amylene, homologues of ethylene; mesityl oxide, $(CH_3)_2C:CHCOCH_3$, an unsaturated ketone; and vinyl acetate, $CH_3COOCH:CH_2$, an ester of an unsaturated alcohol, all gave positive reactions.

Dichloroethylene, CHCl:CHCl; trichloroethylene and tetrachloroethylene failed to give the reaction. This is undoubtedly accounted for by the fact that a substituent on a double bond carbon affects the reactivity of the double bond, which seems to be necessary to produce the reaction.

Acetone, acetaldehyde, diethyl ether and chloroform failed to give the reaction.

From these facts it seems likely that some gaseous or volatile unsaturated hydrocarbon or similar compound is present in Golden Self-Blanching celery during natural blanching of the leaves. The celery, Winter Queen, which is not self-blanching, does not produce such substances. It seems indicated therefrom that the disappearance of chlorophyll from self-blanching celery is accomplished by some process similar to that by which celery is commonly blanched artificially by application of ethylene in low concentrations.[4]

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NEW CANCER-PRODUCING HYDROCARBONS

Two hydrocarbons, belonging to series not hitherto known to be active as agents for cancer production, have been found to cause malignant growths in mice. These two compounds are sym.-triphenylbenzene and tetraphenylmethane. Their action is slower than that of the substances discovered by Cook[1] and coworkers. A specific strain of mice was treated to weekly injections amounting to 1 cc of a 5 per cent. solution of triphenylbenzene in sesame oil. In a year's time 12 out of 60 mice had well-developed tumors of a highly malignant type. In the case of tetraphenylmethane 25 mice were painted twice weekly with a 0.5 per cent. solution in benzene. After the same period well-developed epitheliomata were present in 8 cases. It is interesting to note that the percentage of positive results is relatively high in spite of the long time necessary to induce the growth.

The interest which these results arouse is chiefly in the complete lack of resemblance of these new compounds to the hydrocarbons discovered by Cook. The compounds[2] investigated by him had condensed ring systems containing 4 to 5 aromatic rings in the molecule and possessed a phenanthrene nucleus. He found that the ring system of 1, 2-benzanthracene[3] is present in many cases, although not absolutely necessary. He also observed a possible relationship[4] with the dehydrogenation products of the sex hormones and bile acids. Triphenylbenzene and tetraphenylmethane possess nothing in common with the properties listed above, except that each contains 4 benzene rings. The significance, if any, of this point is not evident at present. Any other structural similarity is lacking, for there is a complete absence of condensed ring systems or a phenanthrene nucleus in the two new agents. Neither may they be derived from the sex hormones or the bile acids.

In triphenylbenzene a single ring holds three other benzene rings attached in the 1, 3, 5 positions, but in tetraphenylmethane no benzene ring is attached to another. The linkages in this last instance are through a central carbon atom. In an effort to find a common ground on which these widely different classes of carcinogenically active agents can stand we may make the tentative assumption that in the hydrocarbons so far discovered the property of producing cancer resides in the benzene nucleus as modified or affected by substituents attached in either the condensed or open manner. Work is now in progress to limit more exactly the nature and position of the substituents. We are also investigating the higher phenyl homologues of the above-named series in the expectation that they may be more active still in causing tumorous growths.

The work is being conducted under a joint program of research of the Evans Memorial Hospital of Boston and the Massachusetts Institute of Technology at Cambridge.

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THE FORMATION OF CARBOHYDRATE FROM GLYCEROPHOSPHATE IN THE LIVER OF THE RAT

GEMMILL and Holmes[1] reported recently that the carbohydrate content of liver slices from butter-fed rats increases during 3 hours' incubation in bicar-

[4] Since the work described was completed for publication, it came to the authors' attention that R. Gane had published in *Nature*, December 29, 1934, Vol. 134, p. 1008, a paper entitled "The Presence of Ethylene in Some Ripening Fruits."

[1] Cook, Hieger, Kennaway and Mayneord, *Proc. Roy. Soc.*, B, 111: 455, 1932; Cook, 485.

[2] Cook, *Proc. Roy. Soc.*, B 113: 273, 1933.

[3] Cook, *Jour. Chem. Soc.*, 1592, 1933.

