

the ordinary approach between aliphatic chains, and compares favourably with the examples given by Bernal and Megaw in their discussion of the 'hydroxyl bond'.

As a conclusion drawn from this calculation, one is forced to question the space group determination of Bernal¹. He has suggested the space group C_{3m}^2 for the α -modification of $C_{12}H_{26}OH$. The two molecules per unit cell are then placed in the equivalent positions $00z$ and $00\bar{z}$. This puts the two molecules on a single triad axis, and does not allow for close packing of the hydroxyl groups. Assuming the tetrahedral angle for the carbon-oxygen bond, then on the basis of this space group determination and the projected OH—OH distance of 1.46 Å., the distance between hydroxyl centres is calculated as 2.09 Å. This distance is much too small to be reasonable for the 'hydroxyl bond'. Perhaps the intensity data of Bernal could be interpreted to give a different space group which would allow for a close packing of the hydroxyl heads.

The calculation of the projected OH—OH distance made by E. Ott and the author is to be seriously considered, because it is based upon 001 reflections from the α -modifications of six members in the series of *n*-aliphatic alcohols $C_{13}H_{27}OH$ to $C_{18}H_{37}OH$, all of which agree.

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¹ NATURE, 136, 755 (1935).

² Proc. Roy. Soc., A, 151, 384 (1935).

³ J. Chem. Phys., 2, 239 (1934).

⁴ Z. Krist., 83, 153 (1932).

Effect of Ascorbic Acid and Indolyl Acetic Acid on Regeneration of Willow Branches and Germination

IN the middle of 1935 we began an investigation into the effect of vitamin C on plants, and the general growth response since claimed by Synnöve v. Hausen¹ was obtained by us with tomato and castor oil plants.

β -Indolyl acetic acid was synthesised after the method of R. Majima and T. Hoshino², and the related indole derivatives β -indolyl propionic acid, β -indolyl methyl malonic acid (which yields β -indolyl propionic acid by loss of carbon dioxide) and β -ethyl indole were synthesised in a manner which precluded any possible contamination with β -indolyl acetic acid. Epinastic curvatures were obtained on a large range of plants with β -indolyl propionic acid, which however gave no positive result with the oat test, so that Thimann and Koepfli³ were mistaken in attributing positive results to persistent traces of the lower homologue.

A selected range of willow branches, 12 inches long, was placed in vaseline-sealed glass containers with the morphologically lower ends in 200 c.c. of Pfeffer's⁴ inorganic nutrient solution containing the compounds mentioned. At a concentration of 1 in 100,000, the order in which both roots and shoots appeared was the following: (1) ascorbic acid, (2) β -indolyl acetic acid, (3) β -indolyl propionic acid, (4) control. With a concentration of 1 in 500,000, the result was: (1) ascorbic acid, (2) control, etc. Evidently the stimulatory action of the ascorbic acid persists even in very low concentration in spite of probable oxidation. With a high concentration of 1 in 2,500, regeneration is retarded considerably by

the ascorbic acid, and almost entirely prevented by the indole derivatives.

Oats, mustard and cress seeds were germinated under sterile conditions in 10 c.c. of Pfeffer solution containing the compounds. At a concentration of 1 in 10,000, both germination and subsequent growth occurred in the following order: (1) ascorbic acid, (2) control, (3) β -indolyl propionic acid, (4) β -indolyl acetic acid. When applied after germination, and following upon a certain amount of root development, the β -indolyl acetic acid took third place. Abnormal length of coleoptiles and hypocotyls occurred with the indole compounds in the case of prostrate seedlings. The indole derivatives at this concentration apparently retard germination and growth, and even at lower concentrations (1/100,000) the result is the same, though ascorbic acid still acts in stimulatory fashion. With higher concentration (1/1,000) the ascorbic acid also showed a retarding effect, while the others were practically lethal.

