Do Tumor Proteins Contain D-Amino Acids?

A Review of the Controversy

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The controversy which forms the subject of this review followed the publication of a paper entitled “Zur Ätiologie der malignen Tumoren” by F. Kögl and H. Erxleben in 1939 (30). In this work evidence was presented that tumor proteins contain certain D-amino acids, particularly D-glutamic acid. These findings have not been generally confirmed, and a controversy arose which has continued to the present. The dispute exists almost entirely between the school of Kögl, a vigorous and able protagonist, and an impressive array of equally vigorous and able dissenters. While it appears to the reviewer that the weight of evidence is still against the view of Kögl and his collaborators, the recent publication of new and challenging positive evidence from the Kögl laboratory makes this problem timely again. The following review is offered in the hope that it will enable oncologists to follow this important controversy more easily as further developments occur.

The occurrence of D-amino acids in nature.—Due to the initial efforts of Emil Fischer and to the extensive studies of Freudenberg, Karrer, Kuhn, and many others, it appears today that all of the amino acids found in the proteins of normal tissues have the same arrangement of substituents about the asymmetric a-carbon atom. These amino acids belong to the group conventionally termed the “L” series. Indeed, so great did the preference of living systems for the L-amino acids once seem that their optical isomers, the D-amino acids, were regarded as the “unnatural” forms. However, since 1935 it has become evident that D-amino acids do occur occasionally in nature, particularly in products derived from certain molds and bacteria (44). Thus, D-proline occurs in a number of the ergot alkaloids, while penicillin contains a derivative of D-cysteine. Similarly, certain cyclic polypeptides contain D-amino acid residues, e.g., gramicidin (D-leucine and D-valine), tyrocidin and gramicidin S (D-phenylalanine), and polymyxin (D-leucine and D-serine). Of special relevance to this review is the occurrence of D-glutamic acid as the sole constituent of the high molecular weight polypeptide in the capsular substance of bacteria of the Mesentericus group. In all of these compounds the acids are bound through amide linkages, and the polypeptides consist largely of a-peptide chains. Thus, sound precedent exists for the supposition that D-amino acids may occur in proteins.

The hypothesis of Kögl et al.—While the original papers (23, 24, 30) of Kögl and his group at Utrecht must be consulted for details on their hypothesis, their basic concept is that tumor proteins are characterized by the presence of certain amino acids in the D-form. They infer further that the initiation and autonomous character of tumor cells are dependent on the formation and maintenance of these D-amino acid units in the cell proteins. Certainly, there are few, if any, a priori arguments that can be marshaled against such a hypothesis. In fact, it is very attractive, for it provides an elementary picture in relatively definite chemical terms of how a cell might very subtly become and remain independent of the controls that govern normal growth. However, after 10 years and much effort the basic premise of Kögl et al. is still in doubt.

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THE ISOLATION PROBLEM.—In the initial work of Kögl and Erxleben (30), the crude proteins (saline soluble and insoluble, both extracted with cold ethanol) from a number of normal and malignant tissues were refluxed in 3 volumes of concentrated HCl for 7 hours, and samples of about fifteen amino acids were obtained from the hydrolysates in a relatively pure state. Four of the acids from the tumor proteins exhibited specific rotations, which indicated that more \( \alpha \)-acid was present than is formed by partial racemization under similar conditions in pure solution. These acids were leucine, lysine, valine, and glutamic acid. The first three of these appeared to contain about 3–4 per cent of the \( \alpha \)-form, while the glutamic acid samples from several tumors were calculated to contain as much as 32–45 per cent of the \( \alpha \)-form. Because of the latter finding and the apparent ease with which glutamic acid hydrochloride can be isolated in the pure state from protein hydrolysates, the controversy that soon developed centered about glutamic acid alone.

