juglone and subsequent injury to capillaries. In conscious rats, massive abdominal contractions were illicited along with movements suggestive of discomfort. This continued for approximately 10 min and was followed by a period of depression. However, these depressed animals were readily aroused when stimulated. There were few reactions from animals that received corn oil only.

Summary. Juglone, administered ip, was found to produce an accumulation of fluid in the abdominal cavity of rats. That the fluid from the circulatory system was accompanied by proteins was suggested by soft clots that formed in the fluid. Changes in hematocrit, plasma proteins, lung weights, and electrolytes suggested that increased capillary permeability resulted from the irritative properties of juglone.

The technical assistance of R. Olson and J. Ries is gratefully acknowledged.

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Received Oct. 12, 1967. P.S.E.B.M., 1968, Vol. 127.

Antigenic Enhancement of Ether-Extracted Influenza Virus Vaccines by $AlPO_4$* (32748)

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It has been shown that the antibody response of humans vaccinated with hemagglutinins prepared by extraction with ether (HA) is equal to or greater than that found after administration of the same dose of intact virus vaccine (1–3). Furthermore, HA vaccines proved to be as potent as virus vaccines when tested in rabbits (4). However, one preparation of HA vaccine that yielded a superior antibody response in man failed to meet minimum potency requirements when tested in mice (3).

The present investigations were carried out to obtain further information on the relative potency of HA vaccines in mice. It was found that dilutions of HA vaccines yielded lower hemagglutination inhibition (HI) antibody titers and lower degrees of protection than equivalent dilutions of virus vaccines, but that addition of a mineral carrier largely restored the antigenic potency of the HA vaccines.

Materials and Methods. Viruses. The strains utilized were selected from the collection of the Virus Laboratory, Department of Epidemiology, School of Public Health, the University of Michigan and are described by currently accepted nomenclature.

Vaccines. Monovalent vaccines containing virus plus $AlPO_4$, HA plus $AlPO_4$, or HA without mineral carrier were prepared with prototype strains of influenza A and B. For each set, a single pool of infected allantoic fluid was employed. Virus was concentrated tenfold by adsorption and elution from chicken RBC. The concentrates were further purified by adsorption and elution from $BaSO_4$ (5). To aliquots of virus, Tween 80 was added to yield a final concentration of 1.25 mg/ml. After the addition of an equal volume of fresh anesthetic ether, the mixture was shaken for 10 min at 4°C. The aqueous phase was reextracted with ether until gelatinous material no longer was visible at the interphase. Five or six treatments generally sufficed. Excess ether was removed by bubbling with nitrogen at room temperature. Virus

* This investigation was conducted under the sponsorship of the Commission on Influenza, Armed Forces Epidemiological Board, and was supported by the U. S. Army Medical Research and Development Command, Department of the Army, under research contract number DA-49-193-MD-2066.

concentrates and ether-treated aliquots were held for 1 week at 4°C after the addition of formalin (1:2000). Inactivation of each suspension was proved by two negative serial passages in eggs. Formalin was then neutralized with sodium bisulphite. Vaccines with mineral carrier were diluted in buffered saline containing $AlPO_4$ (final concentration 3.2 mg/ml in each dilution). Concurrently, HA vaccines without mineral carrier were diluted in buffered saline. All vaccines were held under refrigeration without preservative until used.

Mice. Albino Swiss mice weighing 12–20 gm were obtained from a commercial supplier and used within 3 weeks after receipt.

Mouse protection tests. Groups of six mice were vaccinated intraperitoneally with 0.5 ml of dilutions of vaccines and revaccinated 7 days later. On the fourteenth day the mice were lightly anesthetized and challenged intranasally with 0.05 ml of dilutions of mouse-adapted virus. The titer of the inoculum was determined simultaneously in unvaccinated mice of the same shipment. At the time of challenge separate groups of 3–6 vaccinated mice were bled for antibody determinations. Challenged animals were observed for 10 days. Deaths and lesions were scored in the conventional manner and the data were used to calculate MLD_{50}, MS_{50} values, and protection indices (6).

