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Studies on the serological relationships between avian pox, sheep pox, goat pox and vaccinia viruses

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SUMMARY

By using neutralization, complement fixation and immunogel-diffusion tests, it has been demonstrated that cross-reactions occur between various avian pox viruses and between sheep pox and goat pox viruses. No such reactions were demonstrated between avian pox viruses and vaccinia virus or between avian pox and sheep pox and goat pox viruses. Furthermore, no serological relationship was demonstrable between vaccinia virus and sheep pox and goat pox viruses.

INTRODUCTION

The antigenic relationships within the vaccinia-variola group of viruses have been studied by many workers. It has been shown that vaccinia, variola, alastrim, cow pox and ectromelia viruses are antigenically related (McCartney & Downie, 1948; Downie & Macdonald, 1950; Gispen, 1955; Woodroofe & Fenner, 1962). The antigenic relationships of contagious pustular dermatitis virus and pig pox virus to vaccinia virus have also been reported (Webster, 1958; Datt & Orlans, 1958). However, very little is known about the serological relationship of sheep pox or goat pox viruses to vaccinia or avian poxes. It has been reported that fowl pox and vaccinia viruses are antigenically unrelated (Ledingham, 1931; Burnet & Lush, 1936; Tsubahara & Kato, 1961; Harada & Matamato, 1962), but Takano (1948), by cross-protection tests in rabbits, and Takahashi, Kameyama, Kato & Kamahora (1959), by complement fixation tests and by fluorescent antibody staining techniques, demonstrated strong antigenic relationships between these two viruses. Woodroofe & Fenner (1962) also found that nucleoprotein (NP) antigen extracted from vaccinia or myxoma viruses appeared to contain a group antigen which was common to a wide variety of pox viruses.

This paper describes the study by neutralization, complement fixation and gel-diffusion tests of immunological relationships among avian and mammalian poxes. The avian viruses studied were fowl pox, pigeon pox, canary pox and duck pox and the mamalian viruses were sheep pox, goat pox and vaccinia.

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MATERIALS AND METHODS

Virus strains

Egg-adapted fowl pox, pigeon pox and canary pox virus strains were obtained from the Indian Veterinary Research Institute (I. V. R. I.) Mukteswar, India, the Commonwealth Serum Laboratories, Melbourne, Australia and the National Institute of Animal Health, Japan, repectively. A virus strain labelled as duck pox isolated by Rao (1965) from an outbreak in ducks from Andhra Pradesh (India), was also obtained in the form of scabs. In our experiments, the strain was neither infective to ducks nor could be adapted on chorio-allantoic membrane (CAM) of developing chick embryos. The scabs were therefore used as such in the immunogel-diffusion test.

A strain of vaccinia virus was obtained from Dr B. M. Gupta, Central Drug Research Institute, Lucknow, India.

Sheep pox, Jaipur and goat pox, Mukteswar strains, were obtained from the virus laboratory of I.V.R.I. Mukteswar. These strains have been maintained by regular passages in their respective hosts.

Preparation of virus suspension

The infected CAM of egg-adapted strains of fowl pox, pigeon pox, canary pox and vaccinia viruses were extracted with McIlvaine buffer pH 7·2 (citric acid phosphate–phosphate buffer 0·004 M). Suspensions were centrifuged at 2500 rev./min. for 15 min. and sediments were washed three times with buffer. The supernatants so obtained were centrifuged at 10,000 rev./min. for 1 hr. in a refrigerated centrifuge. Sediments were resuspended in McIlvaine buffer and were kept frozen in small volumes. Viruses were titrated separately in developing chick embryo by the CAM route, the chorioallantoic membranes were dropped in the manner described by Beveridge & Burnet (1946).

For the experiments in which the feather follicle method of inoculation was employed, dry scabs or CAM were ground and suspended in 50 % glycerine phosphate-buffer solution (pH 7·2).

Sheep pox or goat pox nodules, preserved in glycerine phosphate buffer, were washed well in McIlvaine buffer and then macerated in a pestle and mortar with the help of neutral glass powder and resuspended in McIlvaine buffer. The suspension was lightly centrifuged. Antibiotics were added to the supernatant to give a final concentration of penicillin and dihydrostreptomycin of 1000 i.u. and 100 μg./ml. respectively. The treated virus suspensions were stored in the refrigerator overnight and tested for sterility before inoculation. Whenever virus suspensions were partially purified before inoculation, the following procedure was adopted:

A 20 % suspension of infected CAM was made in 0·004 M McIlvaine buffer and was treated with 'Genetron 226' (CF_2Cl, $CFCl_2$) on the lines described by Datt (1964) with slight modifications.