Kögl and Erxleben (30) employed two purification procedures prior to the crystallization of the glutamic acid hydrochloride from the protein hydrolysates. One of these was the familiar Dakin extraction, in which the majority of the monoamino-monocarboxy-amino acids were removed by extraction with butanol and propanol. More generally, however, use was made of the cuprous oxide method (2), in which the slightly acid hydrolysate was treated with an excess of cuprous oxide to remove humin-like substances, filtered, treated with \( \text{H}_2\text{S} \) to remove the copper, and finally concentrated in vacuo. Subsequent to either treatment, the glutamic acid hydrochloride was crystallized in the cold after saturation with HCl gas. Such preparations generally had the correct elementary composition (C, H, N) after one recrystallization. The per cent of \( \alpha \)-acid thought to be present was calculated from the specific rotations of these samples. The analyses of several normal tissues indicated that only \( \lambda \)-glutamic acid was present, whereas \( \alpha \)-glutamic acid appeared to be present in the tumor proteins in varying amounts up to 45 per cent of the sample isolated. These and later extensive analyses by Kögl et al. (16, 25, 34) also indicated that the amount of \( \alpha \)-glutamic acid present was roughly proportional to the malignancy of the tumor in question. As one would expect, many attempts were made to repeat these important findings. Within a short time several notes (3, 5, 43, 55) appeared in which these results were confirmed. These notes contained but few data, and no more was forthcoming from these laboratories after the controversy was in full sway. At about the same time, three notes (9, 13, 20) appeared in which the data of Kögl and Erxleben were not confirmed, either by their methods or with other isolation procedures. Up to 1944, nine extensive papers (1, 11, 17, 18, 22, 42, 53, 54, 56) and several shorter communications (15, 20, 41, 51) were published from many laboratories which contradicted Kögl at nearly every turn. Kögl did not remain silent, and up to 1943 his group published at least ten original papers dealing with more analyses and different attacks on the problem and in which their initial results were repeatedly confirmed.

In the first attempts to confirm the Utrecht workers, Chibnall et al. (9) and Graff (17) used modified Foreman methods for the isolation of glutamic acid and could obtain only the \( \lambda \)-isomer from tumors. With their methods the Ca or Ba salts of the dicarboxy acids were precipitated with alcohol, the free acids subsequently liberated, and the glutamic acid hydrochloride crystallized in the usual way. Kögl et al. (31) replied that these results were to be expected with the Foreman method, since the Ca salt of the DL-acid was over ten times as soluble as the salts of either isomer; thus, any \( \alpha \)-acid present would remain behind in the mother-liquors as the racemate. To demonstrate this, they presented results (32, 36) showing that DL-glutamic acid added to a hydrolysate of normal protein could not be recovered by the Foreman procedure. However, none of these results were confirmed by others (10, 11, 18, 22) who found that the Ca and Ba salts of the DL-, \( \lambda \)-, and \( \alpha \)-acids all had the same solubilities and that added DL-acid could be recovered from protein hydrolysates by the Foreman method. Chibnall et al. (11) list several possible reasons for the failure of this method in the hands of Kögl et al. However, the only rebuttal made by the latter group is contained in a footnote to a paper published in 1943 (37). Here they stated that, while they could obtain the three equally soluble salts of glutamic acid, it had not been possible to define the conditions under which either the easily or difficultly soluble salts could be regularly obtained. Hence, these contradictions remain to be resolved.

Other difficulties were soon encountered even when the methods of Kögl and Erxleben were closely followed. The initial data presented by Kögl et al. show that the amounts of glutamic acid hydrochloride that they obtained from the protein...
hydrolysates were, in general, quite small and represented only a small part (less than 10 per cent) of the total glutamic acid estimated to be present. However, Kögl et al. believed that the optical analyses of these samples would give the minimum amount of D-glutamic acid present, since they found (32) that in pure solution DL-glutamic acid hydrochloride is twice as soluble as the hydrochlorides of either isomer. Hence, they reasoned that the first crop of acid from a hydrolysate would be less racemized than any succeeding crops and on analysis would give a minimal figure for the per cent of D-acid present. While the other workers agree on the solubilities of the pure hydrochlorides in pure solution (11, 18), they did not find that these relationships held in an optically active medium such as a protein hydrolysate. The data of Chibnall et al. (11) and of Woodward et al. (56) demonstrate that in the Dakin and cuprous oxide procedures the specific rotations of successive crops of glutamic acid hydrochloride from tumor hydrolysates either approach or do not deviate essentially from the rotation of the L-acid. Hence, it is not permissible to calculate the amount of D-acid present solely from the specific rotation of the first fraction; instead, the amounts of the fractions obtained must also be considered. When this is done, the amount of D-glutamic acid present in tumor protein hydrolysates appears to be less than 5 per cent of the total glutamic acid present (9, 10, 11, 13, 15, 17, 20, 22, 56). Kögl et al. (36) attempted to answer this criticism by showing that the second crop of acid hydrochloride in several instances had a slightly lower specific rotation than the first crop. Thus, it was inferred from this that the isomers of glutamic acid hydrochloride crystallized from a protein hydrolysate as they do on crystallization from pure solution.