Hemagglutination and hemagglutination-inhibition titrations. Standard pattern methods were employed (7). The concentration of chicken erythrocytes was 0.5%. Mouse sera were pooled by vaccine group and heated at 56°C for 30 min prior to use.

Results and Discussion. The potency of HA and virus vaccines with mineral carrier and of HA vaccines without mineral carrier was assayed in mice using materials prepared with A/Swine/31, A/PR8/34, A_1/FM1/47, A_2/AA/2/60, B/Lee/40 or B/AA/3/62 strains. The results of a typical experiment carried out with A/PR8/34 are shown in Table I. Prior to addition of $AlPO_4$, the hemagglutination titer of the PR8 virus suspension was 12,800 and of the HA suspension 2400. It is apparent from the data that at all corresponding dilutions the virus vaccine containing $AlPO_4$ induced the highest antibody levels and the greatest protection. The HA vaccine induced the lowest levels of antibody and the least protection. However, addition of $AlPO_4$ to HA vaccine markedly enhanced its potency. The levels of antibody and protection conferred were almost equivalent to those found after administration of the intact virus vaccine. Similar findings were obtained with the other strains listed above.

The poorer performance of the unadsorbed HA vaccines in mice is worthy of comment. While the hemagglutination titer of all strains fell after ether treatment, it has been shown that ether-treated virus suspensions contain the same amount of hemagglutinating activity as do untreated suspensions when both are tested by the photometric method of Drescher (8). Nevertheless, degradation of antigenic potency without loss of hemagglutinating activity might have occurred and, if so, that circumstance could be responsible for the weaker responses observed. That explanation however requires the additional assumption that ether extraction caused the same proportionate degradation in each lot of virus treated since the mineral carrier restored the antigenicity of HA vaccines to levels closely comparable to those of the untreated virus vaccines. The occurrence of a fixed increment of degradation in the present experiments seems unlikely since the virus–ether ratio varied over a wide range owing to differences in the concentration of virus present in the different lots. Moreover, it would be expected that different strains would exhibit different sensitivities to ether, in keeping with the known variability of influenza viruses.

Conversely, the marked effect of mineral carrier on the antigenic potency of HA vaccines suggests the hypothesis that particle size may be an important factor for full expression of the antigenic potential of viral envelope proteins. If so, there is apparently an upper limit to the influence of that factor. We, as others have done, found that $AlPO_4$ had only a slight enhancing effect on the antigenicity of intact virus suspensions. Evidently the envelope protein in whole virus suspensions functions at near peak levels. In contrast, the physical fragments of viral envelope protein obtained by ether extraction were consistently

TABLE I. Relative Potency of Experimental PR8 Vaccines Tested in Mice.