Preparation of antisera

Normal control serum was collected from all experimental animals and birds before their inoculation with infective material.

Egg-adapted fowl pox virus in fowls

Fowl pox egg-adapted virus was introduced by the feather follicle method into the thighs of two White Leghorn chickens aged 8 weeks. There was a swelling of the follicles on the third day after inoculation and lesions coalesced by about the eighth day. Fifteen days after infection, each bird was given an intravenous injection of 0·5 ml. of partially purified fowl pox virus suspension. A second similar injection was administered to each bird after a further 7 days. The birds were bled for the collection of serum 1 week after the final injection.

Egg-adapted fowl pox virus in pigeons

Partially purified egg-adapted fowl pox virus was administered to two healthy pigeons by the feather follicle route. A fortnight after initial infection each pigeon was inoculated intraperitoneally with a 0·5 ml. dose of the infective material. Each bird received a similar inoculation 10 days after the first and this was followed, after a further 6 days, by an intravenous inoculation of the infective material at the same dose. Seven days after this final injection, the pigeons were bled and serum was collected.

Egg-adapted pigeon pox and canary pox viruses in fowls and pigeons

Immune sera against these two viruses were prepared on similar lines to those described above except that the first inoculation of canary pox virus was given subcutaneously as this virus, in initial experiments, failed to produce lesions in fowls and pigeons by the feather follicle route.

Egg-adapted vaccinia virus in rabbits

The egg-adapted vaccinia virus was first adapted into rabbits. The material obtained from such rabbits was inoculated into two healthy rabbits intradermally on either side of the flanks. After recovery, the rabbits were given three additional injections of partially purified vaccinia virus intravenously. Rabbits were bled 8 days after the last injection.

Egg-adapted vaccinia virus in fowls

Fowls were immunized by the method of McCartney & Downie (1948) except that the virus was partially purified with fluorocarbon before injecting the fowls.

Sheep pox virus in sheep and goat pox in goats

Two healthy sheep and two healthy goats, 8–12 months old, were selected. The abdominal region of each was shaved, cleaned thoroughly and inoculated intradermally with 0·2 ml. of infectious material in each of 20 sites. Sheep pox virus was

administered at a dilution of 10^{-3} (titre of the virus $10^{6.5}$/ml.) and goat pox virus at a dilution of 10^{-2} (titre of the virus 10^{5}/ml.). Sera were collected 3 weeks after inoculation.

Serological tests

Neutralization test

Preliminary trials were conducted to standardize the test. Selection of a suitable diluent for titration of the virus, the optimum temperature at which the virus and serum mixture should be held and the optimum period of interaction between virus and serum were determined. A known dilution of virus suspension in McIlvaine buffer, pH 7·2, containing *ca.* 700 (600–800) pock-forming units (pk.f.u.) per ml. was added to an equal volume of serially diluted antiserum. The mixture was allowed to react for 4 hr. at room temperature. Five 12-day-old embryonated eggs were inoculated on the CAM with 0·1 ml. of virus and serum mixture containing *ca.* 35 pk.f.u. The highest dilution of serum neutralizing more than 50 % of the pk.f.u. was taken as the titre of the serum.

Complement fixation test

Direct. The technique as reported by Uppal & Nilakantan (1966) in respect of sheep pox antigen-antibody systems was followed.

Indirect. The principle of this test was similar to that described by Rice (1948). Serial twofold dilutions of heat inactivated fowl sera in 0·2 ml. volumes were made. To each dilution of serum was added 0·1 ml. of antigen. The antigen dilution used was the highest dilution which had given complete fixation in the antibody titration. The system was kept overnight at 4° C. This was followed by the addition of 0·2 ml. of complement (2 M.H.D.) and 0·1 ml. of inactivated pigeon or rabbit serum (1 unit). After mixing, the test was kept in a water bath at 37° C. for 90 min.; 0·2 ml. of sensitized sheep red blood cells were then added to each of the tubes which were further incubated for 30 min. Suitable controls of antigen, antiserum and complement were included. The highest dilution of fowl serum showing 50 % haemolysis was taken as the titre of the serum. In the initial stage, 50 % suspensions of infected C.A.M. showing confluent lesions were made in veronal buffer and centrifuged at 3000 rev./min. for 15 min. The supernatant was titrated by box titration and was used as an antigen.

