LETTERS TO THE EDITORS

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NOTES ON POINTS IN SOME OF THIS WEEK'S LETTERS APPEAR ON P. 384.

CORRESPONDENTS ARE INVITED TO ATTACH SIMILAR SUMMARIES TO THEIR COMMUNICATIONS.

Role of Dietary Choline in Neurohumour Production

THE lipotropic action of choline[1] and the role of choline in the formation of acetylcholine have not hitherto been studied in the same experimental animals. It seems reasonable to suppose, however, that, in addition to the production of the familiar fatty liver, lack of dietary choline might result in deficient formation of the neurohumour, acetylcholine. The findings in preliminary experiments[2] suggested that this is indeed the case.

The present experiments were designed to test the hypothesis that a diet poor in choline results in a low level of vagus activity and that this in turn is due to deficient formation of acetylcholine at the nerve endings. Rats were used as experimental animals, and were kept on the various diets for at least nine weeks. Vagus activity was evaluated by observing the slowing of the heart brought about by electrical stimulation (twelve impulses per second at maximal strength) of the distal cut end of the left vagus nerve. The resting heart-rate in rats (170–250 gm.) under urethane anæsthesia on a normal diet is from 300 to 500 beats per minute. The heart rate of animals on a low-choline diet or of those receiving the same ration with added choline fell within these limits. In one series of animals vagus stimulation reduced the heart-rate to approximately 30 per cent of the normal value in rats on a normal diet, to 45 per cent in rats on a low-choline-plus-choline diet and to 75 per cent in the animals on a low-choline diet. In other series many of the rats on the low-choline diet showed a normal vagus effect but others, as seen consistently in the small series cited above, showed very little change in heart rate on vagus stimulation. The most suggestive finding of this investigation is that, in animals on a low-choline diet showing little effect of vagus stimulation, the intravenous administration of choline (0·5 c.c. of 2 per cent choline chloride) increased this effect. This phenomenon was never seen in rats on a normal or low-choline-plus-choline diet.

We conclude that deficient vagus function may be linked with a low intake of dietary choline and this may, in part at least, be rectified by injected choline. It is therefore reasonable to suggest that choline deficiency may result in deficient formation of acetylcholine at the nerve endings. This and other possibilities are to be tested further in subsequent experiments in which it is hoped that dietary conditions producing an even more drastic deficiency of choline may be utilized.

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[1] Best, C. H., and Ridout, J. H., *Ann. Rev. Biochem.*, 8, 349 (1939).
[2] Solandt, D. Y., *Canad. Chem. and Process Indus.*, 23, 280 (1939).

Effect of Lipoid Solvents on Vaccinia Virus

THE elementary bodies of the dermal strain of vaccinia virus can be obtained readily from the skin of the infected rabbit's back in suspensions showing a high degree of physical and immunological homogeneity; the virus contains protein, carbohydrate and ether-soluble lipoid material[1]. We find that, after drying and extraction with benzene or ether, the dry density is increased from 1·26 to 1·31 and it is possible to redisperse the virus residue by mechanical grinding in buffer solution. The appearance by dark-ground examination and the sedimentation constant of the resuspended virus particles are unchanged, and the boundary inhomogeneity is slightly reduced. The infectivity of the virus is unaltered by this treatment and it is therefore probable that the lipoid material extracted with ether (consisting largely of cholesterol and acetone-soluble fat) is derived from the host and adsorbed on the virus.

The ether-extracted virus still contains 9 per cent of lipoid material, containing some 2 per cent phosphorus, which can be extracted only with alcohol or alcohol-ether mixtures. The virus residue after treatment with alcohol has almost entirely lost its infectivity, but it is impossible to say whether this is due to the removal of the lipoid material or to the concomitant effect of alcohol on the virus protein. The virus residue now has a density of 1·36 and suspensions give completely inhomogeneous boundaries in the centrifuge. It contains 15·5 per cent nitrogen and 0·7 per cent phosphorus. Colorimetric carbohydrate estimations (total carbohydrate[2], orcinol-HCl pentose test and Feulgen reaction) indicate the presence of approximately 3 per cent thymonucleic acid and 4 per cent carbohydrate; tests for glucosamine after acid-hydrolysis were positive.

Washing the virus repeatedly with water or dilute buffer, which is known to result in the liberation of specifically precipitable protein[3], was found to remove nitrogen, phosphorus and carbohydrate but no nucleic acid. If the ether-extracted virus is treated with 1 per cent sodium carbonate at 20°, practically all the nucleic acid and 40 per cent of the carbohydrate pass into solution in 1–2 hours. This effect is followed by a gradual disintegration of the residue into particles of widely different sizes, some only slightly smaller than the original.

Though in its chemical and immunological complexity vaccinia virus approaches more nearly to the bacteria than to the plant viruses, there appears to us to be little evidence that the substance of the virus is held within a cell membrane. The evidence which has been given[4] of osmotic swelling (derived indirectly from measurements of sedimentation rates in media of different density) is open to serious objections; and such swelling can be observed in protein gels. There is no evidence of selective ion permeability