d-calcium pantothenate, 0.02 mg pyridoxine, 0.10 mg nicotinamide and 15 mg choline. A test group of 25 animals received the same dietary except that the fat was replaced by a synthetic one, each molecule of which contained one molecule of dihydroxy-stearic acid and two of the same fatty-acids that were present in the control fat. All animals in the control group survived the 70-day test period, gained an average of 170 grams and showed no abnormalities on autopsy. The test group gained weight less rapidly, showed within 20 to 30 days external evidences of blood loss and extensive internal hemorrhage on autopsy. Blood samples were taken from tail incisions for hemoglobin estimations. Incisions in the test group animals failed to heal normally, and many of the animals died from acute blood loss. Only 4 of the 25 test animals survived as long as 70 days.

Since this syndrome resembled that reported for vitamin K deficiency, this experiment was repeated with three test groups. The first group received the same dietary and supplements as the test group in the previous experiment. The second received in addition a daily supplement of 0.10 mg of 2-methyl-1, 4-naphthoquinone and the third a daily supplement of 4 mgs of liver extract (Lilly).

The first and third groups developed the same deficiency syndrome within the same period as before and showed the same internal picture on autopsy. Blood-clotting time[4] was much prolonged in comparison with normal rats of the same age (24 minutes *vs.* 1.5 minutes). The average hemoglobin value and red cell count were low, while the average white cell count was slightly increased. Nucleated red cells were seen in stained blood smears. The therapeutic value of 2-methyl-1,4-naphthoquinone was demonstrated by feeding this compound to deficient animals. Blood-clotting times became normal within three days. Furthermore, none of the rats fed the naphthoquinone throughout the test period developed the syndrome and all showed a favorable weight increase.

In summary we may say that rats normally do not require vitamin K in their dietary because it is synthesized in and absorbed from the intestine. However, in this case, a diet supplying all critical factors known to be necessary to the rat induced vitamin K deficiency when the fat of the diet was replaced by a synthetic fat containing a large proportion of dihydroxy-stearic acid. The deficiency syndrome was prevented and cured by the administration of 2-methyl-1,4-naphthoquinone. Further research is under way to determine the mechanism by which this disorder is produced.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR THE REMOVAL OF BACTERIAL CONTAMINANTS FROM SUSPENSIONS OF INFLUENZA VIRUS[1]

THE isolation of influenza virus commonly involves the inoculation of ferrets or of fertile eggs with throat washings. This source material is of course heavily contaminated with a variety of bacteria, a fact which sometimes complicates the procedure very considerably. Similarly bacterial contaminants may be the cause of trouble in laboratories where influenza virus strains are carried through numerous serial passages in chick embryos. Filtration through candles or ultrafilters does not always solve the problem, for the virus itself may be retained along with the offending organisms. We wish to describe here a method which permits destruction of contaminants without inactivating the associated influenza virus.

In earlier work[2] it was noted that Zephiran, while possessing strong bactericidal powers for the majority of pathogens, does not inactivate type A or type B influenza virus in concentrations as high as 1/10,000 acting for one hour at room temperature. Tests conducted with Zephiran 1/1,000 in physiological saline showed that this relatively concentrated solution does not produce pulmonary irritation when administered to mice by the intranasal route.

For the present experiments a 10 per cent. aqueous solution of Zephiran (a mixture of high molecular alkyl-dimethyl-benzyl-ammonium chlorides) diluted as necessary in nutrient broth or physiological salt solution was used. The effect of Zephiran on 10-day-old chick embryos was tested by inoculating groups of 4 eggs with each of a series of dilutions. 0.1 ml aliquots of 1/5,000, 1/10,000, 1/20,000 and 1/100,000 dilutions of Zephiran in normal saline solution were injected into the allantoic fluid of the eggs, after which the latter were incubated at 37.5° C. All the embryos survived the observation period of 78 hours.

Since throat washings from patients with influenza were not available, artificial mixtures were prepared

[1] The opinions advanced in this paper are those of the writers and do not represent the official views of the Navy Department.

[2] Albert P. Krueger and Unit Personnel, *U. S. Naval Medical Bulletin*, 40: 3, 1942.