Gel-diffusion test

The Ouchterlony double gel-diffusion technique reported by Uppal & Nilakantan (1967) with sheep pox antigen and antibody was followed with slight modifications. Both absorbed and unabsorbed antisera were used. Sera were absorbed with freeze-dried uninfected and infected tissue powder. Wherever CAM was used as an antigen, the membranes showing confluent lesions were triturated in an equal volume of McIlvaine buffer and were used as antigens. Similarly duck pox scabs were suspended in McIlvaine buffer. The method of preparation of sheep pox or goat pox antigen was as described by Uppal & Nilakantan (1967).

RESULTS

Neutralization tests

The results of cross-neutralization tests in respect of fowl pox, pigeon pox and canary pox virus are shown in Fig. 1. The antisera showed their maximum neutralizing power against homologous strains of virus and varying degrees of neutralization could be demonstrated against each of the other strains. The avian pox viruses

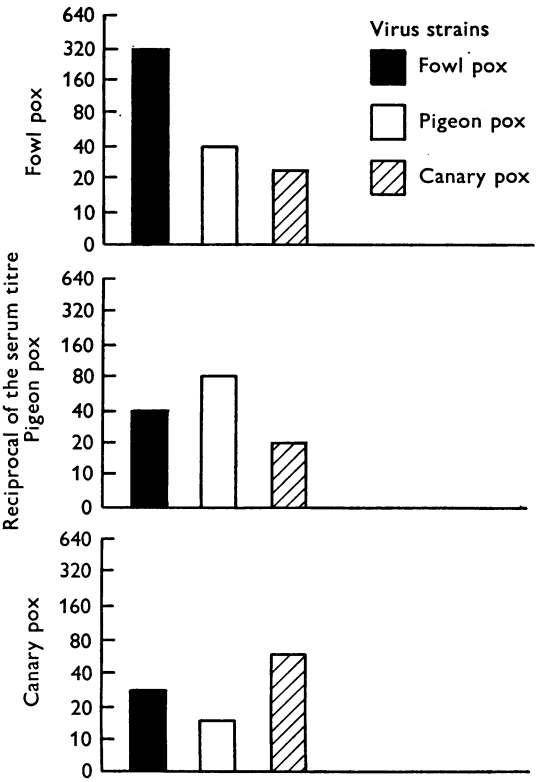


Fig. 1. The neutralization of fowl pox, pigeon pox and canary pox by homologous and heterologous antisera.

were not neutralized by the vaccinia, sheep pox and goat pox sera. The vaccinia virus was neutralized by antisera prepared against it both in fowls (1/640) and in rabbits (1/2560). It was not neutralized, however, by any of the avian pox, sheep pox, or goat pox antisera.

Complement fixation tests

The results of cross-complement fixation tests performed with homologous and heterologous systems using antisera against different avian and mammalian pox antigens are presented in Tables 1 and 2. Among avian poxes, there was cross-fixation between fowl pox, pigeon pox and canary pox viruses but no cross-fixation could be shown with vaccinia virus.

Vaccinial antisera fixed complement with the homologous antigen but there was no fixation when the antigen was prepared either from sheep pox or goat pox viruses. Both sheep pox and goat pox antisera showed relatively high titres with their homologous antigens and lower titres with heterologous antigens. Neither sheep pox or goat pox antisera showed any cross-fixation with vaccinia antigens.

Table 1. *Indirect cross-complement fixation test with vaccinia, fowl pox, pigeon pox and canary pox viruses and their immune sera*

	Antisera			
Antigen	Fowl pox	Pigeon pox	Canary pox	Vaccinia
Fowl pox	64	16	8	—
Pigeon pox	16	32	8	—
Canary pox	8	8	32	—
Vaccinia	—	—	—	128

Results are expressed as the reciprocals of the serum titres.

Table 2. *Direct cross-complement fixation tests with vaccinia, sheep pox and goat pox viruses and their immune sera*

	Antisera		
Antigen	Vaccinia	Sheep pox	Goat pox
Vaccinia	1280	—	—
Sheep pox	—	80	20
Goat pox	—	10	40

Results are expressed as the reciprocals of the serum titres.

Gel-diffusion tests

The results of gel-diffusion tests are presented in Table 3. Fowl pox antisera produced two precipitin lines against fowl pox antigen, one each against pigeon pox, canary pox and duck pox and none against vaccinia, sheep pox and goat pox antigens (Figs. 2, 3). Pigeon pox and canary pox antisera each gave one precipitin line with pigeon pox, canary pox, fowl pox and duck pox antigens but no precipitation lines were visible when these antisera were diffused against vaccinia, sheep pox and goat pox antigens. Similarly, duck pox antigen, diffused against fowl pox, pigeon pox and canary pox antisera, showed one precipitin line of identity but showed none when diffused against vaccinia, sheep pox and goat pox antisera (Figs. 4, 5).

