A NOTE ON THE INFLUENCE OF THE CHEMICAL RAYS OF DAYLIGHT ON VACCINIA IN ANIMALS.

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In view of the observations made of late years on the action of red light in variola, it was thought that it might be of interest to ascertain, if possible, whether the red, or other rays of daylight, exerted any influence in cases of vaccinia; that is to say, with a disease the specific virus of which differs from that of variola probably only in that its pathogenic capabilities are modified, while vaccinia presents clinically many points of resemblance to variola.

As it was conceivable that any influence which rays of daylight might exert *in vivo* might also be exhibited on the virus outside the animal body, in the first instance experiments were made on vaccine material *in vitro*.

Experiments on vaccine emulsion in vitro.

Vaccine emulsions were subjected to the influence of variously coloured rays of daylight in the following manner:—

Vaccine pulp was collected in the usual way from a calf 120 hours after the animal had been vaccinated. A portion of this pulp was emulsified with four times its own weight of sterile distilled water, and was subjected to the chloroform process. A second portion of the pulp was emulsified with four times its own weight of a 50 % mixture of pure glycerine and sterile distilled water.

The chloroformed emulsion was partly used to fill seven small glass vessels of test tube shape each having a capacity of 5 c.c. Each tube was filled with vaccine and tightly corked.

One of each of these seven tubes was placed in a separate glass compartment. The first compartment was made of red glass, the second of yellow, the third of green, the fourth of blue, the fifth of violet, the sixth not coloured, and the seventh, also of uncoloured glass, was itself enclosed in a light-tight tin box.

The remainder of the chloroformed vaccine emulsion was put into *glass capillary tubes* and six of these filled capillary tubes were also placed in each of the seven above mentioned glass compartments. Capillary tubes were used in order that a thin column of vaccine emulsion, as well as the thicker columns of vaccine in the small test tubes, might be subjected to the various coloured rays.

The glycerinated emulsion was placed in small test tubes and glass capillary tubes in a similar manner and these were similarly placed in the compartments.

The compartments themselves were placed in a laboratory window facing north and were all equally subjected to the action of daylight throughout the day. Altogether vaccine from seven calves was treated in this manner, the exposure in the glass compartments lasting continually from the middle of July to the end of February. At the end of this time some rabbits and two calves were inoculated with all the seven vaccines of each compartment, a sample of each vaccine being taken from both a capillary tube and from one of the small test tubes.

It is needless to tabulate the result of these inoculations, for the vesiculations of both calves and rabbits failed to show any difference between the various portions of any one of the seven vaccines. Possibly the vaccine from the small test tubes gave rather better vaccination results than did the vaccine from the capillary tubes, but further than this no difference could be noted.

It would appear from these *in vitro* experiments that the resistance of the specific virus of vaccinia to the germicidal action of daylight is greater than the resistance usually shown by non-spore-bearing micro-organisms, and approximates more closely to the resistance of spore-bearing bacteria.

Experiments on Vaccinated Animals.

The animal experiments were conducted as follows:—Three rabbits, young animals—six to twelve weeks old—and as similar as possible in size and condition, were taken. Each animal had an area, roughly 4×5 millimetres, shaved on its back. The areas of each set of three

rabbits were inoculated in linear incisions with vaccine lymph from the same calf.

Immediately after inoculation the first rabbit was placed in a cage which had been closely covered with a double layer of red glazed cambric such as is used for photographic dark rooms. This covered cage was so situated that it received on its top and four sides direct daylight from sunrise to sunset. The red cover of the cage was only partially and momentarily removed twice in the 24 hours during the first 72 hours after the inoculation of the rabbit, for the purpose of placing the food inside the cage. After the first 72 hours the rabbit was removed for a few minutes to examine the condition of its vaccinated area, after which it was returned to the red light for a further 24 or 48 hours.

The second rabbit of the batch was, immediately after inoculation, placed in the dark, light being admitted to the cage momentarily only when feeding the animal. The rabbit was kept thus for 72 hours after inoculation, at the end of which time—as in the case of rabbit No. 1—it was removed for a few minutes to ascertain the condition of its vaccinated area. After examination it was replaced in the dark for 24 or 48 hours or more.

In experiments with red light in small-pox[1] any advantage which may have accrued in consequence of "red light treatment" may obviously have been due to the red light *qua* red light or to the fact that the chemical rays were occluded. By using a dark cage as an adjunct to the red light the advantage of the latter should be emphasised or detracted from as the case might be.

The third vaccinated rabbit was kept in such a way that it was in direct daylight during the daylight hours of the first 120 hours or more after its inoculation.

The cages of all three rabbits were arranged close together so that apart from the admission or occlusion of light rays, the animals were under the same conditions. The majority of the experiments were made at a time of year when it was daylight during more than 12 of the 24 hours. 31 sets of rabbits were thus treated (93 animals in all).

The results of these vaccinations were noted at varying intervals and are set forth in the accompanying Table.

