Emil Abderhalden: His Contribution to the Nutritional Biochemistry of Protein

GEORGE WOLF

Department of Nutritional Sciences, University of California at Berkeley, Berkeley, CA 94720-3104

Emil Abderhalden (1877–1950)

Emil Abderhalden was a dominant figure in physiological chemistry in Germany between the two world wars. His Lehrbuch der Physiologischen Chemie went through 26 editions. He edited the Handbuch der biologischen Arbeitsmethoden in 107 volumes, with 944 contributors, completed in 1939.

Abderhalden’s principal researches were in the biochemistry and physiology of protein. With hindsight from today, what can one say about his significance in the history of protein research?

Abderhalden was born in Switzerland in 1877. He worked as a medical student in Basel under the physiologist von Bunge. He there became interested in the process of digestion and absorption of protein. Von Bunge held the traditional view of protein digestion and absorption: protein was absorbed from the intestine unchanged, or only somewhat changed by digestion so as to make it diffusible—Nature would not allow complete decomposition, because it would be a “waste” of chemical energy.

After graduating as a medical doctor in 1902, Abderhalden went to work for the great chemist Emil Fischer in Berlin. Fischer had developed an ingenious method for isolation, purification and assay of amino acids. It consisted of esterification followed by fractional distillation of the esters under reduced pressure. At that time already, Fischer (1906) suspected that proteins consisted of amino acids linked by peptide bonds.

In their first paper, Fischer and Abderhalden (1903) describe the hydrolysis of a number of purified proteins—casein, edestin (from hemp seed), hemoglobin, egg albumin, serum globulin, fibrin—by pancreatic trypsin. With all these proteins, they observed the same pattern of liberation of amino acids: tyrosine first, leucine, alanine, aspartic and glutamic acids and the three basic amino acids more slowly, but phenylalanine and proline not at all. Phenylalanine and proline apparently were contained in a polypeptide (called “peptone”) resistant to tryptic digestion. On the other hand, acid digestion liberated all amino acids from both the protein and the enzyme-resistant peptone.
Although not explicitly stated by Fischer and Abderhalden (1903), at the time the idea was in the air (Carpenter 1994) that proteins consisted of a core ("Kern") common to many or all proteins, to which various amino acids were added in varying amounts, as suggested, for instance, by Kossel (1901). Could the enzyme-resistant peptide be this core?

At that time it was known that the intestine could cause disappearance of the peptide, as was thought by direct absorption (Hofmeister 1882) or by reconstitution to new protein within the gut wall. However, Cohnheim (1901) isolated an enzyme, called "erepsin," from intestinal mucosa that split the peptide to amino acids. This discovery, with one stroke, disposed of the "core" hypothesis and demonstrated that proteins can be digested to amino acids in the lumen of the intestine.

About the same time Loewi (1902), by feeding an autolysate of pancreatic protein to dogs, demonstrated that a protein-free (negative biuret test, biuret-free) protein digest could substitute for dietary protein in keeping the animals in positive nitrogen balance. He concluded that animals must be able to synthesize body proteins from the protein breakdown products in the intestine. Loewi's important findings were extensively confirmed by Abderhalden. Approaching the normal digestive processes more closely than Loewi, Abderhalden and Rona (1904) fed trypsin-digested casein to mice. The animals survived and thrived just as well as the casein-fed control animals. On the other hand, feeding an acid hydrolysate of casein resulted in sickness and death of the mice. Similar experiments were performed with dogs, kept in positive nitrogen balance when fed trypsin-digested casein. Upon receiving acid-hydrolyzed casein, the dogs almost immediately suffered a negative nitrogen balance (Abderhalden and Rona 1905). Closely similar results obtained with rats were reported by Henriques and Hansen (1905).

