J. Hyg., Camb. (1971), **69**, 27
Printed in Great Britain

The kinetics of influenza-virus adsorption on iron oxide in the process of viral purification and concentration

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(*Received* 12 *August* 1970)

SUMMARY

This paper reports a study carried out to clarify the mechanisms involved in adsorption of influenza A and B viruses on iron oxide. Accordingly, the amounts of virus that are adsorbed from virus suspensions of varying concentrations per unit surface area of magnetic or non-magnetic oxide at fixed temperature and time have been determined. The principles involved are clearly the same as those involved in multiple equilibria during the interaction of particles with a large number of combining sites with different intrinsic affinity. Consequently, the amount of virus that is adsorbed per unit mass of iron oxide depends on the size of the adsorbent area, not on its magnetic property. Owing to a significant difference between the affinities of influenza A and B particles for the binding sites on iron oxide, unit surface area of the adsorbent is invariably capable of adsorbing significantly greater amounts of influenza A than B particles. The practical implications of these findings are that a better understanding of the mechanisms involved in virus adsorption on iron oxide will permit a more efficient separation of virus particles from impurities. The simplicity and the rapidity of the technique and the cheapness of the equipment required suggest that the iron oxide method is of great value for both small- or large-scale viral purification, whether it is used as a single step procedure or as a primary step followed by zonal separation.

INTRODUCTION

This study is concerned with principles involved in adsorption of influenza viruses A and B on iron oxide. Although no generalized theories can be formulated for the process, some practical rules for the use of this compound for viral purification and concentration can be deduced from the experience of the adsorption kinetics gained in this study. The adsorption of myxoviruses on magnetic iron oxide was originally described by Warren, Neal & Rennels (1966), providing a simple method of virus purification and concentration.

MATERIALS AND METHODS

Viruses

The following strains of influenza virus were used in this study: A0/PR8, A1/England/1/51, A2/Singapore/1/57, B/England/101/62 and B/England/13/65. These strains were obtained as allantoic virus from the Virus Reference Laboratory,

Colindale, and the WHO International Reference Centre for Respiratory Diseases, Salisbury. The substrains WS E720, NWS E714 and NWS E691 of influenza A0/WS virus were obtained as allantoic virus from Dr D. Hobson of the University of Liverpool. Virus was grown in the chorioallantoic sac of 10-day-old chick embryos for 48 hr. at 35° C. and the freshly harvested allantoic fluid was clarified by centrifugation at 2000 rev./min. The allantoic virus was used either fresh or after prolonged periods of storage at −20° or −70° C. Purified virus concentrates were prepared using the iron oxide adsorption-elution method of Warren *et al.* (1966). In order to achieve the optimum pH for virus adsorption (pH 7·0–7·2) the purified virus in elution buffer was dialysed against phosphate buffered saline (PBS) or distilled water. The haemagglutinin titres and the number of virus particles per haemagglutinating units were determined by conventional methods (Davenport & Minuse, 1964; Isaacs, 1957).

Iron oxide

Samples of magnetic (γ) and non-magnetic (α) iron oxides were supplied for this study by Minerals, Pigments and Metals Division, Chas. Pfizer and Co., Inc., Easton, Pennsylvania, U.S.A. The samples used in the study were as shown in Table 1.

Table 1. *Samples of iron oxide*

Batch	Formula	Type	Ultimate particle size (μ)	Surface area ($m.^2/g.$)
RX-2165 D	$\alpha Fe_2O_3.H_2O$	Non-magnetic	0·5	16–18
RX-2165 E	$\gamma Fe_2O_3.H_2O$	Magnetic (made from D)		
RX-2165 F	$\alpha Fe_2O_3.H_2O$	Non-magnetic	0·02	80–100
RX-2165 G	$\gamma Fe_2O_3.H_2O$	Magnetic (made from F)		
Mo 9853	$\gamma Fe_2O_3.H_2O$	Magnetic		

Although the ultimate particle size of the oxide is very small, the material tends to aggregate. To break up the aggregates and to provide a smooth dispersion, the above batches of iron oxide were jet-milled at an air pressure of 75 lb./in.^{2} and a feed rate of 1 c.c./15 sec. to achieve average aggregate size of less than 5 μ

Procedure of virus adsorption-elution

Adsorption. An appropriate amount of powdered iron oxide and virus, used either as crude (allantoic fluid) or purified suspensions, were mixed together in a screw-cap bottle or flask and agitated for 30 min. at room temperature using a Griffin Wrist Action Flask Shaker. The mixture was then centrifuged at 2000 rev./min. to separate virus-coated particles of the iron oxide from the supernatant fluid. This procedure was carried out with increasing concentrations of the adsorbent until the uptake per unit mass became constant. This constant uptake was assumed to correspond to the saturation point of the adsorbent.