[4] R. B. H. Gradwohl, "Clinical Laboratory Methods and Diagnosis," 2nd ed., 406, 1938.

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by having two normal individuals gargle with 20 cc of broth. A 10 per cent. mouse lung suspension of strain W. S. was diluted 1:50 with one of these specimens and the other was used as diluent for a mouse passage strain of type B virus (Lee). The mixtures were added to like volumes of Zephiran (1/10,000 in normal salt solution), thus reducing the virus dilution to 1 per cent. of the original 10 per cent. lung suspension and the Zephiran to a final concentration of 1/20,000.

After standing at room temperature for 20 minutes aliquots were removed from the specimens for inoculation into eggs. Three 0.1 ml amounts of each ten-fold dilution ranging from 10^{-3} to 10^{-8} were injected into the allantoic fluid of 10-day-old eggs. The eggs were incubated at 37.5° C. for 48 hours and the allantoic fluids were then harvested, pooling those from the three eggs of each dilution. Five mice were inoculated with the pooled egg material from each dilution, using 0.05 ml inocula administered intranasally.[3] As animals died they were promptly autopsied and lung lesions noted; those surviving 10 days were sacrificed and the lungs inspected for lesions.

The results of the animal experiments are summarized in Table 1. In the case of the W. S. strain the 10^{-4} dilution employed for egg inoculation contained active virus as did the 10^{-6} dilution of Lee strain material.

TABLE 1

RECOVERY OF INFLUENZA VIRUSES FROM ZEPHIRAN-TREATED THROAT WASHINGS*

Strain	Virus dilution of egg inoculum	Mouse reaction†				
WS (Type 'A')	1:1,000	3	3	3	3	4
	1:10,000	2	3	3	3	3
	1:100,000	K_0	K_0	K_0	K_0	K_0
	1:1,000,000	K_0	K_0	K_0	K_0	K_0
	1:10,000,000	K_0	K_0	K_0	K_0	K_0
	1:100,000,000	K_0	K_0	K_0	K_0	K_0
Lee (Type 'B')	1:1,000	2	3	4	4	4
	1:10,000	2	3	4	4	5
	1:100,000	3	3	3	4	4
	1:1,100,000	3	3	3	4	6
	1:10,000,000	K_0	K_0	K_0	K_0	K_0
	1:100,000,000	K_0	K_0	K_0	K_0	K_0

* Treatment with 1/20,000 Zephiran 20 minutes at 20° C. rendered throat washings free [illegible] were inoculated into eggs and after [illegible] were administered to mice (0.05 ml intranasally).

† Numbers indicate days elapsed between inoculation and death. Subscripts indicate lung lesions if mouse was sacrificed on 10th day. K_4 is complete consolidation, K_0 is none.

Aerobic and anaerobic cultures of the throat washings prior to treatment with Zephiran revealed heavy growths of those organisms commonly found in the mouth and pharynx. After exposure to 1/20,000 Zephiran for 20 minutes no bacteria could be isolated from 48-hour cultures of the throat washings or from 9-day subcultures.

[3] The Unit Personnel of Naval Laboratory Research Unit No. 1, *Jour. Lab. and Clin. Med.*, 27: 1197–1198, June, 1942.

Stock virus suspensions originally harvested from infected eggs and mice, and accidentally contaminated with bacteria during handling were readily freed from contaminants by Zephiran treatment as outlined for the throat washings. Cultures showed that the numbers of contaminating bacteria were smaller than in the case of throat washings, but in every instance the preparations maintained full virus potency and remained free from organisms after exposure to 1/20,000 Zephiran for 20 minutes. Of particular significance is the fact that the method functioned effectively with contaminated mouse-lung suspensions despite the quantities of tissue particles present. The dilution of Zephiran in broth did not lessen the disinfecting action.

Our findings indicate that it is possible to free influenza virus contained in throat washings, egg fluids or ground mouse lung menstrua from adventitious bacterial contaminants by treatment with 1/20,000 Zephiran for 20 minutes at 20° C. This agent does not inactive either type A or type B influenza virus under these conditions.

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