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From soybeans to lectins: a trail of research revisited*

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In the spring of 1942 I was inducted into the U.S. Army after having received a BS degree in Food Technology from the Massachusetts Institute of Technology, a degree which, in the eyes of the army, preeminently qualified me for an assignment as an instructor in the Cooks and Bakers School of the Quartermaster Corp at Fort Knox Kentucky. Most of the rest of my career in the army was spent as a mess officer in European Theater of Operations. Following my discharge from the army in 1946, I entered the Ph.D. program in Biochemistry and Nutrition at the University of Southern California. After devoting one year to intensive course work to get back on track with respect to chemistry, which by this time had become but a very hazy memory, I was afforded the opportunity of returning to active duty, the carrot on the stick being that I would be free to complete the research I needed for my Ph.D. degree. To this end, I was assigned to the Quartermaster Food and Container Institute, then located in Chicago. One of the projects the army was interested in at that time was the increased utilization of soybeans as a possible substitute for meat protein in the army ration program. Thus began my involvement with soybeans.

Based on the early observation by Osborne and Mendel[1], it was well known that the nutritive value of soybean protein did not support the growth of rats unless it had been subjected to heat treatment. It was generally believed at that time that the beneficial effect of heat was due to the inactivation of a trypsin inhibitor which had been previously isolated by Kunitz[2] from raw soybeans. The main objective of my thesis research was to elucidate the mechanism whereby the trypsin inhibitor exerted its deleterious effect on animal growth.

Based on experiments in which the purified Kunitz inhibitor had been added to the diets fed to rats, it became apparent that the trypsin inhibitor did not fully account for the poor growth seen with raw soybeans[3]. Adding the trypsin inhibitor to heated soybeans failed to lower the growth rate of rats to the same extent as a diet containing unheated soybeans. Thus began my search for the presence of some other component in soybeans which might be responsible, at least in part, for its poor nutritive value.

After receiving my Ph.D. degree and my release from the Army in 1949, I joined the staff of the Department of Biochemistry at the University of Minnesota. As is the case with most fresh Ph.D.s who assume a new academic position and are pressured into showing some evidence of productivity as rapidly as possible, I chose an area of research

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with which I was already most familiar, which, in my case, was to continue my research on soybeans. I was still intrigued by the possibility that there was a factor in raw soybeans, other than the trypsin inhibitor, which was acting as a toxin or at least as a growth inhibitor. An indication that this unidentified factor was indeed different from the trypsin inhibitor came from experiments which showed that the intraperitoneal injection of a crude soybean extract into rats caused growth inhibition and ultimate death in a dose dependent fashion, an effect which was not observed with pure trypsin inhibitor administered in a similar fashion[4]. The toxic principle was subsequently purified by salt fractionation to the point of homogeneity as evidenced by moving boundary electrophoresis and sedimentation in the ultracentrifuge[5]. [It should be appreciated that at this time (1952) these techniques were one of the very few available to the protein chemist for evaluating the purity of proteins.] During the course of its purification, it was noted that the toxicity of the various protein fractions closely paralleled their ability to agglutinate the red blood cells from the rabbit. My reason for measuring hemagglutinating activity was based on an observation reported back in 1908 by Landsteiner and Raubitschek[6] that crude extracts of many edible legumes, including soybeans, displayed hemagglutinating activity. While it is true that Stillmark, who is generally credited with the discovery of lectins, had shown over 100 years ago that ricin, the toxic principle of the castor bean, displayed hemagglutinating activity[7], little attention had been paid to the possible toxicity that might be associated with the hemagglutinins present in edible beans. In fact, Goddard and Mendel[8] regarded the hemagglutinins of most legumes to be nontoxic, and because of this they proposed that they might be of "considerable practical importance as an aid in the preparation of antisera".

As an aside, it should perhaps be mentioned that when this toxic hemagglutinin was first isolated it was given the name of "soyin", in accordance with the precedent of naming agglutinins on the basis of their plant origin, *i.e.*, ricin from *Ricinus communis*, abrin from *Abrus precatorius*, and crotin from *Croton tiglium*. It was subsequently brought to my attention that the name "soyin" had previously been used to denote the proteolytic activity which Laufer *et al.*[9] had found in crude extracts of the soybean, but the enzyme responsible for this activity had not been further characterized. But in deference to these authors and to avoid further confusion, the term "soyin" was no longer used in subsequent publications from our laboratory and has since been simply referred to as the soybean agglutinin. It is interesting to note that, at the time we had succeeded in isolating the soybean agglutinin[5], the term "lectin" had not yet been proposed by Boyd and Shapleigh[10].

