eggs and its identity as A virus was established. In one case the total elapsed time from inoculation of the throat washings until the confirmation of the identity of the virus by specific serum was only 9 days. Whether B virus and other as yet undescribed viruses from influenza cases will behave similarly is now being determined.

At present neither the mechanism of the agglutination phenomenon nor its specificity for influenza virus infection is well understood. However, the following facts have emerged:

(a) When infected allantoic fluid, either fresh or stored at −72° C. (from which the red cells had been removed by low-speed centrifugation), was mixed in a test tube with washed normal adult chick red cells, an agglutination phenomenon occurred. This *in vitro* agglutination was somewhat slower than the one previously described. Here a positive agglutination reaction was usually visible in 5 to 20 minutes. The red cells sedimented rapidly and formed a characteristic, ragged, granular pattern on the bottom of the tube. If red cells were added in the test tube to allantoic fluid from uninfected chicks, only the slow sedimentation of red cells occurred with no aggregation, and in settling out the cells formed a sharp round disk in the bottom of the tube. If the allantoic fluid from chicks infected with PR8 virus was diluted before adding the red cells, agglutination was still visible in a concentration of 1:512 (final concentration of allantoic fluid).

(b) When normal chick embryo red cells were added in sufficient numbers to allantoic fluid and allowed to settle out, over 99 per cent. of the virus disappeared from the supernatant fluid.

(c) When the allantoic fluid was centrifuged (45 minutes, 11,500 r.p.m.) the "titer" of the supernatant in terms of agglutinating capacity dropped approximately four times. This fall in agglutinating titer was consistent with the expected drop in virus titer as determined by previous tests in mice with the same fluid which showed that 70 to 90 per cent. of the virus was sedimented.

(d) If, instead of infected allantoic fluid, the supernatant from centrifuged ground mouse lung infected with PR8 mouse passage virus was used, the added red cells agglutinated in a dilution as high as 1:5000 (final concentration of mouse lung).

(e) When influenza A ferret antiserum (PR8) in dilutions as high as 1:1024 was mixed with allantoic fluid infected with the homologous virus, the agglutination phenomenon was inhibited. The inhibition was specific, that is, influenza B ferret antiserum in dilutions as low as 1:8 failed to inhibit the agglutination of red cells by fluids containing influenza A virus.

(f) Such inhibition also occurred with human serum, and in Table I is a titration of acute and convalescent serum from a proven case of influenza A. Dilutions of serum were mixed with a constant amount of W.S. infected allantoic fluid; then a constant amount of chick embryo red cell suspension was added, and the agglutination was read in 1 hour. The change of titer of this agglutination-inhibiting substance following infection is obvious and appears to be of the same order of magnitude as the rise in the patient's neutralizing titer against PR8 virus as determined in the mouse.

TABLE I

COMPARISON BETWEEN IN VITRO INHIBITION OF RED CELL AGGLUTINATION AND MOUSE NEUTRALIZATION TESTS WITH ACUTE PHASE AND CONVALESCENT SERUM FROM A CASE OF INFLUENZA A

Serum	Constant amount of infected allantoic fluid tested against various dilutions of serum*		Results of neutralization test in mice against uniform dose of virus†	
	Dilution of serum	Agglutination after 1 hour	No. died	No. survived
Acute	1:8	++	4	0
	1:16	++++		
	1:32	++++	4	0
	1:64	++++		
Convalescent ...	1:8	−	0	4
	1:16	−		
	1:32	−	0	4
	1:64	−		
	1:128	−	0	4
	1:256	−		
	1:512	+	3	1
	1:1024	+++		
Saline control, no virus		−		
Virus control, no serum		++++		

* The W.S. strain of influenza virus was used for the infection of chick embryos.
† The PR8 strain of influenza virus was used for the protection test in mice.

Such an *in vitro* test as shown in Table I will be of great advantage over the mouse neutralization tests if it can be shown that it measures influenza neutralizing antibodies. Our results so far suggest that the amount of agglutination-inhibiting substances in sera parallels the neutralizing antibody titer more closely than the complement-fixing titer. Whether this *in vitro* test will be sensitive and specific enough to replace the mouse protection method for determining serum neutralizing antibodies, is a problem at present under investigation. A more complete report will be published at a later date.