[4] Cook, *Proc. Roy. Soc.*, B 113: 273, 1933; Cook and Haslewood, *Jour. Chem. Soc.*, 428, 1934.

[1] C. L. Gemmill and E. G. Holmes, *Biochem. Jour.*, 29: 338, 1935.

bonate-Ringer's solution and that this carbohydrate synthesis is associated. with respiratory quotients below 0.7. Since lactic acid was not present in sufficient quantity, they assumed that the newly formed carbohydrate originated from fat.

It was noted in this laboratory that the inorganic phosphate content increases and the phosphate fraction difficult to hydrolyze in N HCl decreases when liver slices of rats are shaken for 3 hours in oxygenated bicarbonate-Ringer's solution. This suggested that glycerophosphate might be a source of carbohydrate in the liver.

Of 3 equal portions of liver slices from fasted rats, one was analyzed after 15 minutes for its total (fermentable) carbohydrate content, while the other 2 portions were incubated for 3 hours, one without and one with added substrate and then analyzed in the same manner as the first portion. Addition of α- or β-glycerophosphate or of glycerol caused in each case a greater increase in fermentable carbohydrate content than incubation of the liver without added substrate. The phosphorylized products were more active than glycerol. Under anaerobic conditions an increase of the carbohydrate content of the liver did not take place.

It was ascertained that during incubation of liver slices with α-glycerophosphate more inorganic phosphate was liberated than during incubation without added substrate. In muscle α-glycerophosphate interacts with pyruvic acid to form dihydroxyacetonephosphate and lactic acid. The mechanism of carbohydrate synthesis in the liver may be different, because addition of pyruvic acid or alanine (which would be deaminized to pyruvic acid) either alone or with α-glycerophosphate has so far not given clear-cut results.

The present experiments emphasize the importance of the glycerol part of the lipid molecule as a source of carbohydrate in the body and they do not lend support to the idea that fatty acids are converted to carbohydrate in the mammalian liver.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

BACTERIOLOGICAL EXAMINATION OF THE CONTENTS OF THE CLOSED ARM IN THE SMITH FERMENTATION TUBE

How often has the curiosity of the bacteriologist been directed to the chemical or biological conditions induced by bacterial flora under anaerobic environment in the closed arm of the Smith fermentation tube, and how often has he been foiled in securing an uncontaminated sample! Attempts to solve this problem have been made in the past, first, by substituting the so-called fish-hook tube with open ends, the longer of which was stoppered; and next, by the introduction by Hill[1] of a modified Smith tube, where the upper end was left opened and into which a glass thimble was made to fit snugly by ground glass surfaces.

In the first case, practical difficulties were encountered, owing to the frequent unexpected loosening of the stopper in the upper end of the fish-hook tube; to inability to readily handle such tubes in racks or other types of holders; and also to the impossibility of utilizing large quantities of inoculum. In the case of Hill's modification of the Smith tube, it was found that by repeated sterilization procedures the upper end of the tube or the thimble would crack and so render the tube useless, and annoyances were encountered in fitting the proper thimble into the end of a tube, where a series of such tubes were being cleaned up after use.

To obviate all these difficulties, and in fact to make the object of the removal of the contents of the closed arm easily carried out, the writer suggests the procedure which follows:

The usual footless type of the Smith fermentation tube (A.P.H.A. model) is taken, and with the thumb held firmly over the opening of the bowl, the end of the tube is brought in contact with a small-sized jet of flaming gas of a blast burner, being careful that the point of the flame impinges centrally on the closed end of the tube. As the glass begins to melt the enclosed air within the tube expands and blows out a small opening in the end of the tube. Everted edges of the opening are now to be held in the flame for a period sufficiently long to produce retraction of the everted lip of the opening to a level with the remaining surface of the glass. It should be the object of the operator to form an opening somewhere close to 3 mm; such an opening will readily accommodate either the passage of a fair-sized hypodermic needle or the drawn-out end of a glass pipette.

The next step involves the use of a method to effectually seal the opening made in the closed arm of the tube, and at the same time to offer later on the minimum of resistance to the passage of the hypodermic

[1] *Jour. Boston Soc. Med. Sci.*, January, 1899.