Cytological Response in Embryonated Eggs to Inoculums from Cases of Common Cold Infection.* (22290)

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Studies on common cold infections have involved principally transmission studies in human volunteers. Frequently, the results of such studies have been difficult to evaluate because of the variable human susceptibility and the indecisive clinical criteria for diagnosis of the common cold syndrome. Human inoculation experiments have served to demonstrate that common cold infection is a transient air borne viral disease(1,2), limited in pathogenicity to man and ape,(3,4). Propagation of the etiological agent in cultures of human tissues has been reported(5). Propagation of common cold agents in embryonated eggs has been affirmed(6-10) and denied (4,11) but in all instances the decisions rested on the response of human volunteers to experimental inoculations.

Recent observations have indicated that the propagation of influenza and mumps viruses in embryonated eggs was accompanied by an increase in numbers of histiocytes in the allantoic fluid (AF). Parallel studies by hemagglutination and histiocyte counts indicated that both of these effects occurred simultaneously. This non-specific cellular reaction was inhibited by antiserums mixed with the viral agents prior to inoculation, thereby affording some basis for identification of the agent. Using this as a reference point, less well recognized pathogens were studied for similar effect in AF. By following the cytological response in serially passaged AF in inoculated eggs, this effect was demonstrated with serum inoculums from acute cases of clinically diagnosed infectious and serum hepatitis(12,13). Nasal washings from clinically diagnosed cases of common cold were similarly studied in embryonated eggs and increased numbers of histiocytes in the AF were associated with such inoculums(14). Control inoculums from cases of allergic rhinitis have been negative. The experimental data on which these observations were based are described below.

Methods. Standard strains of influenza A, A^1, and B viruses† were each inoculated into the AF of six 9 day embryonated eggs. After 2 days of additional incubation the eggs were stored at 4°C overnight and blood-free AF was aspirated by syringe and needle. A 20 ml aliquot of each pooled AF was centrifuged at 2000 rpm for 20 minutes, the supernatant fluid was quickly decanted, and the sediment was resuspended in the residual fluid. A standard "Breed" loopful of the sediment was transferred to a 1 sq cm area on a clean glass slide. Duplicate smears of each specimen were air dried, stained with Wright's stain, and air dried. The total number of histiocytes in 100 consecutive fields was determined with a 5 X ocular and an oil immersion lens. The histiocytes referred to are mononuclear basophilic cells in which a distinct cytoplasm can be detected. In contrast to the rigid cell wall of the erythrocyte line of cell, the histiocyte was undulant. Blood in the AF was minimized by killing the embryos with refrigeration. Groups of 7 day embryonated eggs were inoculated into the chorioallantoic cavity with allantoic fluid containing mumps virus (Enders strain). Four days later the inoculated eggs were stored overnight in the refrigerator and the AF, collected on the morning, was processed for histiocyte content and hemagglutination titer. Controls for such inoculations consisted of normal AF, fluids from eggs previously inoculated with normal AF, and heated influenza and mumps viruses (61°C/30 minutes). The AF specimens were simultane-

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† Obtained from American Type Culture Collection, Washington, D.C.

ously examined for hemagglutinins with chicken erythrocytes(15). Antiserums from chickens, specifically immunized against the 3 influenza types, were mixed equal parts with viral inoculums and their *in ovo* effects on hemagglutinin production and histiocyte counts were determined. Nasal washings were collected from 21 human patients (medical students) having acute afebrile rhinitis, a syndrome coinciding with the conventional description and history of common cold. These cases occurred during the winter season and by careful selection those having a history of allergic rhinitis were excluded. Nasal washings were collected within 12 hours after onset of illness and a second specimen was collected 2 weeks later when the patient was asymptomatic. Five ml of sterile rabbit serum (2%) in physiological saline was instilled into the nostrils of the reclining patient and the fluid was permitted to run from the nostrils into a petri dish when the patient sat up. The nasal washings were treated with penicillin (1200 units per ml) and streptomycin (100 μg per ml), then 0.1 ml was inoculated into the allantoic cavity of six 7-day embryonated eggs. The shell holes were sealed and the eggs were incubated for 4 additional days at 38°C. The live embryos were then killed by overnight storage at 4°C. The allantoic fluid was collected and examined for histiocytes by the technic described above and the number of histiocytes per 100 oil immersion fields was recorded. Serial passages of AF were made with 0.1 ml into the allantoic cavity of 7-day eggs. Control inoculations consisted of normal allantoic fluid, heated inoculums (61°C/30 minutes), and convalescent nasal washings. Additional control inoculums consisted of nasal washings from 23 individuals with acute allergic rhinitis. The diagnosis was based on a history of allergic episodes following exposure to known allergens and on the microscopic detection of numerous eosinophilic leukocytes‡ in the nasal effluent. The nasal washings were collected in physiological saline and stored at −20°C and subsequent manipulation in eggs followed the technic described above.