At first the actual yields of glutamic acid hydrochloride obtained by all the workers were relatively low and of the order of 1–3 per cent of the total protein weight taken. In many cases it was estimated or actually shown (11, 53, 56) that the total amount present was of the order of 8–12 per cent of the protein weight. However, as the arguments continued, each side made greater efforts to obtain higher yields. Kögl’s opponents finally obtained yields up to about 8–10 per cent, but the Utrecht workers claim yields by isolation of up to 19.9 per cent (26) as well as actual contents of up to 17 per cent, as determined by isotope dilution (37).

Another difference between the two opposing groups of workers was soon apparent. While Kögl et al. could not detect any D-glutamic acid in hydrolysates of normal tissue, the other workers could find the same low percentage of D-acid in the hydrolysates of normal tissues that they found for malignant tissues. A similar finding was made in the case of aspartic acid (11), which Kögl et al. (30, 33, 34) claim is present only in the L-form in both normal and tumor tissues. Several workers (11, 51) also found small amounts of D-glutamic acid in the acid hydrolysates of normal plant proteins. There appears to be a general feeling among the opposition that these amounts of D-acid result from the racemization of L-glutamic acid during the acid hydrolysis since the observed racemization of the acid in pure solution is of the same order of magnitude (4, 7, 11, 19, 21, 22, 54). It is of interest here, too, that Kögl’s work prompted the examination of the bacteria which induce plant galls (8) and of one crystalline plant virus (49) for the presence of D-acids; none were found in either case. Similarly, Kögl et al. (27, 28) could not find any D-glutamic acid in the glutathione contained in tumors.

Early in the controversy, Kögl et al. (32) reported a rather arresting result. From the very malignant Brown-Pearce rabbit tumor they had earlier reported (30) the isolation of 10.3 per cent of the protein weight as pure glutamic acid hydrochloride. This sample exhibited a rotation which indicated that 44.5 per cent of the D-form was present. The sample was then resolved by fermentation with yeast to give a sample of pure D-glutamic acid (correct C, H, N analyses and rotation) in a yield of 30.5 per cent of what was presumed to be present originally. Naturally, the opposition has never had the opportunity to check this positive result, but it does represent a minimal content of D-glutamic acid which is still several-fold higher than the levels found by the other workers in either normal or tumor tissues. The detailed presentation of more results like this, but in which the optical purity of the resolved isomer was rigorously proved, would do much for the cause of Kögl et al.

The most recent communications in which the isolation of highly racemized glutamic acid from tumor tissue could not be confirmed were from three German laboratories. Abderhalden (1) obtained D-glutamic acid from tumor tissues but not from normal tissues; however, he could not find more than a few per cent of the D-isomer in the glutamic acid of tumors. His paper is largely concerned with the difficulties encountered in obtaining pure glutamic acid in large yields from protein hydrolysates. Wieland (53) introduced a new

1 A sample of pure D-glutamic acid was also recovered from an ovarian carcinoma, but the yield was low.
method for the isolation of the dicarboxy acids. These acids were adsorbed from the hydrolysate on an acidified alumina column and then eluted with barium hydroxide. Wieland showed that cystine is not present in the dicarboxy acid fraction obtained with this method and that it can be used to recover racemic glutamic acid added to a tumor protein hydrolysate. However, Wieland could only find minimal amounts of D-glutamic acid in hydrolysates of normal or malignant tissue. Klingmüller (22) also could not confirm Kögl’s results, using either the cuprous oxide, Dakin, or Foreman procedures. Klingmüller carefully tested each of his acid samples for cystine and showed that none was present; if a few per cent of cystine dihydrochloride, a possible contaminant, were present, it would not alter the C, H, and N analyses significantly, but the specific rotation of the sample would be lowered greatly because of the high levo-rotatory power of this acid.5

