September 3, and Thursday, September 5, leaving Wednesday and Friday open for inspection trips. The trip to Grand Coulee Dam on Wednesday will be preceded by a general interest talk on the dam at the banquet on Tuesday evening. Friday is being left open for trips to the lumber mill at Lewiston, the mines of northern Idaho, the Columbia River Basin Project, or the scenic lakes of the region.

THE tenth annual meeting of the American Malacological Union was held at the Academy of Natural Sciences, Philadelphia, from June 17 to 21. The program included the presentation of a testimonial of appreciation to Norman W. Lermond, curator of the Knox Academy of Arts and Sciences, Thomaston, Maine, for his preliminary work in the organization of the society; and to Dr. Henry A. Pilsbry, curator of mollusks, Philadelphia Academy of Natural Sciences, its first president. In honoring Dr. Pilsbry on this occasion, the union is issuing a complete bibliography of his published works. This publication will be available for students of Mollusca by the end of August. Officers were elected as follows: *President,* Dr. Harald A. Rehder, U. S. National Museum; *Vice-president,* Frank Collins Baker, University of Illinois; *Corresponding Secretary,* Norman W. Lermond, Knox Academy of Arts and Sciences, Thomaston; *Financial Secretary,* Mrs. Harold R. Robertson, Buffalo Museum of Science. John Oughton, Royal Ontario Museum of Zoology, Toronto, was elected *Councillor at Large* to fill the vacancy occasioned by the resignation of Aurele La Rocque, of the National Museum of Canada. The next meeting will be held in the Knox Academy of Arts and Sciences, Thomaston, from August 5 to 8, 1941.

THE second annual exhibition of photographs of wild life under the auspices of the New York State Nature Association will be held at the Albany Institute of History and Art from October 30 to November 10. A first prize of $20, a second prize of $10 and a third prize of $5 will be awarded to the pictures which best represent the spirit and beauty of living wild birds and animals photographed in their natural surroundings. The prize-winning photographs will become the property of the New York State Nature Association.

THE United States Civil Service Commission has announced an open competitive examination to fill the position of senior engineering aid (topographic) in the U. S. Geological Survey. The salary of the position is $2,000 a year, less a retirement deduction of 3½ per cent. Except for the substitution of experience, applicants must have completed high-school study; and, in addition, must have had responsible civil engineering experience, partly on topographic field surveys. Certain engineering study in a college may be substituted for part of the experience. Applicants will not be given a written test, but will be rated on their qualifications, as shown in their applications, and on corroborative evidence. Applications will be rated as received at the commission's Washington office until December 31.

The Alumni Review of the University of North Carolina states that at a meeting of the trustees on June 7 a settlement proposed by its finance committee and agreed to by the executors and beneficiaries of the Flagler estate was approved. From this fund the university since 1917 has received annually the sum of $75,000 to pay the Kenan professors. The settlement represents a generous interpretation by William R. Kenan, Jr., and his co-executor, Mr. Harris, and the chief beneficiaries of the will. The Kenan Fund was established in 1917 as a memorial to William R. Kenan, father of Mrs. Bingham, and her two uncles, Thomas S. Kenan and James Graham Kenan, all graduates of the university. The will provided that the university should be paid $75,000 annually for twenty-one years, at the end of which period it should be paid an amount sufficient "at the rate of interest then current in North Carolina" to earn $75,000 annually thereafter. Legal advisers to the executors suggested for transfer to the university securities valued at $1,100,000 and yielding the amount of the annual income desired. Mr. Kenan, his co-executor, and Mrs. Graham Kenan and Mrs. Jessie Kenan Wise recognized, however, that funds reinvested in long-term securities by the university might not earn so high a yield. Accordingly, they agreed to a settlement of $1,875,000 which in effect represented an additional gift by the estate, of which they are the chief beneficiaries, of $775,000. The university in appreciation of their action added to the settlement sum an accumulated Kenan Fund reserve of $182,000 together with $43,000 from a pre-consolidation escheats fund to make the endowment of the Kenan Fund $2,100,000.

DISCUSSION

THE NON-SPECIFICITY OF AMINO ACID CONFIGURATION IN MALIGNANT TISSUE HYDROLYSATES

THE development and application of the Krebs *d*-amino acid oxidase to the determination of total *d*-amino acid, described by us in these columns earlier this year,[1] provided a unique opportunity to subject

[1] F. Lipmann, O. K. Behrens, E. A. Kabat and D. Burk, SCIENCE, 91: 21, 1940.

to test both the broad claims and the expectations of Kögl and coworkers[2] in regard to cancer being characterized by proteins containing amino acids of partially unnatural (*d*-) configuration.[3] The uniformly low, randomly distributed values of 1 to 3 per cent. *d*-N of total-N found by us in the hydrolysates of a wide variety of appropriate and representative normal and tumor materials definitely eliminated the main claim of malignancy specificity with respect to total *d*-amino acid. The data obtained excluded a statistically significant difference between the malignant and normal tissue hydrolysate groups studied of greater than 0.5 per cent. in the observed average values of about 1.7 per cent. *d*-N of total-N in each group. Further search might, of course, reveal exceptional values in either group, but scarcely with appreciable bearing upon the generality of the conclusion reached.