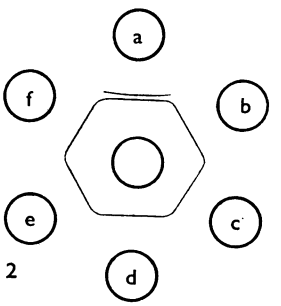
Three precipitating lines were observed when vaccinia antiserum was diffused against vaccinia antigen but there were no precipitin lines when vaccinia antiserum was diffused against fowl pox, canary pox, pigeon pox, sheep pox or goat pox (Fig. 6).

Both sheep pox and goat pox antisera exhibited two precipitin lines when diffused against their homologous antigens and one line when diffused against their heterologous antigens. No lines were observed when these antisera were diffused against vaccinia, fowl pox, duck pox, canary pox and pigeon pox antigens (Figs. 7, 8).

Table 3. *Formation of precipitin lines with homologous and heterologous avian and mammalian pox groups of antigen-antibody systems*

	Antisera					
Antigen	Fowl pox	Pigeon pox	Canary pox	Vaccinia	Sheep pox	Goat pox
Fowl pox	2	1	1	—	—	—
Pigeon pox	1	1	1	—	—	—
Canary pox	1	1	1	—	—	—
Duck pox	1	1	1	—	—	—
Vaccinia	—	—	—	3	—	—
Sheep pox	—	—	—	—	2	1
Goat pox	—	—	—	—	1	2

Results indicate the number of precipitin lines developed.



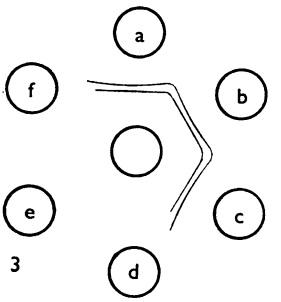
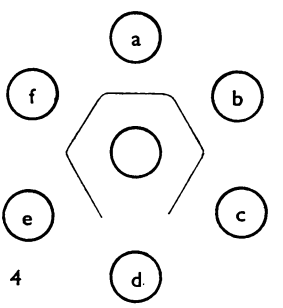


Fig. 2. Fowl pox antiserum in the central well and antigens (a) fowl pox; (b, d) pigeon pox; (c, e) duck pox and (f) canary pox.

Fig. 3. Fowl pox antiserum in the central well and antigens (a–c) fowl pox; (d) vaccinia; (e) sheep pox and (f) goat pox.



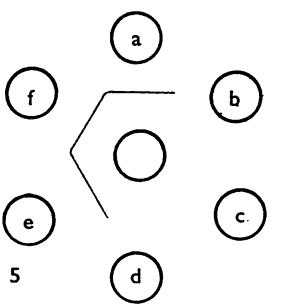


Fig. 4. Pigeon pox antiserum in the central well and antigens (a, e) pigeon pox; (b) canary pox; (c) duck pox; (d) vaccinia and (f) fowl pox.

Fig. 5. Duck pox antigen in the central well and antiserum (a) pigeon pox; (b) vaccinia; (c) goat pox; (d) sheep pox; (e) canary pox and (f) fowl pox.

Fowl pox, pigeon pox, canary pox and duck pox antisera, absorbed with uninfected chorioallantoic membranes, showed the same number of bands as were seen before such absorption. Similar antisera, when absorbed with infected membranes, showed no lines (Fig. 9). Similar results were obtained with vaccinia, sheep pox and goat pox absorbed antisera.

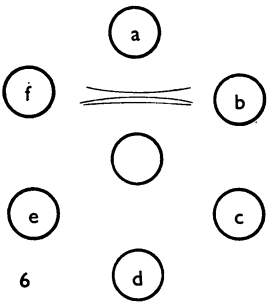


Fig. 6. Vaccinia antiserum in the central well and antigens (a) vaccinia; (b) fowl pox; (c) pigeon pox; (d) canary pox; (e) sheep pox and (f) goat pox.

