spectral characteristics ($E_{420} = 0.203$, $E_{440} = 0.199$, $E_{520} = 0.286$, $E_{540} = 0.320$ per mg; cell length 1 cm) of the color produced in the carbazole reaction do not agree with those of any simple mixture of monosaccharides. Furthermore, after 0.7 saturation with sodium sulfate, the liquid phase contained significantly more activity and carbohydrate per mg of nitrogen than the precipitate.

Our data suggest that the active principle of our preparations is a protein or glycoprotein. It differs in its chemical and physical properties from the active preparation with similar or identical biological effects obtained from leukemic urines by Turner and Miller.[4] The possibility still exists that their product which is regarded as a ketoacid occurs as a prosthetic group in our preparations. However, the loss of activity which we observed on treatment with trypsin would indicate that any prosthetic group present ought to be less potent than the intact molecule.

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14818

A Comparison of Various Methods of Demonstrating Influenza Virus in Throat Washings.*

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The detection of influenza virus in throat washings is an important method, during epidemics, of determining the causative virus as rapidly as possible. Methods of isolating influenza virus in ferrets and mice,[1] in hamsters,[2] and in chick embryos by amniotic[3] or allantoic[4] inoculation have been described, but a comparison of the efficiency of these 4 methods on the same group of throat washings has not been available. During the epidemic of influenza A in 1943-44, 83 throat washings from military personnel[5] were tested in ferrets or hamsters, or in both species, and a smaller number of the same washings were inoculated into chick embryos.

Methods. Ferrets were inoculated intra-

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[3] Burnet, F. M., *Brit. J. Exp. Path.*, 1940, **21**, 147.

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nasally under light ether anesthesia with 2 cc of unfiltered throat washings. Temperatures of the animals were recorded twice daily, and blood specimens were collected from all animals before, and 10 days after, inoculation.

Hamsters were inoculated intranasally under ether anesthesia with 0.5 cc of unfiltered throat washings, and 2 to 3 days after the first inoculation the animals were reinoculated with the same washing according to the method described by Taylor.[2] Blood specimens were collected by cardiac puncture before the first, and 10 days after the second inoculation.

The paired serum specimens from the ferrets and hamsters were tested for an increase in antibodies by means of the Hirst chicken cell-agglutination technic,[6] using the PR8 and several recently isolated strains of influenza virus A, and the Lee strain of influenza virus B. No attempt was made to carry out passages in ferrets or hamsters. The diagnosis of influenza in most of the cases was also established by the complement fixation and chicken cell-agglutination tests on the human serums.

Initially 6 to 24 chick embryos 11 or 12 days of age were inoculated by the amniotic route with each throat washing filtered through Berkefeld N filters. With most specimens the window method of Burnet[3] was used, but with some the stab method described by Hirst[7] was also employed. Three to 5 amniotic passages at intervals of 2 to 5 days were carried out with each throat washing before the result was considered negative. Virus was detected in the amniotic or allantoic fluids by agglutination with human, guinea pig, or chicken erythrocytes[8] and was identified by inhibition tests with known type A and B antisera.

Allantoic inoculations were done with unfiltered throat washings centrifuged for 20 minutes at 4,000 R.P.M. in an angle head and also with duplicate specimens treated with Zephiran‡[9] at a dilution of 1:20,000 for 20 to 30 minutes. Only 1 passage was done because of the overgrowth of bacterial contaminants in almost all of the embryos inoculated by this method. The virus was detected and identified by the method used for the amniotic passages. From the third to the sixth day after inoculation allantoic fluids were withdrawn daily by a needle and syringe from the living embryos and tested for agglutinins so that they might be harvested or passed if positive.

Results. The results of inoculating ferrets with throat washings from 62 patients are summarized in Table I. None of the throat washings from 23 patients with negative serological tests for influenza A produced any antibody response to that virus after intranasal inoculation into ferrets. Of the 30 throat washings from patients with positive serological tests for influenza A, 17 (56.7 ± 9.05%) produced in the ferrets an increase in antibodies to influenza virus A. With 2 exceptions all of the ferrets showing an increase in antibodies also developed a febrile reaction 2 to 3 days after inoculation. Four of the 41 ferrets showing no increase in antibodies had a similar febrile response. All ferrets were also tested for antibodies to influenza virus B, and none showed an increase.

Each of 55 throat washings was inoculated into 2 hamsters. The results are presented in Table II. Not included are 2 throat washings from serologically negative patients which were also negative in hamsters. Of the 20 throat washings that were positive in ferrets, shown in Table II, 17 (85.0 ± 7.98%) were also positive in hamsters. Thirty, or 56.6 ±

TABLE I.
Results of Inoculating Ferrets with Throat Washings.

Serological tests for influenza A	Antibody response to influenza virus A in ferrets	
	No. positive	No. negative
Positive	17	13
Not done	4	5
Negative	0	23

‡ Zephiran, manufactured by Alba Pharmaceutical Co., Inc., is a mixture of high molecular alkyl dimethyl benzyl ammonium chlorides.

[6] Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

[7] Hirst, G. K., *J. Immunol.*, 1942, **45**, 293.

[8] Burnet, F. M., and Bull, D. R., *Australian J. Exp. Biol. and Med. Sc.*, 1943, **21**, 55.

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TABLE II.
Results of Inoculating Hamsters and Chick Embryos with Throat Washings.