It would appear, in retrospect, that too little attention has been paid by the Utrecht school and by some of the other workers to the criteria of purity necessary to demonstrate that the optical rotation of any given sample of glutamic acid is actually a measure of the D-glutamic acid contained therein. Some authors, for example, have reported only melting points (3, 5, 12, 13, 14, 45); this criterion is unaffected by impurities such as NaCl, even up to 50 per cent (42). Other authors note that both NaCl and NH₄Cl (1, 11, 22) and other amino acids (42) are difficult to remove by recrystallization from glutamic acid hydrochloride. This would be particularly the case when the recrystallization is carried out with the small weight losses (10 per cent or less) recorded and recommended (31, 32) in the papers of Kögl and his collaborators. Such contaminants would obviously alter the specific rotation, and a substance such as NH₄Cl would escape detection in the usual ash determination. While C, H, N, and ash determinations have been used most frequently as criteria of purity, it is particularly apparent from the work of Klingmüller (22) and Abderhalden (1) that further criteria must be applied to eliminate the possibility that contaminating amino acids contribute to the observed optical rotations. Very likely, the techniques of partition and paper chromatography and of microbiological analysis could be applied with considerable advantage. Even so, it is difficult to explain the differences observed by Kögl et al. between normal and malignant tissues as due to contaminants.

While it is difficult to summarize the work on the isolation of glutamic acid hydrochloride from tumor and normal tissues, two points should be considered. When Kögl et al. obtained small yields of the acid, the preparations probably were optically pure. Even their opponents could also obtain first crops with high degrees of racemization; but the disagreement at this point centered about Kögl’s assumption that such preparations were representative of the total glutamic acid present. However, when Kögl et al. obtained large yields of the acid hydrochloride, they did not prove that the low rotations of these preparations could not have been due to contaminating amino acids and salts which quite possibly would not have been discovered by the criteria of purity which were applied. In any case, the isolation, in one instance, of a relatively large amount of “pure” D-glutamic acid from a malignant tumor remains to confront Kögl’s opposition, although it is clear that this one example does not prove Kögl’s general thesis.

Enzymatic Studies.—Lipmann et al. (42) employed D-amino acid oxidase to test tumor protein hydrolysates for the presence of D-amino acids, since the specificity of this enzyme for these isomers is very great. While they recognized the fact that D-glutamic acid is rather slowly attacked by the enzyme, they were able to recover D-glutamic acid added as the racemate to hydrolysates of normal tissue. In no case could they detect more than 1–3 per cent of D-acid-nitrogen in the total nitrogen of the hydrolysates of tumor proteins. However, when Kögl, Herken, and Erxleben (39) employed D-amino acid oxidase, they could not recover added D-isomer from protein hydrolysates and concluded that it could not be used in such complex mixtures, particularly for an acid as slowly oxidized as D-glutamic acid. However, Kögl et al. could determine this acid in pure solution with the enzyme. This is interesting, since with this technique Lipmann et al. still could not detect any D-acid in the glutamic acid isolated from tumors.

It seems likely that several enzymes could be employed to destroy a large part of the L-glutamic acid present in tumor hydrolysates. If any considerable quantity of D-glutamic acid were present, the remaining glutamic acid would be very highly racemized and presumably could be isolated by the methods already mentioned. The L-glutamic acid decarboxylases, oxidases, and transaminases from various sources could be considered here.

Kögl et al. (35) have also published data on the digestibility of tumor proteins in vitro. They noted that the feces and urine of dogs fed cooked tumors

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5 The contamination of the glutamic acid hydrochloride by cystine dihydrochloride would be unlikely if the cuprous oxide procedure were applied as given by Bailey et al. (6). The [α]D of L-cystine in 3.6 per cent HCl is approximately −220° (22).
contained large amounts of peptides, which on hydrolysis yielded highly racemized glutamic acid. The feeding of normal tissues yielded only small amounts of peptides, which contained no d-glutamic acid. Aside from the results on the racemization of the glutamic acid in the peptides presumably derived from the tumors, the great differences observed in this work between normal and tumor tissues in terms of the excretion of bound glutamic acid should receive some attention from other workers.

Isotope Dilution Studies.—The first application of the powerful analytical method of isotope dilution to this problem was made by Graff, Rittenberg, and Foster (18) in 1940. They employed n-glutamic acid labeled with N14 and added this to the hydrolysates of six different tumors. After boiling the acid hydrolysates for 8 hours to equilibrate the added acid with any a-pyrrolidone carboxylic acid formed in the original hydrolysis, samples of pure L- and D-glutamic acid were isolated and their isotope contents determined. In no case did the isotope analyses indicate that more than 1 per cent of n-glutamic acid could have been present in the total glutamic acid found in the tumor proteins.