In the same year, Abderhalden published his "Habilitationsschrift" (thesis to allow him to become a university teacher) (Abderhalden 1905). In this work he proposed the important idea that proteins must be degraded in the digestive tract to a series of nonspecific building blocks and that it is only from these that specific proteins can be built up. The building blocks could be the amino acids. He stated that the protein decomposition products—the building blocks—are qualitatively the same for different proteins but differ in quantity for different proteins. He presented the quantitative composition of serum globulin, casein, elastin and other proteins in respect to 16 amino acids, using the Fischer distillation method (Table 1). He drew attention to the fact that a suckling infant obtains only casein, lactalbumin and lactoglobulin as dietary proteins, but produces proteins specific for blood, muscle, skin, etc. He proposed, therefore, that a transformation must take place whereby the dietary proteins are decomposed into nonspecific building blocks, the amino acids, from which the specific proteins are then synthesized. How to obtain evidence for this hypothesis? After Cohnheim's discovery (1901) of erepsin, which digests protein all the way to amino acids, Abderhalden attempted to detect free amino acids in the digestive tract of dogs fed protein, but he failed (Abderhalden 1905).

The reason for the absence of detectable amounts of free amino acids in the gut, Abderhalden argued, is their rapid absorption as soon as they are formed from protein. For instance, in vivo digestion of protein is complete in 6 to 8 h, whereas in vitro, with trypsin and erepsin, the digestion takes several days. He postulated that the high rate in vivo was caused by the constant removal of the products of digestion, whereas in vitro their presence would inhibit the process (Abderhalden 1912a). In an experiment, he added excess alanine to an in vitro digestion, and indeed found that the process was slowed considerably. As early as 1898, Fischer had suggested that enzymes combined with substrates through a specific enzyme-substrate interaction (Frunton 1972). Abderhalden proposed the hypothesis that the protein decomposition products (i.e., amino acids) inhibited formation of the enzyme-substrate complex, what we would now term a competitive inhibition by product of the substrate-binding sites on the enzyme.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Abderhalden1,2</th>
<th>Harvey3</th>
<th>Abderhalden1</th>
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<td>0.4</td>
<td>0</td>
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1 Abderhalden (1905).
2 Chibnall (1939) comments that it was realized that the amino acids discovered at that time represented only part of the protein molecule, this is seen in the sum of the values reported by Abderhalden being so much less than 100%.
3 Harvey (1970).
5 ND = not determined.
Note that this was before the famous Michaelis-Menten paper [1913] on the mathematical treatment of the enzyme-substrate interaction and competitive inhibition.

Another route for confirmation of the hypothesis had to be taken, because the end products of digestion could not be detected in the intestine during protein digestion. By that time (1909), the use of erepsin in addition to trypsin enabled Abderhalden to prepare a protein digest consisting only of amino acids. He could then find out whether animals could be maintained in a healthy state and in positive nitrogen balance when fed such mixtures. Of course, Loewi already in 1902 and later Henriques and Hansen [1905] had found that animals remained healthy and in positive nitrogen balance when fed a protein-free (i.e., biuret-free) protein digest. However, Abderhalden had observed that "biuret-free" does not mean free of polypeptides, and a rigorous and quantitative proof was needed to show that the trypsin-erepsin digests contained only the ultimate building blocks, the amino acids. This was done [Abderhalden 1912a] by use of the Fischer method of quantitative assay of individual amino acids by fractional distillation of their esters (esters of polypeptides would not, of course, distil). It should be emphasized that Abderhalden validated the method by applying it separately to each of the 16 amino acids then known to occur in protein, to determine exactly what recoveries could be obtained. He then assayed a portion of the enzymatic digest for amino acids; another portion was hydrolyzed by hydrochloric acid and the amino acids were again assayed. The results showed that there were only amino acids and no polypeptides present in the trypsin-erepsin digest. This digest, when fed to dogs, kept them in positive nitrogen-balance (Abderhalden 1909). For instance, by feeding a young dog the trypsin-erepsin digest of meat, the animal gained weight normally and was steadily in positive nitrogen balance for 3 wk. Similarly fed, a pregnant bitch even delivered healthy puppies on this diet. To quote Abderhalden [1912b]: "[W]e established without question that the mixture of amino acids obtained from protein could substitute completely for protein." A rigorous proof had been provided that dietary protein is digested to amino acids and that these are the building blocks for the synthesis of new body proteins.

