The Nature and Importance of Physiologic Specialization in Phytopathogenic Fungi

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PHYSIOLOGIC SPECIALIZATION IN PLANT pathogenic fungi is an old subject that is always new. It has long been known that within species of most pathogenic fungi there are many biotypes or physiologic races that differ principally or solely in physiologic rather than morphologic characters. It also has been known for about a quarter of a century that it is necessary to know the number, geographic distribution, and pathogenic potentialities of races to understand the development of epidemics and the regional and seasonal variation in the disease resistance of many crop plants. Moreover, it has become increasingly apparent that knowledge of physiologic specialization is important in formulating plant quarantine regulations and in the breeding of disease-resistant varieties. There are indications that it may be important also in the control of diseases by the use of fungicides and soil management practices.

It is not the purpose of this paper to catalogue specific facts about physiologic specialization but rather to evaluate what we know and indicate what we need to know. As practical needs must be met by special investigations in the multitudinous special cases, it seems desirable to discuss general principles.

The term physiologic race usually is used to designate intraspecific biotypes or groups of biotypes that can be distinguished by consistent behavior in pathogenicity. It is implied that races are essentially alike morphologically; but there may be some differences in morphology also, as is certainly true of haploid races within *Ustilago zeae*, which comprises sporidial, mycelial, and many intergrading types. In general, intraspecific lines are designated as physiologic races if morphologic characters are neither sufficiently great nor consistent to justify designating the lines as varieties. It is the usual practice, then, to apply the term physiologic race to lines or collections of plant pathogenic fungi if the most conspicuous or most important differences between them are in pathogenicity, even though there may be appreciable morphologic differences also.

Physiologic races may differ in growth rate, size, color, topography, and other characters of colonies on artificial media; in sex and mutability; in nutritional and temperature requirements; in enzyme activity; in tolerance to pH concentrations, poisons, fungicides, or toxins; in ability to produce substances toxic to plants; in pathogenicity; and in many other characters. The number of races within many species, the abundant production of new ones, and the number of characters in which they may differ require many criteria for distinguishing between closely related races or sometimes even between distantly related ones.

Puccinia graminis tritici, stem rust of wheat, which is itself a variety of *P. graminis*, comprises, for example, an indefinite number of pathogenically different biotypes. Each of the 189 described races may comprise one or more of these biotypes. For practical purposes these races are identified by inoculating about 20 seedling plants of each of 12 so-called differential varieties of *Triticum* spp. These were selected as differentials a number of years ago because they seemed to be representative of several hundred varieties that had been tested for reaction to the rust races then known. But plant breeders have subsequently developed many new varieties of wheat, and consequently there are many potentially new differentials that may enable the recognition of racial differences not formerly apparent. Naturally, therefore, it is necessary to test these new varieties against available races. But not enough races are available, because the process of identification, description, and recording has been in progress for about 30 years, and some races that were common then are no longer found. Also, there can be no certainty that a rust collection that was identified as race 8 a quarter of a century ago is identical genotypically with one that is so identified today. Unless all races are maintained permanently in living condition and in pure form under standardized conditions, which is practically impossible, a collection identified as race 8 today must be compared with the record of performance of race 8 many years ago; it is a comparison with a record, not with a type specimen. Despite this difficulty, the agreement of the records often is so remarkable that doubts regarding genotypic identity seem unimportant for practical purposes.

Regardless of the inherent difficulties in identifying physiologic races of an obligate parasite like *P. graminis tritici*, relative precision can be attained. Seedlings of the 12 differential varieties are inoculated in the greenhouse with rust collections, placed in a moist chamber for 48

Vice-presidential address delivered at Boston, December 27, 1946, before Section G, AAAS.