Elution. The virus-coated iron oxide was suspended in 10% $Na_2HPO_4.7H_2O$ (pH 8·9) and the suspension was agitated for 30 min. at room temperature using

the flask shaker. All the adsorbed virus was recovered in the supernatant after the iron oxide was deposited by light centrifugation.

Protein estimations. The method described by Lowry, Rosebrough, Farr & Randall (1951) was used throughout this study.

RESULTS

Basic observations and expression of results

In this present study the availability of surface area of magnetic and non-magnetic iron oxides, γFe_2O_3 and αFe_2O_3 respectively, was expressed as the ratio of the number of virus particles to unit surface area of the adsorbent. The effect of 'favourable' ratios was that all virus particles were adsorbed, whereas a range of 'unfavourable' ratios gave rise to a pattern in which the amounts of adsorbed virus decreased in proportion to the decrease of available surface area of the adsorbent.

All adsorption-elution experiments employing iron oxide and suspensions of allantoic or purified virus used a batch procedure in which measured amounts of virus and oxide were mixed together under conditions where virus adsorbed quantitatively. The virus-coated iron oxide was then separated by low-speed centrifugation and the virus was eluted under conditions near zero adsorption. Each adsorption-elution experiment was always carried out in triplicate.

Table 2. *Adsorptive power of iron oxide;* tentative observations*

Biological particles (molecules)			
Identity	Size (mμ)	Weight	Adsorption-elution results
E. coli	800×2000	$1{\cdot}5 \times 10^{11}$	Excluded
Influenza virus	80–120	$2{\cdot}8 \times 10^{8}$	Adsorbed-eluted
Rhinovirus 2 (HGP)	20–30	—	Adsorbed-eluted
Haemoglobin (fowl)	3×15	$6{\cdot}8$–$7{\cdot}6 \times 10^{4}$	Adsorbed-eluted
Ovalbumin	—	4×10^{4}	Excluded

* Relates to the iron oxide amounts as used in influenza virus adsorption.

In general, the adsorption of the virus was very dependent on pH and salt concentration, but did not significantly change when virus particles were suspended in allantoic fluid, distilled water or a 0·1 M phosphate buffer solution over a range pH 6·8–7·3. Tentative observations concerning the adsorptive power of iron oxide indicated that the adsorbable range of biological particles was within 3×15 to 800×2000 mμ (Table 2). It may be emphasized here that the rate at which particulate material is adsorbed is influenced by the rate of contact between the adsorbate and the adsorbent during the adsorption procedure. When allantoic or purified influenza virus was adsorbed on iron oxide at room temperature under the conditions for interaction and binding between the virus and the iron oxide as provided by the Griffin Shaker, the adsorption percentage for two strains of influenza virus was as shown in Table 3.

Egg infectivity titrations showed that the adsorption on iron oxide followed by elution did not affect virus infectivity.

https://doi.org/10.1017/S0022172400021215 Published online by Cambridge University Press

Table 3. *Adsorption times of two influenza virus strains on iron oxide using a Griffin Flask Shaker*

Adsorption time (min.)	Virus particles adsorbed (%)	
	A 2/Singapore/1/57	B/England/13/65
5	50	12·5
10	75	37·5
30	100	100