While the experiments of Graff et al. seemed very convincing, the Utrecht group performed isotope experiments with the opposite results. These were published by Kögl, Erxleben, and van Veersen (37) in a paper which contains a large number of experiments with deuterio-glutamic acid. Except for a somewhat lower degree of racemization in the glutamic acid of tumor tissues by this technique, their results were in general a complete reaffirmation of their earlier stand. They suggested that the negative results of Graff et al. were probably due to incomplete mixing of the labeled glutamic acid with the acid already present in the hydrolysates. While Kögl et al. did not provide any rational basis for this extraordinary suggestion, they did present some data which tended to support this explanation. If the tagged acid was added after the main hydrolysis had occurred, but before the equilibration period, they could only partially account for any added n-glutamic acid; on the other hand, the addition of the marked compound before the acid hydrolysis was performed permitted good recoveries of the added n-glutamic acid. This surprising finding was soon checked by Wieland and Paul (54), who employed N14-glutamic acid in hydrolysates of the very malignant Brown-Pearce tumor employed in much of the work of the Utrecht school. Wieland and Paul added the labeled acid before the acid hydrolysis according to Kögl et al. However, their results were essentially negative and showed that not more than 5 per cent of the glutamic acid present in these tumors could have been n-glutamic acid. They suggest that the results of Kögl et al. may have been due to labile deuterium atoms in the marked acid used by these workers. However, the papers of Ratner et al. (47) and Kögl et al. (40) on the lability of certain deuterated glutamic acids in acid do not seem to clarify this point.

Somewhat later Rittenberg and Shemin (48) also criticized the paper of Kögl et al. (37) on the basis that it appeared to contain errors in calculation and reasoning in the application of the techniques of isotope dilution. While these specific criticisms were answered recently by the Utrecht group in an appendix to a paper on another subject (38), the main contradictions apparent between the work of Graff et al. and of Wieland and Paul with that of Kögl's group remain unanswered to date.

The New Data of Kögl et al.—Despite the many contradictions that remain to be clarified, the Utrecht school has recently published two papers on biochemical experiments which they feel again support their original contention that n-glutamic acid exists in tumor proteins but not in normal tissue proteins.

The first of these recent communications is a note published in 1948 (29). In this paper use was made of the observation of Ratner (46) that about 75 per cent of single doses of n-glutamic acid appeared in the urine of rats as d-a-pyrrolidone carboxylic acid; l-glutamic acid did not yield the corresponding lactam in the urine. Kögl et al. reasoned that the feeding of tumor proteins should produce a similar result if n-glutamic acid actually existed in these tissues and was liberated therefrom in the body. They report that after feeding cooked rat tumors (induced by benzpyrene) to a dog, a small amount of pure d-a-pyrrolidone carboxylic acid could be isolated from the urine. The amount obtained was estimated to be about 10 per cent of the theoretical on the basis of the usual optical analyses of the tumor proteins employed. The same dog was later fed beef protein, and the urine was extracted as before. However, no attempt was made to isolate any a-pyrrolidone carboxylic acid; instead a sample of the 1-lactam labeled with deuterium was added to the extract and then reisolated. This material did not show a lowered isotope content, so it was concluded that no d-a-pyrrolidone carboxylic acid was present in the urine obtained after feeding the beef protein. The 1-lactam was also obtained (as n-glutamic acid after hydrolysis; yield not stated) from the urine of rats fed with similar tumors.
The second paper appeared in 1949 (26). This is largely a résumé of the work by the Utrecht group, although it contains several tables of new data obtained with the aid of isotopes. At first Kögl attempts to show that the hydrolysis procedures used by other workers were unable to release the D-glutamic acid present in tumor proteins. This claim apparently does not agree with the earlier statement (37) that the amount of D-glutamic acid obtained from tumor proteins is independent of the time of hydrolysis for periods of 7–20 hours. Now Kögl contends that their particular method of heating allows the concentration of acid (HCl) during the hydrolysis to remain for a short time higher than that in the methods of his opponents, and produces a higher amino-N/total-N ratio. In all fairness to the large number of workers whose results have been so uniformly negative in this controversy, this attempt by Kögl to explain their results appears to be very weak. His data on the amino-N/total-N ratios in protein hydrolysates made in various ways are not to the point. Detailed experiments on the liberation of D-glutamic acid from tumor proteins are necessary if this explanation is to be used to explain the difficulties so widely experienced in the efforts to confirm the Utrecht school.