hours, kept on a greenhouse bench; and in about two weeks, the exact time depending on light intensity and temperature, identification can be made. The degree of susceptibility of the differential varieties may range from immune to completely susceptible, corresponding with well-defined infection types ranging from 0 to 4, with a variable type X, produced by certain races on certain varieties. Infection types 0, 1, and 2 are clear-cut indications of resistance, and types 3 and 4, of susceptibility. Type X indicates a "mesothetic" or variable reaction. Races can then be identified by means of a trichotomous key based on resistance, susceptibility, or a mesothetic reaction of the differential varieties. But there are degrees of resistance and susceptibility. Thus, all of the differential varieties except Khapli emmer are susceptible to race 15, first identified more than 25 years ago. But there are degrees of susceptibility. Several years ago there was obtained from Brazil a collection to which some of the differentials were decidedly more susceptible than to one from the United States. On the other hand, several varieties were less susceptible to a collection from Japan than to that from the United States. According to the key for the determination of races, all three collections were race 15. They were, however, different, and accordingly were designated as races 15, 15A, and 15B. From a scientific standpoint it would be justifiable to designate them as distinct races; from a practical standpoint there is advantage in considering them as biotypes of the same race. Many others of the 189 numbered races are known to comprise several biotypes. In reality, then, there are many more races than the 189 that have been numbered.

It is becoming increasingly evident that *P. graminis tritici* comprises an indefinite number of biotypes that differ in pathogenicity and other physiologic characters. It is equally clear that the differences between many of them are so slight as to defy easy and certain identification by the methods that are available. Phenotypic variability sometimes may obscure genetic differences between closely related biotypes, or even between certain closely related races, in so far as genetic relationship is indicated by the reactions of the relatively few wheat varieties that can be used because of limitations of time and space. Light, temperature, varietal purity, and vegetative vigor of the inoculated wheat plants cause considerable variation in infection types. As an extreme example, certain varieties of wheat may be highly resistant to a given race at 65° F. and completely susceptible at 85°. Differences in light intensity may have almost as great an effect. Were it possible to make large numbers of precise studies at the same time, at constant temperatures and light intensities and with standardized test plants, it would be easier to recognize the multitudinous biotypes that are known to exist. But it would even be necessary to have several sets of standardized conditions, because different biotypes may behave alike under certain sets of conditions and differently under others. The best that can be done, then, is to study large numbers of biotypes crudely and smaller numbers intensively. In identifying races for practical purposes it is necessary to lump rather than split. Although this lumping does not reveal the great complexity of biotypic components of a single variety of *P. graminis*, it does satisfy certain practical requirements in understanding and combating a destructive pathogen.

The physiologic races of *P. graminis tritici*, as the expression has been defined, can be identified with reasonable facility and certainty. Although isolations can be made from different types of pustules if two or more races occur in a mixture, still greater assurance of purity can be attained by isolating single urediospores and establishing monosporous lines, which can be propagated indefinitely as dicaryotic clones. As observable mutation for pathogenicity seems to be rare in the rusts, it is not particularly difficult to maintain the essential genetic purity of lines. But this is not true of many other fungi, such as smut fungi and many of the Fungi Imperfecti.

In the smut fungi the term physiologic race is usually used to designate chlamydospore collections that are relatively consistent in pathogenicity on certain varieties of plants. The original inoculations usually are made with a considerable number of spores, and successive crops of chlamydospores are then tested to determine the consistency of their behavior. As chlamydospores are diploid, and sexual reproduction intervenes between one generation and the next, a "chlamydospore line" comprises a changing population of biotypes. The pathogenicity of lines is, however, often remarkably consistent in replicated tests in the same year and in successive years. Thus, the writer and associates several years ago inoculated several varieties of corn in the field with collections of *U. zeae* from a number of different states of this country; the degree of infection, as measured particularly by size of smut galls, ranged from light to heavy. Chlamydospores produced by each collection were then inoculated into corn the next year, with almost exactly the same results. The same procedure was followed a third year, and again the results were similar to those of the first two years. Theoretically, this would not be expected, because of the opportunity for changes in biotypes within each smut collection. In this and other cases, however, it appeared that the changes were usually not great enough, within the relatively short duration of the experiments, to become perceptible on corn plants. An industrious investigator could, however, spend a lifetime in studying biotypes within any one of these collections. There would be distinct differences between many of the biotypes within each collection, but the populations of biotypes apparently retain their distinctive characters for some time when judged by their pathogenicity on corn. Further study is being made of the geographic distribution and the stability of such "chlamydospore populations."

The corn smut fungus, then, comprises an indefinite number of haploid biotypes that may differ in so many physiologic characters that they could be considered as physiologic races. Since haploid lines cannot cause infection singly, however, they are usually not so designated. But monosporidial diploid lines also can be isolated. These can propagate asexually by budding and thus maintain themselves indefinitely as saprophytes; they can also cause infection in corn, where they produce normal smut galls and chlamydospores. Diploid lines may differ decidedly in pathogenicity, and yet they are not called physiologic races. This term is usually reserved for the "chlamydospore lines."

