among the complicated processes involved in a tuberculin skin test. Thus, if we may assume that the rate of diffusion of dye is approximately an indicator of the diffusion of the protein, an animal in which the tuberculous infection has induced a delay in the rate of diffusion in the tissues is able to react to a smaller amount of tuberculo-protein, because a larger proportion of the material is retained in contact with the cells instead of being rapidly diffused throughout the body. Thus the rate of diffusion affects the concentration of material at the point of injection. The converse of this phenomenon is illustrated in the observations of Duran-Reynals[3] and of Thomas and Duran-Reynals,[4] in which they showed that an animal positive to a given amount of tuberculo-protein can be rendered less reactive by the introduction of a spreading factor with the protein.

[3] Duran-Reynals, F., *J. Exp. Med.*, 1933, **58**, 451.

[4] Thomas, R. M., and Duran-Reynals, F., *J. Exp. Med.*, 1935, **62**, 39.