In the same year, Folin and Denis [1912a and 1912b] arrived at a similar conclusion by a different route: they infused amino acids or a pancreatic autolysate into the intestines of cats. Methods for the determination of amino acids in blood were not then available. Instead, they were able to observe the successive rise, peak and fall of non-protein, non-urea and non-ammonia nitrogen in blood and tissues. Their conclusions, though of necessity based on indirect, negative evidence, were essentially correct (Folin and Denis 1912c): "We reported experiments which showed that amino acids are absorbed as such from intestine and are transported unchanged to all the different tissues of the body."

At the same time Abderhalden speculated that proteins were also degraded to amino acids in tissues. As evidence he mentions [Abderhalden 1905] the excretion of cysteine in urine in the disease cysteinuria and phenylalanine derivatives in phenylketonuria.

As early as 1912, Abderhalden [1912a] proposed the hypothesis that different proteins are distinct because of differences in the sequence of their amino acids and realized that different combinations of the then-known 16 amino acids occurring in protein could result in a very large number of different proteins.

Abderhalden became professor in the Veterinary University in Berlin in 1908 and professor and department head of physiology and physiological chemistry in Halle, Germany, in 1911, where he remained until 1945.

Tryptophan was discovered by Hopkins and Cole (1902) and isolated from a pancreatic digest of casein. These investigators were the first to show that tryptophan was destroyed during acid hydrolysis of protein. Later, Willcock and Hopkins [1906] fed mice a diet of zein as a protein source. This plant protein lacks tryptophan; the mice rapidly lost weight and died, whereas the mice survived twice as many days when given a tryptophan supplement. They lost weight, however, even when given tryptophan, a fact ascribed by Willcock and Hopkins to the lack of lysine or other amino acids in zein. Thus, these authors were the first to recognize that an amino acid was essential. However, they open the question of what the amino acid was essential for: "...it [tryptophan] serves as a basis for the elaboration of a substance absolutely necessary for life—something, for instance, of an importance equal to that of adrenaline. . . ."

Abderhalden [1909] greatly expanded the work of Willcock and Hopkins. He fed dogs a digest of meat protein consisting only of amino acids. When he removed tryptophan from the mixture by precipitation with mercuric sulfate and fed the remaining mixture to dogs, he observed a rapid drop in nitrogen balance to the negative side. He then succeeded in restoring positive nitrogen balance by re-adding tryptophan. He found that dogs, when fed a diet lacking tryptophan, excreted increased amounts of amino acids in urine. Abderhalden [1909] states explicitly that tryptophan was an essential building block for newly synthesized protein.

By 1912, Abderhalden [1912b] had reported the essentiality of phenylalanine, because a digest of gelatin, which is low in this amino acid (gelatin: phenylalanine, 0.8%; casein: phenylalanine 5.6%), could not sustain nitrogen balance, unless phenylalanine [and tryptophan] were added to the digest. He observed that a digest of the plant protein gliadin, low in lysine, could not maintain nitrogen balance, and he suggested (1912b) that adding animal protein to plant protein might protect against inadequate utilization of a pure plant protein (i.e., vegetarian) diet.
In the same series of experiments, Abderhalden showed that glycine and proline were nonessential and speculated that proline is perhaps derived from glutamic acid, itself nonessential.

Abderhalden (1905) fed complete digests, after amino acid analyses, of serum albumin, egg albumin, casein, serum globulin, silk fibroin and gelatin, and from the nitrogen balance results concluded that there are minimal requirements of the essential amino acids.