It is essential to the accuracy of the test procedure that eggs of superior quality be incubated under carefully controlled conditions, especially with regard to temperature and humidity. Abnormal number of histiocytes were encountered in the AF of uninoculated eggs when they were exposed to high temperatures during the shipment to the laboratory. The eggs used during the cool time of the year were uniformly satisfactory. Experimental procedures were performed at least in duplicate and all fluids were cultured for bacterial content in thioglycollate medium.

Results. The propagation of influenza and of mumps viruses was accompanied by the appearance of hemagglutinins and of histiocytes in the AF (Tables I and II). The cell counts significantly exceeded the normal levels (Fig. 1). Both histiocyte and hemagglutinin production were specifically reduced by treatment of inoculums with homologous antiserums (Table I). Histiocyte counts and hemagglutinins were negative at the same titration end-point in eggs (Table II).

Antibiotic-treated nasal washings from 15 of 21 common cold patients induced increased numbers of histiocytes in the AF of inoculated eggs. Hemagglutinins could not be detected. The embryos did not die as a result of such inoculations. The counts ranged from borderline with 5 specimens to marked increases with 10 specimens (Fig. 1). Subsequent inquiries of the donors revealed that 3 of the negative specimens were collected later than 24 hours after onset of illness. The normal limit of 15 cells per 100 fields was not exceeded with AF from passage of "normal" AF and with AF from uninoculated eggs. The

TABLE I. Serum Neutralization of Influenza Viruses. Detection by histiocyte count and by hemagglutination technics.

Virus	Without serum	+ antiserum for influenza A	A′	B
A	360/ 640*	0/ 0	177/320	140/320
A′	104/2560	224/ 640	1/ 0	143/640
B	192/ 160	312/1280	316/640	0/ 0

* No. of histiocytes per 100 fields/reciprocal of hemagglutination titer.

‡ Stained with "Hansel" stain, Lide Laboratories, St. Louis, Mo.

TABLE II. Titration of Inoculums in Embryonated Eggs.

Agent	No. of histiocytes per 100 oil immersion fields							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Influenza A	—	200+/1280*	180/2560	266/2560	200/2560	0/ 0	—	—
" A'	—	200+/2560	237/2560	210/2560	288/2560	152/1280	102/320	0/0
" B	—	182 /1280	151/1260	154/ 640	3/ 0	—		
Mumps	46/160	47 / 160	3/ 0	12/ 0	—			

* Histiocyte count/hemagglutinin titer.

effect of nasal washings from the 11 convalescent patients who were available contrasted markedly with that of their specimens collected during the acute stage of illness (Fig. 2): none of the convalescent specimens engendered counts in excess of the normal limit. These were collected and processed by the same method as the acute specimens. Simultaneous inoculations into groups of eggs of untreated and of heated nasal washings indicated that the agent responsible for the high histiocyte count was thermolabile (Fig. 3).

Smears from allergic rhinitis tissues revealed numerous eosinophilic leukocytes, whereas smears from the common cold cases contained numerous epithelioid mononuclear cells, and varying numbers of neutrophilic leukocytes, the latter predominating during the later stages of illness. Nasal washings

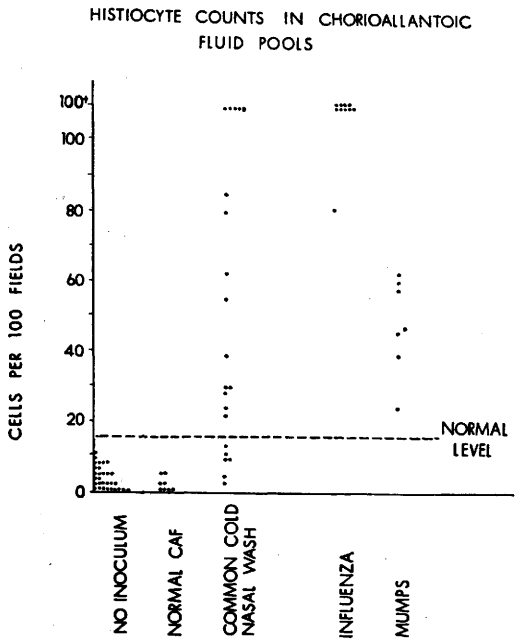

FIG. 1.

