as controls, but reference was made to forthcoming determinations based upon isotopic analysis with deuterium, a second method of *d*-amino acid analysis not involving the incomplete or unrepresentative isolation procedures employed by Kögl or Arnow. Details must still await full publication, but we may briefly report here that the samples B and D of human normal liver and liver carcinoma earlier found[1] to contain 2.4 and 1.7 per cent. total *d*-amino acid-N of total-N both showed upon analysis with deuterium the definite presence of small, approximately equal quantities of *d*-glutamic acid of the order of several tenths of a per cent. of the original dry weight of tissue. These results agree very well in order of magnitude with the bulk of values reported by others for normal or malignant tissues by various methods, and in particular by Chibnall *et al.*[6] for fairly complete isolation,[8] by Graff, Rittenberg and Foster[7] from isotopic nitrogen analyses, and from expectation based upon glutamic acid racemization[9] now to be reported.

Probably the most direct, simple and conclusive evidence against *d*-glutamic acid malignancy specificity is the fact now definitely established that—contrary to earlier indications (2a, p. 73; 6, p. 294)—*l*-glutamic acid itself is racemized in hot hydrochloric acid. The rates under varying usual conditions are small but readily measurable. They are of an order needed to account for essentially all the isolated or analyzed quantities of *d*-glutamic acid reported in hydrolysates of protein, normal or tumor tissue by Kögl *et al.*,[2a-d] others since,[5, 6, 7, 10] and ourselves above. In our experiments *l*-glutamic acid, when refluxed under the same conditions as employed in our earlier protein and tissue hydrolyses,[1] was converted to *d,l*-glutamic acid at an average, nearly constant rate of 0.3 per cent. per hour up to at least 50 per cent racemization. The formation of *d*-glutamic acid, measured by the decreasing specific rotation of samples removed from time to time, was further confirmed and completely established by both *d*-amino acid oxidase analysis and isolation of analytically pure partially racemic glutamic acid hydrochloride in good yield. Kögl *et al.*[2a-d] have reported 29 isolations of glutamic acid from human, rabbit and rat tumors, with an average yield of 0.4 per cent. *d*-glutamic acid of tissue dry weight. This, assuming an average of about 10 per cent. total glutamic acid in tissue dry weights,[6, 7] is an average of about 4 per cent. racemization, or of the same rough order that might be expected from simple racemization of *l*-glutamic acid during hydrolysis. Any close comparison here would obviously call for a detailed consideration of actual concentrations of reactants during hydrolysis, isolation yields, the effect, if any, of combination of glutamic acid in protein linkages, etc. Two of Kögl's tumors yielded no glutamic acid, whereas only four gave more than 1 per cent. *d*-glutamic acid of dry weight, two of these attaining 3.7 and 4.2 per cent. These latter two values, obtained by Kögl early in his work, can well be regarded, even in the light of Kögl's own work, as definitely not typical or characteristic of tumors. It would seem unprofitable or futile, therefore, to make much point concerning them, as do Arnow and Opsahl in their last note[9] on the subject at hand, in an attempt to maintain malignancy specificity on the basis of these two atypical cases, now that virtually all other cases, to the number of several score, can be readily understood on a basis of simple glutamic acid racemization during hydrolysis.

Conclusion

The view of malignancy specificity proposed by Kögl and supported by Arnow *et al.*, that cancers but not normal tissues or proteins are composed of partly unnatural (*d*-)amino acids, is clearly no longer tenable, both as regards *d*-glutamic acid as well as total *d*-amino acid.[11]

We again take pleasure in expressing our appreciation and indebtedness to Professor Vincent du Vigneaud for his continued counsel during these investigations.

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DIOXYMALEIC ACID OXIDASE

We have been much interested in the dioxymaleic acid oxidase of Banga and Szent-Györgyi[1, 2] and in

[8] Concerning the unsuccessful isolations of *d*-glutamic acid from normal tissues by Kögl, Arnow and others, or from tumors by still others, further reference may be made to the detailed discussion and explanation by Chibnall *et al.*[6]

[9] L. E. Arnow and J. C. Opsahl, *Jour. Biol. Chem.*, 133: 765, 1940; J. M. Johnson, *Jour. Biol. Chem.*, 134: 459, 1940.

[10] (*a*) J. White and F. R. White, *Jour. Biol. Chem.*, 130: 435, 1939; (*b*) C. Dittmar, *Zeits. f. Krebsforsch.*, 49: 441, 1939; J. M. Johnson, *Jour. Biol. Chem.*, 132: 781, 1940; H. Ottawa, *Zeits. f. Krebsforsch.*, 49: 677, 1939.

[11] Added to galley proof: In an article just come to hand by F. Kögl, H. Herken and H. Erxleben (*Zeits. physiol. Chem.*, 264: 220, 1940), the applicability of the *d*-amino acid oxidase method employed by us in our earlier communication (footnote 1) is questioned in several respects. In our full publication, now in preparation, we are presenting a complete description of the method and of the data obtained, which will demonstrate in detail the validity of the earlier results and conclusions reached by us.