Vaccine given	Dil. of vaccine	10^{-4}	10^{-5}	10^{-6}	MLD_{50}	MS_{50}	Protective index	Titer HI
Virus + $AlPO_4$	10^{-1}	7 0 0 0 0 0	5 0 0 0 0 0	0 0 0 ∓ ∓ [a]	$<10^{-4}$	$<10^{-4}$	>724.4	1024
	10^{-2}	8 0 0 ∓ ∓ ∓	0 0 ∓ ∓ + +	0 0 0 0 ∓ ++	$<10^{-4}$	$<10^{-4}$	>724.4	1024
	10^{-3}	8 0 ∓ ∓ + ++++	9 0 0 0 ∓ +	8 0 0 ∓ ∓ +++	$<10^{-4}$	$10^{-4.25}$	407.4	384
	10^{-4}	7 7 8 + + [a]	5 0 0 ++ ++ ++++	0 0 0 0 + ++	$10^{-4.15}$	$10^{-4.74}$	131.8	96
HA without mineral carrier	10^{-1}	7 8 8 + ++ ++	7 7 8 ∓ + +++	0 0 ∓ ∓ ++ +++	$10^{-4.5}$	$10^{-5.11}$	56.23	96
	10^{-2}	5 5 6 0 + ++	5 5 5 6 6 0	8 0 0 ∓ ++++ ++++	$10^{-5.2}$	$10^{-5.84}$	10.47	4
	10^{-3}	5 5 5 6 6 0	5 5 5 7 8 0	7 8 8 8 8 ++	$>10^{-6}$	$>10^{-6}$	<7.24	8
	10^{-4}	4 5 5 7 7 9	5 6 6 6 6 7	5 7 7 8 8 8	$>10^{-6}$	$>10^{-6}$	<7.24	8
HA + $AlPO_4$	10^{-1}	0 0 0 0 0 [f]	0 0 0 0 + ++++	0 0 0 0 0 ∓	$<10^{-4}$	$<10^{-4}$	>724.4	512
	10^{-2}	0 0 0 0 0 +	7 0 0 0 ∓ ∓	0 0 0 0 0 ∓	$<10^{-4}$	$<10^{-4}$	>724.4	512
	10^{-3}	7 7 7 8 0 ++++	7 7 0 0 0 +++	8 9 0 0 0 +	$10^{-4.5}$	$10^{-5.12}$	54.95	256
	10^{-4}	5 5 7 +++ +++ ++	5 5 8 0 +++ [a]	8 0 0 0 ++ +++	$10^{-4.6}$	$10^{-5.38}$	30.20	24

Virus titrations:	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
Controls	4 4 4	4 5 5	8 9 0	8 9 0	9 0 ∓	10^{-6}	$10^{-6.86}$
	5 5 5	6 7 0	+ + +++	+ ++ ++++	∓ ++ ++++		

[a] Mouse missing.

less antigenic if unadsorbed. Since mineral carriers are believed to exert their antibody enhancing effects by increasing the particle size of the antigen given in vaccines, the most likely explanation for the present findings would seem to be that use of the mineral carrier achieved a near optimal particle size with undegraded HA antigens(9). Since the amount of $AlPO_4$ used in the present investigation adsorbed 50% or less of the hemagglutinating activity of the vaccines, a search for more efficient adsorbants might prove rewarding.

The fact that HA vaccines behaved as weaker antigens in mice but strongly in rabbits and humans remains unexplained. In considering this discrepancy it is noteworthy that in the separate experiments with the different HA vaccines a very large dose was used for the primary and booster inoculations of rabbits and that a large dose was given to adult human subjects who as a class generally exhibit booster responses (1–4). In contrast, the dosage of vaccines given to mice was deliberately lowered in order to determine antigenic extinction titers. The differences noted, therefore, may be dose dependent. However, the possibility that different species might respond differently to HA vaccines cannot be ignored. Clearly further studies are needed if the results of testing HA vaccines in animals are to be used as standards for predicting the performance of such products in man.

Summary. Virus vaccines with $AlPO_4$, HA vaccines and HA vaccines with $AlPO_4$ were prepared as monovalent suspensions using 4 strains of influenza A and 2 of influenza B. In all cases, dilutions of the HA vaccines induced lower antibody levels and lower levels of immunity than their virus counterparts. Addition of $AlPO_4$ restored the antigenicity of HA vaccines to levels almost equal to those of whole virus vaccines. The significance of these findings is briefly discussed.

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Received Oct. 12, 1967. P.S.E.B.M., 1968, Vol. 127.

Critical Exposure Time for Androgenization of the Rat Hypothalamus Determined by Antiandrogen Injection* (32749)

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It is well established that there is a postnatal steroid sensitive period during which testicular or exogenous androgen may induce a permanent masculinization of the hypothalamic mechanisms which regulate pituitary gonadotrophin secretion in the rat, i.e., androgenization (1–3). Recent studies indicate that pentobarbital can protect the 5-day-old female from this action of androgen, provided the barbiturate is administered within ap-

* Supported in part by USPHS Grant HD-01182, and a Training Grant from the Ford Foundation.

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