As early as 1872, Ritthausen (1872) had speculated that differences in the proportion of the amino acids between animal and plant proteins may be the cause of the differences in their nutritive value. This idea was confirmed by Abderhalden (1912b), who held that the proportions of the quantities of dietary amino acids from protein were more important than their absolute amounts.

Abderhalden hoped to provide the ultimate proof that the animal organism could use free amino acids to build its own proteins, by feeding a completely synthetic diet of 16 amino acids, with glucose, fructose, fatty acids and glycerol (1912b). We now know that methionine and threonine were missing. Of course, the diet was very badly tolerated. Nonetheless, Abderhalden reported that he had obtained positive nitrogen balance when feeding the mixture to dogs for 6 d. Because of frequent vomiting and diarrhea, he then gave a meat digest for 2 d to allow the dogs to recover, then gave the mixture again for 6 d, with positive nitrogen balance. Abderhalden comments that “common street dogs” tolerated the diet better than dogs used to good food, and that the animal feeder had to establish “friendly relations” with the experimental dog. With “verbal persuasion (‘Zureden’), a dog can be made to eat anything” (1912b).

We now know that Abderhalden erred in supposing that a positive nitrogen balance could be obtained with his synthetic, protein-free diet that lacked methionine and threonine. The experiment was performed under extreme conditions, with small numbers of animals, for very short times. Osborne and Mendel (1912/1913), who review Abderhalden’s work, state “...the necessity of long-continued experiments calls for particular emphasis. The fact that a satisfactory nutritive balance can be maintained for a week or two ...is no guarantee of the ultimate success of the dietary. ...” Nonetheless, it was a first attempt at a synthetic diet and a precursor of later work, primarily in the United States, using vitamin concentrates, minerals and the missing amino acids that allowed for long-term feeding of purified, synthetic diets. Abderhalden correctly predicted that a synthetic mixture of amino acids with other essential nutrients would one day be developed as a means of parenteral nutrition.

With some reservations, then, one could say that Abderhalden established the following: 1) specific dietary proteins are digested to a nonspecific mixture of amino acids; 2) the body synthesizes specific proteins from the nonspecific amino acid mixture absorbed from the gut; 3) some amino acids must be obtained from the diet, whereas others are made by the organism; 4) the kind of protein fed is irrelevant, provided the kind and quantity of amino acids and their proportions are adequate for the proteins to be made by the organism.

Although the experiments of Loewi (1902) and Henriques and Hansen (1905) had strongly suggested that body protein could be synthesized from nonprotein precursors such as polypeptides and amino acids, it was Abderhalden who provided rigorous, quantitative proof of this hypothesis. Abderhalden (1912a) admitted that we know very little about the ability of the body cells to synthesize amino acids, although we know that glycine can and tryptophan and the aromatic amino acids cannot be made by cells of the body. Abderhalden could therefore be regarded as the discoverer of the distinction between essential and non-essential amino acids. The reservations are that almost all Abderhalden’s experiments depended on short-term nitrogen balance experiments in dogs. Osborne and Mendel (1911) state “...a favorable N-balance over a short period of time is in no sense an adequate index to a satisfactory nutritive condition.” They remark that positive nitrogen balance results can often be erroneous due to losses of urinary nitrogen, leading to an erroneous assumption of greater retention of nitrogen than actually occurred. The long-term growth experiments with rats by Osborne and Mendel, starting in 1911, and the later work of Rose put the concept of essential and non-essential amino acids on a firm footing.

In the years after the first world war, Abderhalden worked on many topics, amongst others the metabolism of cholesterol, thyroid hormone and the vitamin B complex. His “favorite child,” however, was the “Abwehrreaktion” or defense reaction. Abderhalden postulated, and appeared to find evidence to show, that foreign proteins, when injected into animals, elicited the appearance in blood of specific proteinases that degraded the foreign protein. Starting in 1909, he worked on these putative plasma enzymes as possible early indicators of pregnancy, infection and cancer, until his retirement in 1945. His findings could not be confirmed and the concept of “defense enzymes” in plasma has now disappeared without trace. Abderhalden made other mistakes, such as his reported discovery of dictopiperazine linkages in protein (Abderhalden and Komn 1924). Perhaps these errors were made in consequence of his enormous output of publications (over 1,250 titles between 1897 and 1950). It is possible that his standing in the history of nutrition would have been higher if the mistaken concepts had not been published or had been corrected in subsequent publications. Abderhalden died in Zurich in 1950.