The effect of the ascorbic acid agrees with the recently published result of Havas⁵, and points to the familiar hyper-effect of the vitamins; but the retarding effect of the indole derivatives seems in main part due to the prevention of root growth, which occurred very strikingly in these cases. In high concentration the roots grown in β -indolyl acetic and β -indolyl propionic acids were short and cone-shaped with swollen bases, F. A. F. C. Went's⁶ suggestion of transverse growth in the root caused by the auxins seeming to be verified. The abnormal length of the stems of prostrate seedlings was probably due to direct contact with the growth compounds.

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¹ Synnöve v. Hausen, NATURE, 136, 516 (1935).

² R. Majima and T. Hoshino, Ber., 58, 2043 (1925).

³ K. V. Thimann and J. B. Koepfli, NATURE, 135, 101 (1935).

⁴ Pfeffer; see Knudson, Bot. Gaz., 73, 1-25 (1922).

⁵ L. Havas, NATURE, 136, 435 (1935).

⁶ F. A. F. C. Went, Biol. Rev., 10, 2 (1935).

Ascorbic Acid as a Precursor of Serum Complement

PARTICULARLY in septicæmic conditions affecting man, the importance of "alexine" (Bordet) or "complement" (Ehrlich) in immune processes, although (so far) inexactly defined in scope, is becoming very generally recognised^{1,2}. Frequent attempts to analyse and define this substance, generally by chemical means, have resulted in the recognition of at least four components or "end" and "middle-pieces"^{3,4}.

I have recently obtained evidence (to be published in detail elsewhere, pending the permission of the Anglo-Iranian Oil Co., Ltd.) that the "complement" complex, as it exists in the circulating blood of the guinea pig, following coagulation and separation of serum, and in the form demonstrable by a standardised hæmolytic system, disappears or suffers reduction in titre, when ascorbic acid is withdrawn, completely or partially, from the food of the experimental animal; and the concentration of "complement" (obtained in the same manner) can be restored to normal, or slightly supernormal, level by the consumption of a diet rich in vitamin C.

These effects can be produced in seven days or less, too soon for qualitative malnutrition to affect the animal's physical well-being; indeed it remains in perfect health, being supplied with everything necessary for healthy functioning, except, during a few days only, ascorbic acid. It follows that reduction or disappearance of "complement" from the circulating blood must be one of the earliest signs (in guinea pigs, and perhaps in man) of a state of affairs which, if prolonged for twenty to forty days (in the cavy) or four to eight months (in man) will result in scurvy.

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- ¹ Cadham, F., "Septicaemia: a Method of Treatment", *Amer. J. Med. Sci.*, **188**, 542 (1934).
² Whitby, L. B. H., and Britton, C. J. C., "Disorders of the Blood", 45 (J. and A. Churchill, London, 1935).
³ Gordon, J., and Wormald, A., "The Action of Ultra-Violet Rays on Complement", *Biochem. J.*, **22** (4), 909 (1928).
⁴ Gordon, J., and Thompson, F. C., "The Relationship between the Complement and Osmotin of Normal Serum", *Brit. J. Exp. Path.*, **16** (1), 101 (1935).

A Satisfactory Substitute for the Osmium Tetroxide Golgi Apparatus Methods

OSMIUM tetroxide at 18s. a gram is the most expensive substance used in biological research. It is principally used in the Kolatchew and Weigl methods for the Golgi apparatus, and in the F. W. A. and Champy methods for mitochondria. The nearest cheap substitutes for Weigl and Kolatchew methods are the formalin-silver methods of Cajal and Da Fano. The trouble with these latter methods is that there is usually distortion and often incrustation of the Golgi bodies, banana-shaped elements, for example, often becoming beads, or spheres, and different from what can be seen *intra vitam*. The general fixation is often very poor.