[1] I. Banga and A. Szent-Györgyi, *Zeits. physiol. Chem.*, 255: 57, 1938.

[2] I. Banga, E. Philippot and A. Szent-Györgyi, *Nature*, 142: 874, 1938.

the evidence of Theorell and Swedin[3,4] which indicates that this new enzyme is really identical with peroxidase. We find that in the presence of fresh alcoholic dioxymaleic acid, acetate buffer of pH 4.5, partially purified horse-radish peroxidase and hydrogen peroxide, methyl red/o-carboxy-benzene-azodimethylaniline/ is rapidly oxidized and decolorized. In the absence of dioxymaleic acid the oxidation is slow. Moreover, peroxide need not be added, provided that the solution of dioxymaleic acid, methyl red, acetate buffer and peroxidase is shaken with air. In nitrogen there is no decolorization.

We find that peroxidase, as shown by testing with guaiacol and hydrogen peroxide, is rather rapidly inactivated by buffered dioxymaleic acid, and that this inactivation is retarded by aeration. Inactivated peroxidase is less effective in oxidizing dioxymaleic acid and in decolorizing methyl red than is active peroxidase.

Old alcoholic solutions of dioxymaleic acid give different results from fresh solutions. Here, a mixture of dioxymaleic acid, acetate buffer and peroxidase decolorizes methyl red even in nitrogen, while aeration largely prevents decolorization.

Our explanation of the rapid bleaching of methyl red in the presence of fresh dioxymaleic acid, peroxidase and hydrogen peroxide is that the peroxidase and peroxide convert the dioxymaleic acid into diketosuccinic acid and that the methyl red is oxidized by a coupled reaction. If one shakes dioxymaleic acid solutions in air, it is not necessary to add peroxide, since dioxymaleic acid is spontaneously oxidized and forms peroxide. This is in agreement with the evidence of Theorell and Swedin. Old solutions of dioxymaleic acid already contain peroxide, so that they require no shaking with air. We are not able to tell why shaking with air should retard the decolorization of methyl red in the presence of old dioxymaleic acid.

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THE EFFECT OF GROWTH SUBSTANCES ON THE ROOTING OF BLUEBERRY CUTTINGS

THREE experiments have been conducted to study the effect of growth substance on the rooting of summer cuttings of blueberries, *V. corymbosum*. These experiments were carried on in 1937 and 1938, using in all over 2,500 cuttings from eleven different varieties of blueberries. Indole-3-acetic acid and indole-3-proprionic acid were each used dry and in solutions of 5 and 10 mg per liter. Phenyl acetic acid was used only in solutions of 10, 25 and 50 mg per liter. Auxilin was used at the recommended concentration No. 3. Phenyl acetic acid was the only one of the growth substances used which significantly increased the percentage of rooting and the greatest increase was at the medium concentration, 25 mg per liter. The results obtained with auxilin were very poor at the concentration used. The results of the above experiments are in agreement with those of Stanley Johnston's investigations independently conducted at the same time and reported in *Michigan Station Quarterly Bulletin,* 21: 255–8, 1939. From the results thus far obtained it does not seem advisable to recommend the use of growth substance for the rooting of blueberrry cuttings.

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THE IDENTITY OF THE TREE "ANNEDDA"

FROM your review of "The Englishman's Food"[1] it appears that the authors think the tree (called by the Indians "Annedda") which cured Jacques Cartier's men of scurvy when they were wintering at Stadacona in the winter of 1535–36, was *Sassafras officinale*. The sassafras does not grow anywhere in the Province of Quebec. Its only station in Canada is a relatively narrow strip in southern Ontario and there is no reason to suppose that it ever ranged farther north.

In support of the sassafras as against an evergreen, the authors state, according to the reviewer, that Cartier's notes particularly refer to the fact that the Indians had to wait for the leaves to appear in the spring. Perhaps authority is given for this statement, but no such passage occurs in Biggar's edition of "The Voyages of Jacques Cartier." Furthermore, Cartier says[2] that it was while he was walking on the ice that the Indians told him of the tree which would cure the sickness and two squaws went with him to gather some of it. Nine or ten branches were brought back, and Cartier adds: "They showed us how to grind the bark and leaves and to boil the whole in water."

The identity of the tree Annedda has been much disputed, but from considerations not necessary to discuss here, it seems likely that it was the hemlock, *Tsuga canadensis*.

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THE KIT FOX

IN the summer of 1911, T. A. Rocklund and I were camped for one month on the Pennell Ranch in Wallace County, Kansas, adjoining the George A. Allman

[3] H. Theorell and B. Swedin, *Naturwissenschaften,* 27: 95, 1939.
[4] B. Swedin and H. Theorell, *Nature,* 145: 71, 1940.

[1] SCIENCE, 91: 217, 1940.
[2] H. P. Biggar, "The Voyages of Jacques Cartier," p. 212–215. Ottawa: The King's Printer, 1924.