In a recent note in SCIENCE Arnow and Opsahl[4] raise the point that *d*-glutamic acid, even if not total *d*-amino acid, might still be specifically characteristic of malignancy, providing the *d*-glutamic acid were of the same order of magnitude as the amino acids known to be racemized chemically during hydrolysis, and hence experimentally difficult to distinguish therefrom by the oxidase method. This suggestion, however, obviously introduces a *double* claim of malignancy specificity. For to assume that the malignant hydrolysate material contained some half of its *d*-amino acid-N specifically as *d*-glutamic acid-N—or much more than half as the literal 1.6 per cent. value proposed by Arnow and Opsahl would suggest—logically introduces the further implicit claim of a corresponding *deficiency* in the malignant material of half or more of that *d*-amino acid-N found in the normal material. Such a claim would, to say the least, be very difficult to maintain.

In regard to the experimental data of Arnow *et al.* presented in three other notes[5] held by them to be confirmatory of malignancy specificity, it may be pointed out that the purity of the glutamic acid hydrochloride samples claimed to have been isolated from tumor or normal tissue was not independently established by indispensable N or C and H (or ash) analyses, but only by melting point. The latter criterion, however, is of essentially no significance here, for we have observed that the melting point of *l*-glutamic acid hydrochloride is unaffected ($\pm 2°$ C) by NaCl impurity up to at least 50 per cent. With the Kögl procedure employed, we have frequently isolated materials of low rotation that proved upon analysis by the oxidase method to contain no *d*-glutamic acid, or far less than that indicated by the rotation; inorganic chloride and even small amounts of other occluded amino acids could be found which were difficult to eliminate upon one or two recrystallizations if these were carried out, as recommended by Kögl, with a weight loss of less than 10 per cent. It is obviously impossible, in the absence of adequate analytical control, to deduce the presence of *d*-glutamic acid merely from rotation values low for *l*-glutamic acid. The yields of materials isolated by Arnow *et al.*, where reported at all, were very scanty, and hence almost certainly stereochemically unrepresentative;[6] they were but one tenth to one hundredth the quantities of glutamic acid found in tissues upon complete isolation by the Foreman-type procedure,[6] by ourselves employing a considerably improved Kögl procedure involving vigorous stirring during crystallization or by isotopic analysis.[7] It is difficult to see that the data of Arnow *et al.*[5] have much if any bearing on the question of malignancy specificity at hand.

As to the possibility that *d*-glutamic acid occurs specifically in malignant material as a *small* fraction of the total *d*-amino acid, there is now developing abundant evidence that, providing appropriate methods of isolation or analysis are employed, *d*-glutamic acid is to be found in hydrolysates of both normal and tumor material, and to about the same small extent in each. As indicated in our earlier communication on the oxidase method,[1] no attempt was made to determine individual *d*-amino acids, in particular *d*-glutamic acid, in hydrolysates, except *d*-amino acids intentionally added

[2] F. Kögl and H. Erxleben, *Zeits. physiol. Chem.*, (*a*) 258: 57, 1939; (*b*) (with A. M. Akkerman) 261: 141, 1939; (*c*) 261: 154, 1939; (*d*) (with H. Herken) 263: 107, 1939; (*e*) 264: 108, 1940.

[3] In any consideration of the Kögl claim it is important to bear in mind that this claim was made with respect to a variety of amino acids, including not only glutamic acid, the most prominent and easiest amino acid to isolate in this connection, but also at least leucine, lysine, valine, hydroxyproline, hydroxyglutamic acid and arginine, and even possibly to some extent those amino acids known to be racemized in part by the acid hydrolysis employed but provisionally excluded by Kögl from closer quantitative analysis because of the experimental finesse required. In his early papers Kögl laid frequent emphasis on the presence in malignant tumors of what he called "partially racemic protein," of "a derangement of stereochemical specificity possibly propagated from glutamic acid to other amino acids," or "*d*-forms of still other amino acids (even indispensable ones) that might be found in residual mother liquors." In the opening sentence of a recent paper[2d] Kögl has continued to stress his broad claim, adding a note of reproof, however, for the exclusive attention being paid by others to glutamic acid: "The discovery of partially racemic amino acids in tumor protein has quite understandably induced various workers to check our findings, but with restriction, above all, to the extreme case of glutamic acid."

[4] L. E. Arnow and J. C. Opsahl, SCIENCE, 91: 431, 1940.

[5] L. E. Arnow and J. C. Opsahl, (*a*) SCIENCE, 90: 257, 1939; (*b*) (with C. J. Watson), *Proc. Soc. Exp. Biol. Med.*, 43: 766, 1940; (*c*) (with W. G. Clark) *Proc. Soc. Exp. Biol. Med.*, 43: 767, 1940.

[6] A. C. Chibnall, M. W. Rees, E. F. Williams and E. Boyland, (*a*) *Nature*, 145: 311, 1940; (*b*) *Biochem. Jour.*, 34: 285, 1940.

[7] S. Graff, D. Rittenberg and G. L. Foster, *Jour. Biol. Chem.*, 133: 745, 1940.

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.

Otto K. Behrens
Fritz Lipmann
Mildred Cohn
Dean Burk

Department of Biochemistry, Cornell University Medical College, New York City; and National Cancer Institute, U. S. Public Health Service

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.