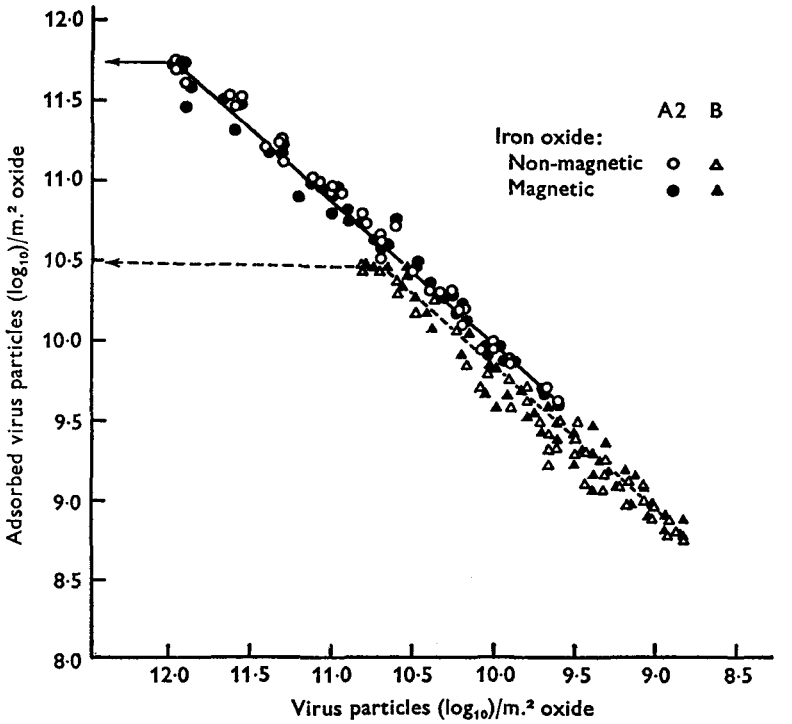

Fig. 1. The patterns of adsorption curves at constant temperature with influenza A 2 and B viruses.

Comparison of the adsorptivity of α and γ iron oxide

Adsorption kinetics of α and γ iron oxides

Originally, the purification of several viruses was accomplished with the use of γFe_2O_3 (magnetic iron oxide) by Warren *et al.* (1966). When it was found in our preliminary experiments that the magnetic property of iron oxide did not influence the rates of virus adsorption and elution and that virus purification could be readily accomplished using non-magnetic iron oxide, the mechanism involved in virus adsorption became of general interest. Work was undertaken therefore to investigate adsorption–elution kinetics of several batches of γ and α oxides and strains of influenza A and B viruses. The effect of six different ratios of virus to oxide surface area on the rate of virus adsorption was determined in triplicate experiments. Figs. 1 and 2 are representative of the results obtained with A 0/PR 8, A 1/England/

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1/51, A2/Singapore/1/57, B/England/101/62 and B/England/13/65 strains of influenza virus. The following explanations seem to be sufficient to account for the pattern of adsorption curves plotted in Fig. 1:

(1) It is evident that the virus adsorptivity of magnetic and non-magnetic oxides is virtually identical. Consequently, it appears that the process of virus adsorption is due to forces other than the magnetic property of the adsorbent.

(2) Operations near the saturation point of the adsorbent result in a series of adsorption values indicating that unit surface area of iron oxide is invariably

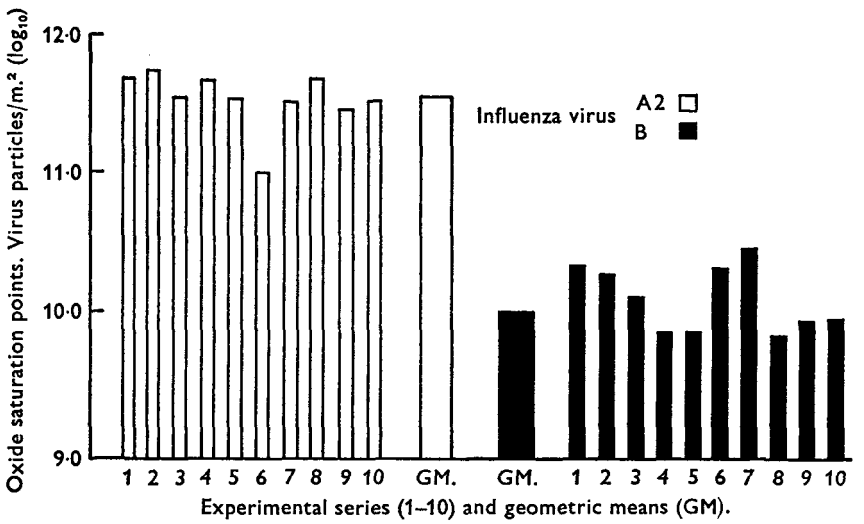

Fig. 2. The uptake of influenza A 2 and B virus particles per unit surface area of iron oxide at the saturation point of the adsorbent.

capable of adsorbing a greater amount of influenza A than B particles. As can be seen in Fig. 2, the amount of influenza B virus adsorbed is only 3·7 % that of A 2 virus. A study of the adsorption kinetics of a limited number of influenza A (including WS and NWS substrains) and B strains showed that strain variations did not affect the adsorption rate of crude or purified virus.