I have been astonished at the beauty of silver nitrate formalin preparations made according to F. Aoyama's modification of Cajal's method¹. This method is as follows: Fix small pieces of tissue in cadmium chloride 1 part, formal neutral 15 parts, distilled water 85 parts, for three to four hours. Rinse quickly in two changes of distilled water, and transfer to 1.5 per cent solution of silver nitrate for 10 to 15 hours at 22° C. Rinse quickly in two changes of distilled water, preferably in a darkened room, and transfer for 5 to 10 hours to the reducing solution (hydroquinone 1 part, neutral formal 15 parts, distilled water 85 parts, 0.1 to 0.15 parts of sodium sulphite, sufficient to produce yellowish tinge). Wash thoroughly in tap water ($\frac{1}{2}$ to $\frac{3}{4}$ of an hour), upgrade, imbed and section. Counterstain in carmine or hæmatoxylin and eosin. Cold-blooded animals may need longer impregnation and fixation.

This method has been tried in this laboratory on the following material: rat pancreas and dorsal root ganglion, *Helix* ovotestis and cerebral ganglion, midgut of mealworm. In every case the preparations were superior to those got by Da Fano's method, and very nearly approached the best Weigl preparations for morphology of Golgi bodies. The non-cellular elements of tissues were bright yellow, and quite different from the Cajal or Da Fano effect. The rat and snail preparations were perfect enough for the most precise research on cell inclusions. In

only a few cases did mitochondria impregnate, and the method is probably more specific than Weigl, and much more specific than Da Fano.

The preparations were made by Mr. R. Brown and Miss M. Daniels. The formalin used was unneutralised, but from a fresh supply; the silvering was done in a Hearson incubator at the proper temperature.

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W. Z. Wiss. Mikr., (1930).

Induction of Mammary Ducts

THE mammary gland of the pig has normally two primary ducts which open separately on the surface of the nipple. Embryologically, the two ducts proliferate from the base of a cone-shaped depression of the stratum germinativum known as the mammary pocket or mammary bud. A similar depression or epithelial ingrowth, variable in depth, surrounds the nipple at its juncture with the body wall. Among several foetal glands sectioned, a few cases of aberrant ducts, similar histologically to the primary ducts, were noticed to have sprouted from the same relative position to the apex of the epithelial ingrowth at the base of the nipple as the primary ducts do to the apex of the mammary pocket (Fig. 1).

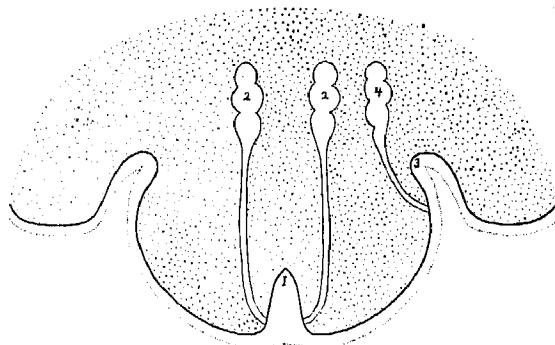


FIG. 1. Diagrammatic section through the nipple of a foetal pig. (1) Mammary pocket; (2) primary ducts; (3) epithelial ingrowth; (4) aberrant duct.

These aberrant ducts would appear to furnish evidence that an inducing stimulus is responsible for the development of the primary ducts, similar in principle to Spemann's demonstration¹ of the underlying mesoderm providing an inducing stimulus for neural plate development in Urodela. Recently, Waddington² suggested that some, if not all, inductions are due to the diffusion of active chemical substances into the reacting tissues. It would appear reasonable therefore to infer that an active substance is present in the nipple which stimulates duct development in specific locations relative to apices of epithelial ingrowths. Furthermore, that the stimulus is specific for particular groups of animals. Thus in the horse, in common with the pig, there are normally two primary ducts which develop respectively from the anterior and posterior walls of the mammary pocket; ruminants have only one duct, which proliferates from the apex of the mammary pocket, and in man there are several primary ducts.