The fact that soybean agglutinin proved to be toxic when injected into an animal does not in itself indicate whether it is of any nutritional significance when raw soy fluor is consumed as a dietary ingredient. To prove this point, it became necessary to develop a method for the large-scale preparation of the soybean agglutinin so as to obtain sufficient quantities which could be incorporated into a diet fed to rats[11]. When the purified soybean agglutinin was added to autoclaved soy flour at level that was equivalent to that present in raw soy flour, the growth rate of rats was reduced to about

one-half of that obtained with autoclaved soy flour alone. It was noted, however, that this growth depression was accompanied by a corresponding decrease in food consumption when the rats were allowed free access to their feed. When feed consumption was equalized, there was no significant difference in the growth of rats with or without the added soybean agglutinin. More definitive proof for the relatively innocuous effect of the soybean agglutinin on growth came from a much later experiment in which we showed that a crude soybean extract, from which the soybean agglutinin had been removed by affinity chromatography, supported no better growth than the untreated extract[12]. The failure of the soybean agglutinin to inhibit the growth of rats upon oral ingestion may perhaps be explained by the observation that it is readily inactivated by pepsin[13] and by the hydrolases of the brush border membrane of the intestines[14].

Although it appeared that the soybean agglutinin played only a marginal role in the nutritive properties of the soybean, we nevertheless turned our attention to more detailed studies of its biochemical properties. During the ensuing years (1953–1958), we determined some of its important physicochemical parameters, including its molecular weight[15], amino acid composition[16], requirement for metal ions[13], and the effect of chemical modification[17]. Quantitation of hemagglutinating activity was refined by developing a photometric technique for measuring hemagglutination[18]. End-group analysis revealed for the first time that the soybean lectin was comprised of subunits consisting of several polypeptide chains[16], a feature which later turned out to be characteristic of the quaternary structure of most other lectins. Another unique feature of the soybean lectin was the fact that it contained a sugar component[16] (glucosamine), suggesting that it was a glycoprotein. This finding does not appear to be particularly surprising to modern day biochemists, but at the time this was regarded as a most unusual observation because it had been thought up until then that glycoproteins were only of animal origin. We now know of course that most lectins and plant proteins in general are glycoproteins. We also reported that the soybean agglutinin was capable of inhibiting the *in vivo* growth of a transplanted tumor in rats[19], an observation which suggested that lectins might be of some therapeutic value against cancer, although their clinical application in this regard has not been fully explored to date.

Although most of our earlier studies had been devoted to the soybean agglutinin, my lingering interest in nutrition prompted me to study the possible nutritional significance of the hemagglutinins known to be present in edible legumes other than soybeans. It was well known for example that kidney beans (*Phaseolus vulgaris*) were very poorly tolerated by animals unless subjected to heat treatment. This observation led us to the purification of the kidney bean lectin which, unlike the soybean agglutinin, proved to be a very potent inhibitor of the growth of rats[20] and chicks[21]. A detailed study of its physicochemical properties revealed that it was a glycoprotein[22,23] comprised of several subunits[24], and that it had a requirement for metal ions for hemagglutinating activity[25]. The lectin from the black bean, another variety of *Phaseolus vulgaris*, was also found to be lethal to the larva of the bruchid beetle[26], thus providing the first direct piece of evidence that lectins probably play an important role in protecting plants from insect predators. A more recent study has shown that the navy bean lectin exerts its toxic effect

on monogastric animals by binding to the ephithelial cells of the small intestines[27]. As a result of the damage thus inflicted on the brush border, this lectin leads to a nonspecific impairment of the absorption of nutrients from the intestinal tact.

Going back in time, I am reminded of the fact that, following our report on the isolation of the soybean agglutinin[5], I received a request from Dr. Nathan Sharon for a sample of the material which we had isolated. I like to think that this simple act of professional courtesy may have been the spark which ignited his interest in lectins. During the ensuing years, he and his collaborators greatly expanded our knowledge of the properties of the soybean agglutinin. To mention but a few of his contributions in this regard, these included: (a) determination of its sugar specificity[28], (b) its purification by affinity chromatography using a sugar ligand[29], (c) subunit structure[30], (d) metal requirement[31], (e) elucidation of the structure of its carbohydrate component[32], and (f) crystallization and preliminary X-ray diffraction data[33]. In terms of a biochemical application, the finding that the soybean agglutinin could be used to separate B and T lymphocytes[34] provided the basis for the usefulness of this lectin in bone marrow transplantation[35]. After depletion of its T-cells, histoincompatible bone marrow could be succesfully transplanted into patients with severe immune deficiency or into lethally irradiated leukemia patients without causing graft *vs.* host rejection[36].

Space does not permit a more comprehensive coverage of all of the other contributions which Sharon and his colleagues have made to the field of "lectinology". The many excellent reviews which he and his long time associate, Dr. Helena Lis, have published are well known to scientists who have come to appreciate the important role that lectins play as cell recognition molcules[37] and as a valuable research tool in carbohydrate chemistry as well as in many diverse areas of biology and medicine[36].

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