The various degrees of development have been classified, somewhat

[1] *vide* Finsen, *Brit. Med. Journ.*, 1903, Vol. I. p. 1297, and *Lancet*, 1904, Vol. II. p. 1272. Schamberg, *Journ. Amer. Med. Assoc.*, 1903, Vol. XL. p. 1183, and 1904, Vol. XLIII. p. 1641. Ricketts and Byles, *Lancet*, 1904, Vol. II. p. 287, and 1904, Vol. II. p. 816.

roughly, into four classes. Class 1 includes all vesicles containing lymph of high virulence, Class 2 all vesicles containing lymph of secondary virulence, Class 3 vesicles containing lymph of virulence next below that of 2, and Class 4 all vesicles containing lymph of low virulence. Absence of vesiculation is recorded as 0. This classification takes no note of any sign other than the appearance of the vesicle itself.

The appearance of each areola was recorded and, according to its degree of intensity, was noted under one of three headings, the most extensive being 1, the medium 2, and that below the medium 3, while absence of areola was noted as 0.

TABLE.

	Class of areolae				Class of vesicles				
Nature of Exp.	1	2	3	0	1	2	3	4	0
Red light	8	8	8	7	23	1	2	4	1
Dark	11	14	4	2	17	1	5	8	0
Daylight	10	12	6	3	15	2	4	10	0

Dealing in the first instance with the tabulated results of the areolae, it will be observed that the largest number of the most severe areolae occurred in those animals protected from all light rays. Those animals which were exposed to red light had the smallest number of the severest type of areola, while the "daylights" had an intermediate number. Again, in the case of those animals which had least extensive areolae, if the "red lights" and the "daylights" are examined it will be seen that the former have the greatest numbers, whereas those animals protected from all light rays had a smaller number of mild areolae than had the "daylights." The same contradiction of results is seen if one examines the totals of those aninals which showed no areolae, for of these the "red lights" are in the greatest number, and the "darks" in the least, the "daylights" being intermediate.

It is clear that in the case of these experiments no deduction can be drawn as to the action of any of the rays of daylight in the production of vaccination areolae, unless it be that the degree of areola is uninfluenced by the presence or absence of daylight.

Proceeding to the examination of the vesicles it will be seen that by taking the actual total numbers of the results, it would appear that a distinctly larger number of cases exposed to red light developed vesicles of a highly virulent type than did those cases exposed to daylight; while those cases left in the dark, and therefore also excluded from the chemical rays, gave a somewhat larger number of virulent vesicles than did the cases exposed to daylight.

These figures are corroborated by examining the numbers of vesicles of low virulence developed in red light, dark, and daylight respectively. It will be seen that the proportion of vesicles of low virulence was rather larger among the "daylight" than among the "dark" animals, and much larger than among the "red lights."

In order to gain additional information on this point three goats were vaccinated and treated in a manner similar to that adopted in the case of the rabbits. The goats were placed in red light, daylight, and dark respectively, and the results of the vaccinations were noted from the sixth to the tenth days. In respect to vesiculation the results were in complete accordance with the results of the experiments with rabbits, for the goat exposed to red light developed vesicles of first-rate quality, the goat left in the dark developed vesicles almost as good, while the goat left in daylight yielded only poor vesicles.

As a further experiment four more goats were vaccinated and treated in a manner similar to that of the former three, except that it was not found practicable to use red light. One of the animals was placed in the dark, and the other three were tethered during the course of the experiment in an unshaded field. This experiment was carried out in the middle of June, 1906.

On the sixth, eighth, and tenth days after vaccination the goat excluded from daylight showed exceptionally good vesicular development, while none of the three animals kept in daylight showed any appearance of real vesiculation, dried lines of crust only appearing along the lines of inoculation.

A few days later a further experiment was made with two calves. Each of these calves was vaccinated from the same batch of vaccine lymph, following the procedure of the former experiments. One of the calves was placed in the dark, while the other was kept in the open field.

On the fifth day after inoculation the calf protected from daylight showed well-developed, typical vesicles, while the calf kept in the field failed to show more than the merest trace of vesiculation. This second animal was kept under observation under the same conditions for three weeks, during which time no further vesiculation appeared.

From these experiments it would seem at least to be strongly indicated :—

(1) That chloroform water emulsions, and glycerine water emulsions of vaccine lymph *in vitro* are not appreciably affected with regard to their potency by exposure to or protection from daylight. The vaccine

virus indeed would appear to resemble in this respect ordinary bacterial spores rather than the usual non-spore-bearing bacteria.

(2) The development of the areola in vaccinated rabbits is apparently unaffected by the exposure of these animals to the chemical rays of daylight, or by protecting them from such rays.

(3) That vaccinia in rabbits, goats, and probably in calves, *as a specific disease* is influenced in such a way by the prolonged exposure of the vaccinated animals to the chemical rays of daylight that its development is prevented to a greater or lesser extent.

Should this last point be established, the advantage in a vaccine establishment of protecting the animals used for the production of vaccine lymph from the rays of daylight is obvious.