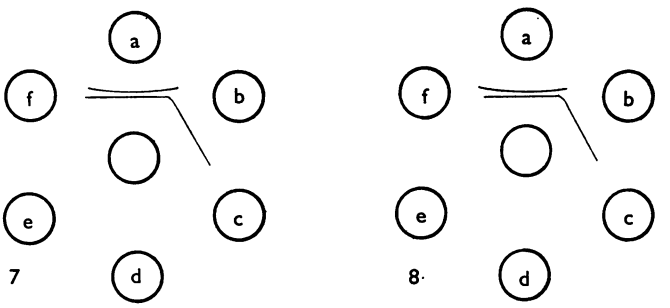


Fig. 7. Sheep pox antiserum in the central well and antigens (a) sheep pox; (b) goat pox; (c) vaccinia; (d) fowl pox; (e) pigeon pox and (f) canary pox.

Fig. 8. Goat pox antiserum in the central well and antigens (a) goat pox; (b) sheep pox; (c) vaccinia; (d) fowl pox; (e) pigeon pox and (f) canary pox.

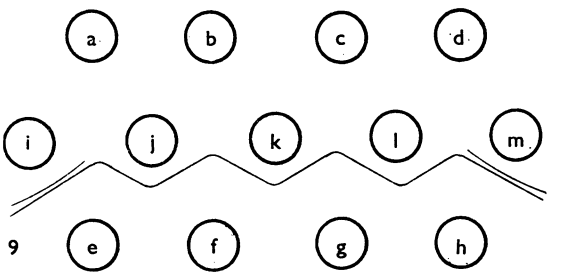


Fig. 9. Antigens in the central wells (i, m) fowl pox; (j, l) pigeon pox and (k) canary pox. Absorbed antisera with infected CAM in the top peripheral wells (a, d) fowl pox (b) pigeon pox and (c) canary pox. Absorbed antisera with uninfected CAM in the bottom peripheral wells (e, h) fowl pox (f) pigeon pox and (g) canary pox.

DISCUSSION

Uppal (1963) employing both complement fixation and immunogel-diffusion tests, reported that different strains of sheep pox virus (Jaipur, Bengalor and Mysore) isolated in India were antigenically related. In the present study it has been shown that sheep pox and goat pox viruses are also serologically related. With sheep pox or goat pox antigens, two precipitin lines were observed when diffused against their homologous antisera. The later findings are in agreement with the early observations of Uppal (1963); Sharma, Nilakantan & Dhanda (1966); Uppal & Nilakantan (1967) and Sen & Datt (1968) but are contrary to those of Bhambani & Murty (1963), who could not demonstrate any precipitin lines when sheep pox antigen was diffused against either sheep pox or goat pox convalescent sera. Nevertheless these workers considered sheep pox and goat pox viruses to be antigenically related on the basis of results obtained in gel-diffusion tests with antisera prepared in rabbits against goat pox scabs obtained from a natural outbreak of goat pox. Uppal & Nilakantan (1967), however, found that antisera prepared in rabbits against sheep pox scabs, even when partially purified with fluorocarbon, gave as many as three non-specific lines in gel-diffusion tests due to normal components of sheep skin or other tissues and they therefore regarded this method of study as unsatisfactory. In the present work, therefore, antisera were prepared against goat pox and sheep pox viruses in their natural, susceptible host. While the results indicate relationship between sheep pox and goat pox viruses, they further indicate that these two show no serological cross-reaction with either vaccinia virus or with avian pox viruses.

Woodroofe & Fenner (1962), obtained an NP antigen by alkaline extraction from vaccinia and myxoma viruses which appeared to contain a group antigen common to all pox viruses he examined. As they did not include sheep pox and goat pox viruses in their studies it would be interesting to investigate whether this group antigen is in fact common to them.

The lack of serological relationship between fowl pox and vaccinia as demonstrated by neutralization (Burnet & Lush, 1936), gel-diffusion (Gispen, 1955; Tsubahara & Kato, 1961) and complement fixation tests (Harada & Matamato, 1962; Woodroofe & Fenner, 1962) has been confirmed in the present study by concurrent application of all these serological tests. It was also found that pigeon pox, canary pox and duck pox viruses were unrelated to vaccinia virus.

The results indicate that there exist serological relationships between fowl pox, pigeon pox, canary pox and duck pox viruses. These findings are contrary to those of Miyamoto (1959) who claimed that there was no immunological affinity between canary pox virus and the viruses of fowl pox and pigeon pox. This claim, however, was based on the failure of cross-protection tests in immunized birds. The present findings are in agreement with those of Burnet & Lush (1936), who reported relationship between canary pox and fowl pox by neutralization tests, with those of Harada & Matamato (1962) who showed cross-reactions with avian poxes by the complement fixation test, and with Tsubahara & Kato (1961), who found at least

one common antigenic component in fowl pox, pigeon pox and canary pox viruses by the immunogel-diffusion test.

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