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ON THE SPECIFIC ADSORPTION OF ALCOHOLS AT THE SALICYLALDEHYDE/WATER INTERFACE

DURING a recent investigation by one of us (D.F.C.) of the interfacial tensions between water and mixtures of isoamyl alcohol and salicylaldehyde, it was observed that, over a wide range of isoamyl

alcohol content, the interfacial tensions were so low as not to be measurable with the Du Noüy tensiometer. In order to study this phenomenon in another aspect, the E.M.F.s of the cell—

					A_1			
Hg	Hg_2Cl_2	0.0001 N. KCl						
			B_1			B_2		
Salicylaldehyde (1)				Sat. KCl			Salicylaldehyde (2)	
	A_2							
0.0001 N. KCl		Hg_2Cl_2		Hg				

—were investigated. Alcohols were titrated by means of a microburet into the oil phase (2). Precautions were taken to ensure proper mixing. The E.M.F.s were measured by means of an electrometer valve circuit and a compensating potentiometer.

A large series of alcohols was thus investigated. It was found that, in comparison with most primary normal alcohols, isobutyl and isoamyl alcohols caused a much larger initial drop in the E.M.F. curve, whereas secondary octyl alcohol showed a markedly smaller effect (see Fig. 1). The most surprising results of the investigation were, however, the larger effects shown in general by alcohols containing odd numbers of carbon atoms in the chain and the distinct specificity shown by normal heptyl and nonyl alcohols. The changes in the E.M.F. of the cell occasioned by the addition of 4 per cent. by volume of homologous primary normal alcohols to the oil phase (2) are shown in Fig. 2, where the E.M.F.s are plotted against the numbers of carbon atoms in the alcohol chains. It will be observed that the heptyl and nonyl alcohols give effects several times greater than those of the other primary alcohols, while the nonyl effect is the greater of the two. The point X indicates the E.M.F. for a similar system containing secondary octyl alcohol, which differs from the heptyl alcohol by a methyl group in the 1-position.

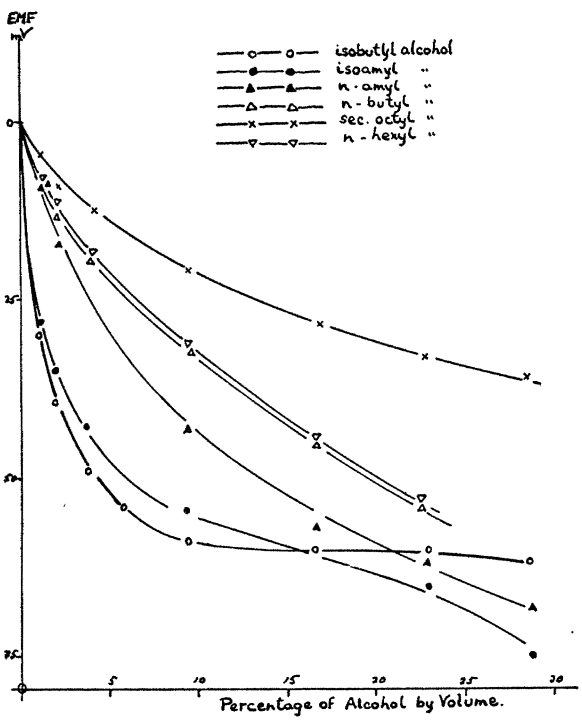

FIG. 1

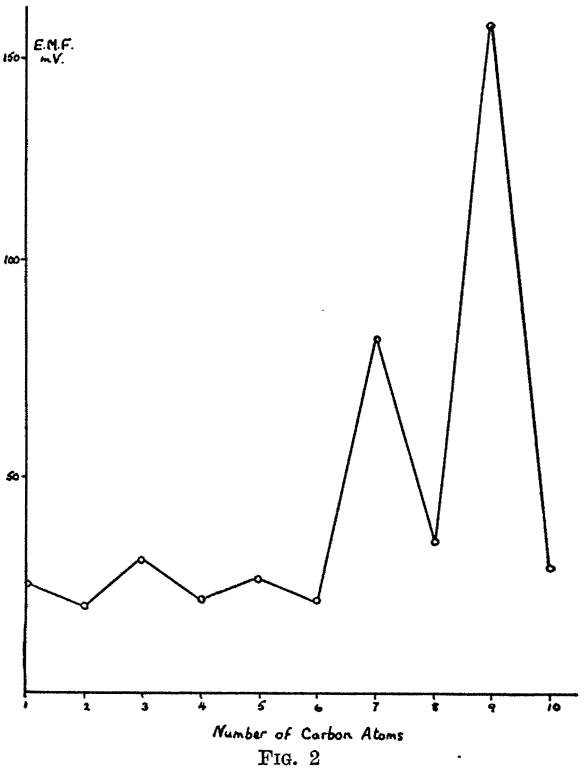

FIG. 2

The primary alcohols benzyl and phenyl ethyl alcohols give effects of the same order of magnitude as those of most normal alcohols, whereas cyclohexanol gives a curve whose slope is much greater.