Determination of overall concentration and purification factors with α and γ iron oxide

In this series of experiments, specific activities expressed as HA units per μg protein were observed in the successive purification steps of two influenza virus strains. The results of this experiment revealed that by the use of iron oxide, virus can be efficiently purified and concentrated greater than 19 times as is shown in Table 4.

A comparison was also made between results obtained from the purification and concentration of two strains of influenza B virus, with an eightfold difference in virus particle content. The results, summarized in Table 4, show that purification factors, calculated on haemagglutinin and protein content, are higher with the allantoic fluid containing a greater number of virus particles. This may be due to the high background level of egg protein in virus allantoic fluid compared with virus protein.

https://doi.org/10.1017/S0022172400021215 Published online by Cambridge University Press

Table 4. *Viral material monitored for haemagglutinin and protein content during iron oxide purification*

Strain	Material	HAU/ml.	Protein μg./ml.	HAU/μg. protein	Purification factor
A 2/Singapore/1/57	Crude allantoic virus	5,144	360	14·2	—
	Concentrated eluate from iron oxide	98,304	42	2,340·5	164·8
	Eluate centrifuged at 35,000 rev./min., the virus pellet suspended in PBS, the same volume as the removed supernatant	98,304	36	2,730·6	192·2
B/England/13/65	Crude allantoic virus	128	255	0·5	—
	Concentrated eluate from iron oxide	1,536	80	19·2	38·4
	Eluate centrifuged at 35,000 rev./min., the virus pellet suspended in PBS, the same volume as the removed supernatant	1,200	24	50	100·0
B/England/101/62	Crude allantoic virus	1,024	353	2·9	—
	Concentrated eluate from iron oxide	12,288	32	384	132·4
	Eluate centrifuged at 35,000 rev./min., the virus pellet suspended in PBS, the same volume as the removed supernatant	12,288	24	512	176·6

DISCUSSION

The use of viral adsorption on iron oxide and elution in purifying and concentrating of influenza virus was demonstrated by Warren *et al.* (1966). These authors employed γFe_2O_3, permitting the use of a magnet for the separation of virus-coated particles of iron oxide. The present experiments were conducted (*a*) to study the mechanisms involved in viral adsorption on the iron oxide employing magnetic (γFe_2O_3) and non-magnetic (αFe_2O_3) iron oxides with different surface areas per unit mass for comparison and (*b*) to determine the amounts of influenza A and B viruses adsorbed per unit surface area of γFe_2O_3 and αFe_2O_3 at fixed temperature and time. The data presented show that the adsorption of influenza virus on iron oxide is clearly not associated with its magnetic properties, the mechanisms involved being of the same order as those observed in multiple equilibria of suspended particles with a large number of combining sites. Examination of the adsorptivity of influenza virus in a series of its ratios to iron oxide established that, although the adsorption rates of the same virus were virtually identical with γFe_2O_3 and αFe_2O_3,

there was, in contrast, a significant difference between the adsorption rates of influenza A and B virus particles with each oxide employed. Estimates made on the uptake of virus particles per unit surface area of iron oxide at the saturation point of the adsorbent showed that the uptake of influenza B virus was 3·7 % (P = 0·001) of that of influenza A virus. This finding indicates that these viruses differ significantly in their affinities for iron oxide and suggests a significant difference in surface properties between the viruses in question. Practical implications of all these findings are that they permit a better separation of virus particles from impurities and thus improve the iron oxide method, which offers a practical and useful technique for both small- or large-scale viral purification and concentration.

We are indebted to Dr J. Warren and Mr H. S. Greiner for advice, to Dr R. J. Merrills for protein estimations, to Mr J. E. Jeffries for jet-milling the iron oxide used in these studies, and to Mr J. L. Wood for information concerning the adsorption of *Escherichia coli* on iron oxide.

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