TABLE III. Histiocyte Response to Inoculums from 23 Patients with Allergic Rhinitis.*

Date nasal wash		Histiocytes in AF/100 fields
Collected	Inoculated	
9–13–55	9–16–55	11
10– 1	10– 5	4
1	5	3
13	14	5
14	14	4
14	14	8
14	14	3
14	18	0
14	18	10
14	25	6
24	26	10
24	26	5
21	26	7
24	26	4
27	11– 9	5
11– 1	2	4
4	9	15
4	9	4
4	9	3
8	9	3
8	9	4
8	9	4
1– 9–56	1–10–56	15

* Numerous eosinophilic leukocytes noted in smears of nasal effluent.

from 23 cases of acute allergic rhinitis failed to induce histiocytes in excess of the normal limit (Table III). Nasal washings collected during the same period from common cold patients showed in 7 of 8 instances increased numbers of histiocytes in inoculated eggs. (Table IV).

TABLE IV. Histiocyte Response to Inoculums from 8 Cases of Afebrile Rhinitis (Common Cold).

Date nasal wash		Nasal smear	Histiocytes in AF/100 fields
Collected	Inoculated		
10–24–55	10–26–55	Epithelial cells	64
3	11– 9	Mononuclear cells	62
11– 8	9	Rare eosinophiles	100+
11	16	Epithelial cells	17
18	18	No cells	30
21	23	Epithelial cells	70
12– 8	12– 9	*Idem*	75
1– 5–56	1–10–56	"	65

Passage of AF, from eggs in which high counts had been engendered with acute common cold specimens revealed high counts during the succeeding 5 and 6 passages. Thereafter the majority of cell counts declined abruptly to normal levels. Continued passage was accompanied by irregular rises and falls in cell counts. Serial passages of AF from uninoculated eggs induced no significant cell counts in AF. Hemagglutination could not be demonstrated in any of the passaged AF which originated from the acute common cold cases. Bacterial agents could not be demonstrated in any of the fluids by microscopic and by culture technics.

Discussion. The cytological content of AF appears to reflect viral activity, as has been demonstrated in parallel studies for hemagglutinin and histiocyte counts in influenza and mumps-infected allantoic fluids. Both technics were equally sensitive. A similar interpretation might be ascribed to this cytological response by inoculums from acute common cold cases. When the acute nasal washings were treated at 61°C for 30 minutes, this response was negated. Furthermore, the histiocyte response did not occur

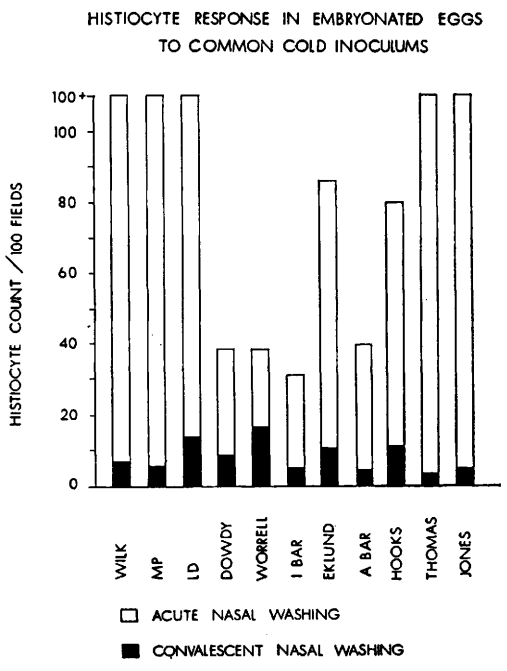

FIG. 2.

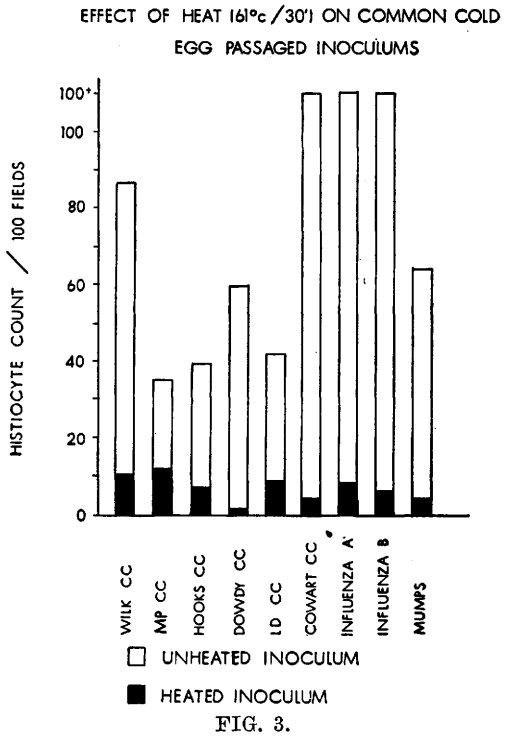

FIG. 3.

with nasal washings collected from the same patients during convalescence. The agent responsible for the histiocyte response may not be the etiological agent of common cold infection; however, its chronological association with the syndrome is suggestive of that possibility. If latent viral agents were responsible for the histiocyte response, they should have been demonstrable in the convalescent nasal washings.