For a man who occupied a position of such eminence in Germany during the Nazi years, including the sec-
ond world war, the question must inevitably be asked: what was Abderhalden's attitude towards the regime?

Abderhalden had a strong social conscience: all his life he fought against the use of alcohol and tobacco. In 1915, he founded an association to provide smallholdings for poor working families, who could rent them and also obtain seed and fertilizer for a nominal fee, to grow food for themselves and to enjoy gardening in their leisure time. By 1949, there were almost 3,000 tenants occupying 1 sq. km near Halle. In 1919, Abderhalden organized transports for undernourished German children for 3 mo of recuperation in Switzerland; ultimately 60,000 children participated. He founded a home for babies and infants with 80 beds and sought ways to provide food, clothing and fuel for the elderly in the hard years in Germany, 1922–1924, in his city of Halle.

As a Swiss citizen, Abderhalden was not forced to join the Nazi party. His son Rudolf, in Abderhalden's obituary (Anonym. 1951/1952), quotes from a party document, discovered after the war, that describes the efforts made by the party to remove him from his university chair, because his "Weltanschauung" was "intolerable" to the party. Rudolf Abderhalden states that Abderhalden frequently intervened in favor of colleagues in concentration camps and achieved their release or at least a mitigation of their condition. However, neither the obituary nor a biography (Gabathuler 1991) quotes actual names or details.

In his position as a university professor and as an eminent physician he knew the facts about concentration camps and must have been informed first about the killing ("euthanasia program") of retarded children and mentally defective adults (estimated at 5,000 children and 100,000 adults) (Gabathuler 1991), which was stopped in 1941 as a result of protests by the Protestant church; later, he must have been aware of the systematic killing of the Jews. Yet not the slightest word of protest, public or private, is reported, nor any thoughts of emigration. It would presumably have been easy for him to obtain a university post in his native Switzerland. Ironically, he was forced to leave Halle and his position there in consequence of the American occupation of parts of Germany, and he thereupon chose to emigrate to Switzerland in 1945.

Gabathuler's biography (1991) makes the usual plea that he could do more to help those suffering from the regime while remaining in his high position than if he had given it up, to be replaced by a Nazi party member. Abderhalden writes after the war (Gabathuler 1991): "With open eyes I saw how the German people was led towards the abyss. . . . Already in 1934, I came under surveillance of the Gestapo. I stayed completely away from the Nazi party from the beginning. I had contacts with circles who opposed privately and often also in public what happened under the Nazis."

This essay is best closed with a quotation from a book, written by Abderhalden (1947) in Switzerland during his retirement: "The unimaginable has become reality: a people that was once described as one of poets and thinkers . . . has heaped upon itself immeasurable guilt, a people led by irresponsible persons who, without hindrance, accomplished the realization of their criminal, often pathological predispositions."

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EDITORIAL NOTE FROM KENNETH J. CARPENTER

We are grateful to Dr. Wolf for having prepared the preceding paper about the work of Emil Abderhalden that related to the nutritional value of proteins. It is clear from the papers of Osborne and Mendel on the same subject that they were building on the earlier work of European scientists, particularly Abderhalden and his colleagues, that had been conducted in the early years of the present century. However, there is little or no reference to this work even in the standard works on the history of nutrition (McCollum 1957, Gugenheim 1981). As Dr. Wolf explains, this may be due in part to Abderhalden having in his later work promoted ideas that others were unable to confirm. Nevertheless, it is appropriate that a gap in the historical record of our subject has now been filled.

LITERATURE CITED


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