Measurements on the cell— Hg		Hg_2Cl_2		Sat. KCl				
Salicylaldehyde (1)			Salicylaldehyde (2)			Sat. KCl		
	Hg_2Cl_2		Hg					

—indicated that the titration of alcohol into the oil phase (2), which was separated from (1) by either a porous membrane or a long diffusion path, caused only minor potential changes. In the original cell, therefore, the principal phase boundary effect must have been located at the interface A_2, the interfaces A_1, B_1 and B_2 being almost constant.

We have been led to the conclusion that the effect is due, directly or indirectly, to the interfacial adsorp-

tion of the alcohols at the salicylaldehyde/water interface. We consider that changes in the polar characteristics of the interfacial layer of salicylaldehyde molecules and water dipoles are induced by the alcohol molecules in accordance with their steric disposition. These changes at the A_2 interface will lead in turn to electrical asymmetry of the cell, due to different states of ionic distribution at the A_1 and A_2 interfaces. The salicylaldehyde/water interface may therefore be considered as having the properties of a specific receptor for alcohols with certain arrangements of the carbon chain.

The phenomena described above may conceivably lead to a clarification of certain selective phenomena in biological systems, such as, for example, the changes in the properties of local anesthetics caused by different carbon chain arrangements and the specificity shown by the olfactory chemoceptors.

Further observations are proceeding, and their results, together with a more detailed account of the above, will appear in the *Arkiv för Kemi,* Stockholm.

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EXPERIMENTAL VITAMIN P DEFICIENCY

RUSZNYÁK and Szent-Györgyi[1] were the first to find that flavones (citrine) behave like vitamins in man. The new vitamin was named P vitamin, because of its effect on the permeability of capillaries. Later Bentsáth, Rusznyák and Szent-Györgyi[2] found that scurvy in guinea-pigs is not only due to vitamin C deficiency, but is a mixture of deficiency in C and P vitamins. Zilva[3] could not confirm these later experiments, and Szent-Györgyi[4] did not succeed in reproducing them. Two years ago Zacho[5] showed that the diminution of capillary resistance in guinea-pig scurvy has no connection with a lack of ascorbic acid, and can only be made to cease with citrine. It seemed that with the help of a method based on this result vitamin P deficiency could be studied and the efficiency of various citrine preparations controlled. Our own experiments are in agreement with those of Zacho, and we succeeded in showing that those citrine preparations which have a therapeutic action in man, cause the diminished capillary resistance to disappear in the guinea-pig. As it appeared that the scurvy diet is not only deficient in ascorbic acid, but in flavones also, we have studied the effect of a scorbutogenic diet on rats. It is well known that the rat does not develop scurvy even on a diet lacking ascorbic acid. It appeared that under the influence of a scorbutogenic diet the rats did not, in fact, develop scurvy even after a long period of time, but their capillary resistance, measured with the Borbély method, diminished considerably in 5 to 6 weeks. When we gave such rats with diminished capillary resistance 3 to 4 mgm. of citrine per day subcutaneously, their capillary resistance became normal in 10 to 14 days. It became clear, therefore, that one can study vitamin P avitaminosis and control the efficiency of citrine preparations on guinea-pigs with scurvy and rats kept on a scorbutogenic diet. These animal experiments are in entire agreement with the results of Scarborough,[6] who has recently published observations which prove the occurrence of isolated P avitaminosis in man.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PREPARING NITRATE-FREE SEA WATER

IN the photometric or colorimetric determination of nitrate in sea water by the "reduced strychnine" method, nitrate-free sea water of the same chlorinity as the water being analyzed is required for the preparation of the standard solutions used in the estimation of the unknown solutions or calibration of the photometer.

Harvey[1] first mentioned the difficulty of obtaining nitrate-free sea water. However, he made the observation that surface water from the English Channel during the spring months usually contained less than ten microgram atoms of nitrate-nitrogen per liter. (A microgram atom is a millionth of a gram atom.) Riddell[2] also observed that at the time of extensive diatom flowering certain waters from the Georgia Straits were nitrate-free. Unfortunately, naturally occurring nitrate-free sea water is not always available when needed. Because of this sea water is often freed of nitrate by conversion of the nitrate to ammonia by boiling for several hours with amalgamated

[1] *Nature,* 138: 27, 1936.
[2] *Ibid.,* 138: 798, 1936; 139: 326, 1937.
[3] *Biochem. Jour.,* 31: 915, 1488, 1937.
[4] *Hoppe-Seylers Zeits.,* 255: 126, 1938.
[5] *Acta path. scand.,* 16: 1411, 1939.
[6] *Lancet,* 2: 644, 1940.

[1] H. W. Harvey, *Jour. Mar. Biol. Asn. United Kingdom,* 14: 71–88, 1926.
[2] W. A. Riddell, *Jour. Biol. Board Canada,* 2: 1–11, 1936.