The significance of these results is further supported by the failure of nasal washing from allergic rhinitis cases to engender a significant histiocyte response in AF. Such allergic manifestations were often clinically indistinguishable from the acute cases of common cold. The conditions under which the specimens were collected had no influence on the results, since all specimens were collected and stored under the same conditions (Tables III and IV). The agent(s) do not appear to be influenza-like, since egg passaged material failed to agglutinate chicken erythrocytes.

The histiocyte response is associated with inoculums collected during the acute stage of common cold illness. Although this is a non-

specific response, it might be rendered significant through inhibition by specific antiserums as was demonstrated with influenza virus (Table I). Neutralizing antiserums, prepared in chickens with several strains of common cold agent thus far studied, have demonstrated more than one antigenic strain.

It would be premature to say that even one common cold agent has been adapted to or propagated in the tissues of the embryonated egg. The decline of cytological response with passage gives no support to this proposition. Further studies on optimal passage interval and on tissue susceptibility may provide a means of adapting such agents to avian tissues and the process may be revealed through the cytological response in the AF of inoculated eggs. This would provide a technical advantage over the use of human volunteers for the detection of virus.

Summary. (1) Influenza and mumps viruses induced increased numbers of histiocytes in the allantoic fluids of embryonated eggs. The significance of this response was enhanced by coincident hemagglutinin production and by an interference effect on both responses by specific antiserums. (2) Nasal washings from acute common cold (afebrile rhinitis) patients engendered increased numbers of histiocytes in the allantoic fluid of embryonated eggs. The agent(s) responsible for this cytological response were thermolabile, were not inhibited by penicillin and streptomycin, and produced no detectable growth in bacteriological media. The agent(s) were not demonstrable in nasal washings collected from the same patients during convalescence. (3) Nasal washings from cases of acute allergic rhinitis did not induce increased numbers of histiocytes in the allantoic fluid of inoculated eggs.

1. Kruse, W., *Munch. Med. Wochschr.*, 1914, v61, 1547.

2. Foster, J. B., Jr., *Inf. Dis.*, 1917, v21, 451.

3. Dochez, A. R., Shibley, G. S., and Mills, K. C., *J. Exp. Med.*, 1930, v52, 701.

4. Andrewes, C. H., *Brit. Med. Bull.*, 1953, v9, 206.

5. Andrewes, C. H., Chaproniete, D. M., Gompels, A. E. H., Pereira, H. G., and Roden, A. T., *The Lancet*, 1953, Sept. 12, 546.

6. Kneeland, Y., Jr., Mills, K. C., and Dochez, A. R., PROC. SOC. EXP. BIOL. AND MED., 1936, v35, 213.

7. Pollard, M., and Caplovitz, C. D., *Science*, 1947, v106, 243.

8. Topping, N. H., and Atlas, L. T., *ibid.*, 1947, v106, 636.

9. Ward, T. G., and Proctor, D. F., *Am. J. Hygiene*, 1950, v52, 91.

10. Pollard, M., and Caplovitz, C. D., *Am. J. Hygiene*, 1948, v47, 106.

11. Andrewes, C. H., Lovelock, J. E., and Sommerville, T., *Lancet*, 1951, Jan. 6, 25.

12. Pollard, M., and Diserens, L. T., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 236.

13. ———, *Fed. Proc.*, 1955, v14, 416.

14. ———, *Bact. Proc.*, 1955, 61.

15. Hirst, G. H., *J. Exp. Med.*, 1942, v76, 195.

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Inhibition by Aminopyrine of Adrenocortical Activation Caused by Pyrogenic Reaction.* (22291)

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Like a number of other stressful stimuli, typhoid vaccine has been shown to cause stimulation of the pituitary-adrenal axis in rats (1). In human subjects, Arendshorst and Falls(2) showed the occurrence of eosinopenia following typhoid vaccine administration, and Rosen(3) and Conn, *et al.*(4) demonstrated increases in urinary 17-ketosteroid and "corticoid" excretion after injection of

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