200 *S* was obtained, which corresponds to a molecular weight of 10 million or more, depending on molecular shape. These experiments also revealed that, after complete sedimentation, the virus could not, in general, be recovered without some loss in titer, due presumably to inactivation or to mechanical difficulty in completely resuspending a very small sediment.

Comparison of the observations recorded here for SK murine poliomyelitis virus with the results obtained by Gard and Pedersen,[4] who worked with Theiler's virus of spontaneous mouse encephalomyelitis, suggests that the two viruses are not significantly different in the physical sense. Thus, Theiler's virus was found to have a sedimentation constant of 160; a molecular weight of 52 million and molecular dimensions of 640 mμ for the long diameter and 14 mμ for the short diameter were estimated from sedimentation and optical diffusion measurements; these data were obtained with a fraction which represented 0.5 mgm out of 100 gm of infected mouse brain, and which carried practically all the original infectivity. The preliminary results reported here for SK murine poliomyelitis virus are therefore in fair agreement with those of the Swedish workers. For human poliomyelitis virus obtained from monkey cords, a slightly lower sedimentation constant of 62 has been recently reported.[5] The activity of the viruses belonging to the poliomyelitis group, therefore, regardless of the strain of virus considered, appears to be always associated with material of very heavy molecular weight. On the other hand, these viruses are able to pass through membranes of small porosity, and particle diameters of about 10 mμ have been obtained from the filtration of all three strains.[6,7,8] The apparent contradiction between sedimentation and filtration measurements is not completely resolved if one makes the permissible assumption that all three strains consist of long thread-like molecules, since the lengthwise progression of such molecules through collodion membranes of low permeability does not seem quite plausible. It is probably more rational, therefore, to suppose that the rapid sedimentation of these viruses is due to the fact that they are present mostly as large aggregates of elementary particles, conceivably in linear association, which are able to break up, to some extent, into smaller active units. This view would be supported by analytical measurements of the diffusion of Theiler's virus.[9]

[4] S. Gard and K. O. Pedersen, SCIENCE, 94: 493, 1941.
[5] H. S. Loring and C. E. Schwerdt, *Jour. Exp. Med.*, 75: 395, 1942.
[6] W. J. Elford, I. A. Galloway and J. R. Perdrau, *Jour. Path. and Bact.*, 40: 135, 1935.
[7] M. Theiler and S. Gard, *Jour. Exp. Med.*, 72: 49, 1940.
[8] M. Sanders and C. W. Jungeblut, *Jour. Exp. Med.*, 75: 631, 1942.
[9] J. Bourdillon, *Jour. Gen. Physiol.*, 25: 263, 1941.

The essential purity of the materials obtained by Gard and Pedersen or by us can not be assessed as long as none of the criteria usually employed for chemical purity can be applied. It may be remarked, however, that the protein present in one lethal dose of the ultracentrifuged preparation described above must have weighed about 3×10^{-15} gm, which is equivalent to 180 molecules with a molecular weight of 10 million. If one assumes that an appreciable proportion of these molecules were in an inactive state, only a comparatively small number of active molecules could have been present to constitute one lethal dose for mice. This in turn may be taken as a suggestion that "pure" virus represented a substantial fraction of the material isolated.

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DIHYDROXY-STEARIC ACID AND VITAMIN K DEFICIENCY

THE fat depots of animals show relatively high acetyl values, indicating the presence of considerable hydroxy-fatty acids. These acids, therefore, are normal constituents of omnivorous dietaries.

It has been reported that butterfat is superior nutritionally to vegetable oils.[1] The appearance of this report prompted the suggestion that possibly this superiority was due to the hydroxy-acids in butterfat, since butterfat is relatively rich in these acids and their presence in animal tissues may be presumed to indicate a nutritional significance. Later it was reported[2] that the active factor in butter is a long-chain saturated fatty acid.

To test our thesis a synthetic fat containing a proportionally large amount of dihydroxy-stearic acid was prepared and fed in the dietary of albino rats. The data on the nutritive values of hydroxy-acids will be presented later but we wish at this time to report interesting abnormalities observed during a part of this investigation.

A control group of 25 weanling rats was put on a diet containing 18 per cent. casein (Labco), 53 per cent. sucrose, 25 per cent. hydrogenated fat (Spry) and 4 per cent. mineral salts.[3] Each rat received a daily supplement of 60 I.U. vitamin A, 6 I.U. vitamin D, 0.02 mg thiamine, 0.025 mg riboflavin, 0.10 mg

[1] E. J. Schantz, C. A. Elvehjem and E. B. Hart, *Jour. Dairy Sci.*, 23: 181, 1940.
[2] E. J. Schantz, R. K. Boutwell, C. A. Elvehjem and E. B. Hart, *Jour. Dairy Sci.*, 23: 1205, 1940; R. K. Boutwell, R. P. Geyer, C. A. Elvehjem and E. B. Hart, *Jour. Dairy Sci.*, 24: 1027, 1941.
[3] R. B. Hubbell, L. B. Mendel and A. J. Wakeman, *Jour. Nutrition*, 14: 273, 1937.

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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