Much of the new data presented in the second paper are concerned with the administration of D2O and various deuterated amino acids to normal and tumor-bearing rats. While few experimental details are given, the data indicate that the D-glutamic acid present in tumors originates by a different metabolic pathway from that employed in the biosynthesis of the L-isomer. In conclusion, Kögl presents a few data on what he regards as an “experimentum crucis.” In this case, D-glutamic acid labeled with C14 in the carboxyl-carbon and a-carbon atoms (38) was injected into normal and tumor-bearing rats. The expectation was that the latter rats would normally excrete a small amount of D-a-pyrrolidone carboxylic acid derived from the tumors, which would dilute any of this compound formed from the administered D-glutamic acid. The data indicate that a small but definite dilution of the isotope occurred in the acid derived from the urine of the tumor-bearing rats but not in the acid from the urine of the normal rats. Furthermore, each isomer of glutamic acid was isolated from the tumor proteins, and only the D-isomer was found to be radioactive.

The most recent paper (38) from Kögl's laboratory is concerned with the synthesis of the D-glutamic acid-1,2-C14 used in the work just mentioned. Kögl et al. report a yield of the D-acid of 102.5 per cent by resolution (through recrystallization) of a mixture of 2 parts of the radioactive DL-acid with 1 part of inactive L-acid. This result is apparently due to the presence of a few per cent of the racemate. While it seems unlikely that the small amount of L-acid (with twice the specific activity) present could have played a role in the biochemical experiments, it would appear that the yield should be sacrificed for greater purity.

It is apparent that publication of the full details on these new experiments, as well as their repetition in other laboratories, will be necessary for their evaluation.

Possibilities Emphasized by the Controversy.—While it is hoped that this dispute will yet produce some positive information of value to oncology, it probably will stimulate interest in the general problem of the presence or absence of D-amino acids in proteins. Thus, even though it is generally held that the rates of racemization of L-glutamic acid under various conditions of hydrolysis are sufficient to account for the low levels of D-glutamic acid found in protein hydrolysates, these findings do not exclude other possibilities. For it is also possible either that low levels of the D-acid actually exist in some proteins or that some of the L-residues in certain proteins are in peptide sequences which would be easily racemized upon hydrolysis. Indeed, some evidence exists that acid hydrolysis may bring about the racemization of L-glutamic acid in certain synthetic peptides (52). Nor is it inconceivable that amino acid racemases may exist. The presence of such enzymes would complicate the interpretation of the biochemical experiments reported by Kögl et al. On the other hand, it is interesting to speculate on the mode of action of carcinogens if one assumes that the claims of the Utrecht group are even partially correct. Such agents might induce either racemization within peptide chains or they may cause the formation of easily racemizable sequences of amino acids in proteins. It would appear that the great variety of the known carcinogenic agents would cause little difficulty in such concepts. Finally, the controversy may perhaps focus more attention on the symmetry of the synthesis of amino acids in vivo. The data of Shemin and Rittenberg (50) seem to indicate clearly that the biosyntheses of glutamic acid and tyrosine are totally asymmetric in the rat. However, the biosyntheses of the other amino acids need to be examined in this regard. Such studies may give a clue as to the function of D-amino acid oxidase.

In any event, the reviewer feels that few scientists would share Kögl's conviction in 1949 (26) that the existence of D-glutamic acid in tumor proteins is now finally established. The burden of proof still lies with the Utrecht school.
APPENDIX

After this review was submitted for publication, a note by P. Boulanger and R. Osteux (Compt. rend., 229:311-13, 1949) on “Caractérisation et dosage de l’acide d-glutamique dans les protéines de tissus normaux et néoplasiques et les protéines microbiennes” came to the author’s attention. These workers isolated glutamic acid from hydrolysates of the proteins by adsorption on acidified alumina and elution with dilute acetic acid. The purity of the eluate was verified by paper chromatography, and the L-glutamic acid present in the total glutamic acid was determined with a bacterial L-glutamic acid decarboxylase. Following chromatography, and the L-glutamic acid present in the total glutamic acid was determined with a bacterial L-glutamic acid decarboxylase. Following the destruction of the L-glutamic acid by the enzyme, the preparations were chromatographed on paper to obtain concentrates of the D-glutamic acid. Boulanger and Osteux found 0.6–2.4 per cent of the D-acid in the glutamic acid from a variety of both normal and tumor proteins. As expected the glutamic acid from Bacillus anthracis contained a high level (approx. 40 per cent) of D-glutamic acid. Four other bacterial species were examined and 1.2–7.6 per cent of the glutamic acid was found to be in the D-form.

REFERENCES

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