Serology of cases	Responses of ferrets to throat washings	Antibody response in hamsters		Amniotic inoculation		Allantoic inoculation	
		No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.
Positive	Positive	13	3	3	8	2	7
Positive	Negative	0	12	0	3	0	1
Positive	Not done	13	8	0	2*	0	0
Not done	Positive	4	0	4	0	0	3
Total		30	23	7	13	2	11

* These 2 throat washings were positive in hamsters.

6.81%, of the 53 specimens from known cases of influenza A were positive in hamsters, a result which is identical with that obtained in ferrets. Twelve throat washings that were negative in ferrets produced no antibody response in hamsters.

For the purpose of determining the effects of antigenic variations the pre- and post-inoculation serums from all of the ferrets, and a large proportion of the hamsters, receiving throat washings were tested with 2 or more strains of influenza virus A isolated during the epidemic of 1943-44 in addition to the strain PR8. Two ferrets failed to develop a definite antibody response to the strain PR8, but did show an increase in antibodies to the recently isolated strains. This was also true of 2 sets of hamsters inoculated with other throat washings. In one or two instances the antibody response of the animals was negative with one of the recently isolated strains, but positive with others and with PR8.

The results of inoculating chick embryos are summarized in Table II. Seventeen, or all but 3, of the throat washings inoculated by the amniotic route, had been shown to contain virus by tests in ferrets or hamsters. Of these 17 specimens, 7 (41.2 $\pm$ 11.9%) infected chick embryos amniotically. Virus was demonstrated in 4 throat washings in the first passage and in 3 on the second passage, but usually 1 or 2 additional amniotic or allantoic passages were necessary before the virus reached sufficient titer to be positively identified as influenza A. In the early amniotic passages of 2 strains the titer of amniotic fluid was higher with guinea pig cells than with chicken cells. This is in accord with the observations of Burnet.[8]

Virus was demonstrated in 2 out of 13 unfiltered throat washings on the first allantoic passage. Ten of the same 13 throat washings were treated with Zephiran 1:20,000 before inoculation into the allantois of chick embryos. In one case virus was isolated from both Zephiran treated and untreated throat washings. The strain of virus isolated from the Zephiran treated throat washings was carried for 4 allantoic passages with the addition of Zephiran. A streptococcus contaminated each of the passages and it was necessary to filter the fourth passage material before carrying the virus further in chick embryos. A similar procedure was followed with the other strain isolated from an untreated throat washing.

Discussion. The results indicate that hamsters and ferrets are probably equally reliable as experimental animals for the demonstration of influenza A virus in human throat washings, as judged by the antibody response of these animals. Chick embryos may have been less reliable, but they afforded the advantage of more rapid isolation of virus.

Demonstration of virus in ferrets and hamsters required 10 to 14 days, a period of time equal to that required for serological diagnosis with the human serum. Isolation of virus from the first passage ferrets could be accomplished by passing nasal washings to mice or chick embryos according to the method of Burnet,[8] and from hamsters by passage of lungs and turbinates to mice according to the method of Taylor and Parodi.[2]

With chick embryos the required time for

identification of the virus was 3 to 10 days, depending on the number of passages before positive agglutinations of red cells were obtained. With about 5 to 10% of the throat washings it was possible to identify the virus within 3 to 4 days, using either the amniotic or allantoic inoculation. Zephiran at a concentration which would not inactivate the virus was not consistently effective in removing bacteria. It is quite possible that different results in ferrets, hamsters, and chick embryos would be obtained with throat washings from patients with influenza B.

Summary. During the epidemic of influenza A in 1943-44, throat washings from military personnel were tested in ferrets or hamsters, or in both species, and a smaller number of the same washings were inoculated into chick embryos.

None of the throat washings from 23 persons in whom response to serological tests for influenza A virus was negative produced antibodies to that virus after intranasal inoculation in ferrets.

Of 30 throat washings from persons showing positive response to serological tests for influenza A virus, 17 (56.7 ± 9.05%) produced in ferrets an increase in antibodies to the virus. With 2 exceptions all ferrets showing an increase in antibodies also developed fever 2 to 3 days after inoculation. Four of 41 ferrets showing no increase in antibodies had similar febrile reaction.

Thirty, or 56.6 ± 6.81% of 53 throat washings from persons with known cases of influenza A were positive in hamsters, a result identical with that obtained in ferrets. Twelve throat washings that were negative in ferrets produced no antibody response in hamsters.

Seventeen throat washings shown to contain influenza A virus by tests in ferrets or hamsters were inoculated into chick embryos by the amniotic route. Of these, 7 (41.2 ± 11.9%) infected the embryos. Virus was demonstrated in 4 throat washings in the first passage and in 3 in the second, but usually 1 or 2 additional amniotic or allantoic passages were necessary before the virus reached sufficient titer to be positively identified as influenza A.

In 2 of 13 unfiltered throat washings inoculated into the allantois of chick embryos, virus was demonstrated on first passage.

14819 P

Electrocardiographic Changes in Epidemic Parotitis (Mumps).*

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In January, 1943, a 20-year-old white sailor entered the hospital with epidemic parotitis and on the fifteenth day of his illness complained of constant retrosternal pain and of dizziness on arising. His pulse rate was 35 per minute and the electrocardiogram revealed complete heart block with prolongation of the QRS interval (0.12 sec.). He improved rapidly and by the eighth day following the onset of his angina the electrocardiogram was normal. Early this year, another 20-year-old white sailor, under treatment for mumps, developed precordial pain and palpitation on the seventh day of illness. The pulse rate fell to 28 per minute and the electrocardiogram showed 3:1 heart block. Three days later, another electrocardiogram revealed complete auriculo-ventricular dissociation. His condition slowly improved and by the 77th day following the onset of his cardiac pain the elec-

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