Clearly, then, physiologic races are different in the Uredinales and Ustilaginales. In most of the rusts they are determined by the behavior of dicaryotic clones. A culture of a given race may be maintained indefinitely in the asexual uredial stage, and some homozygous ones maintain their identity through the sexual stage also. Others, however, are so heterozygous that many segregates appear even after "selfing." In the smuts, on the other hand, it is the chlamydospores, the counterpart of rust teliospores, that are used in determining races. As the dicaryophase does not produce spores, except perhaps in a few cases, it cannot be propagated in successive generations. Moreover, as this phase appears not to thrive on artificial media, there are difficulties in studying physiological characters other than pathogenicity. But the saprophytic haplophase of many smuts can be propagated on artificial media, and the physiologic characters can be studied as in bacteria or yeasts. The pathogenicity of haploid lines, however, must be studied in paired combinations. From certain crosses between monosporidial haploid lines of *U. zeae* monosporidial diploid lines can be obtained, thus making it possible to study on artificial media the haploid parental lines singly and the diploid lines derived from them; and the pathogenicity of the dicaryophase can be studied in the host plant. Thus, studies of rusts and smuts supplement each other and furnish a rather clear picture of the continually changing diversity and complexity that may exist within species.

Physiologic specialization has been studied extensively also in such taxonomically distinct plant pathogens as *Actinomyces scabies, Phytophthora infestans, P. faberi, Sclerotinia fructicola, Venturia inequalis, Fomes lignosus, F. igniarius, Alternaria* spp. *Colletotrichum lindemuthianum, Fusarium* spp., *Helminthosporium* spp., *Rhizoctonia solani*, and many others. Since all of these can be grown readily on artificial media, certain characters can be studied that cannot be studied in obligate parasites. In many of these species a wide variety of monosporous cultural races can be isolated. Some look alike but differ in pathogenicity, and others differ in appearance but are essentially the same in pathogenicity.

Helminthosporium sativum, H. gramineum, and *Fusarium lini* illustrate especially well the fact that there may be an indefinite number of races, ranging from 0 to 100 per cent in pathogenicity for a single variety of wheat, barley, or flax, respectively. The order of pathogenicity may differ on other varieties, however, thus illustrating again the high degree of specificity between races and varieties of crop plants.

A. scabies, the organism that causes common scab of potatoes, is made up of a motley array of cultural races that may differ almost spectacularly in cultural characters. There are the usual differences in pathogenicity, with considerable specificity between races and potato varieties; but the scab organism is especially interesting because of the ability of different races to grow at different pH concentrations, as shown by Schaal. Potato scab usually is most abundant in alkaline soils, but the fact that some races of *A. scabies* can grow at a pH as low as 5.4 suggests that the situation might be reversed where such races predominate.

R. solani has a wide host range, including such important crop plants as potatoes, sugar beets, soybeans, beans, tomatoes, peas, alfalfa, clovers, cereal grains, and flax. There are numerous physiologic races, some of which seem to be generalized in pathogenicity, but apparently some of them are specific, attacking some plants heavily and others weakly. Some also produce diffusible substances that cause stunting or wilting of noninfected tomato and certain other plants, while others do not. This suggests that certain physiologic races of this and other soil fungi may differ in injurious effects on crop plants because of differences in ability to produce toxic substances that can act at a distance.

There is evidence also that races of some plant pathogens, such as *A. scabies*, may differ in their susceptibility to antibiotic organisms that are common in the soil. Extensive investigations are needed to determine whether the differential ability of different races of various pathogenic fungi to survive in the soil may be due partly to their ability or inability to compete with other soil organisms. The ability to produce substances antibiotic to other organisms and to withstand those produced by competing organisms may be important in survival and success as pathogens. The complex series of interactions between the large numbers of physiologic races of plant pathogens and nonpathogens in the soil may partly explain the variable results often obtained in pathogenicity tests in nondisinfected soils.

Physiologic races, as would be expected, differ in temperature relations. As an example, race 56 of *P. graminis tritici* is most pathogenic at fairly high temperatures, while races 34 and 36 are most pathogenic to certain varieties at lower temperatures. The effect of temperature varies with the rust race and the variety of wheat on which it is growing; hence, there may be a complex series of interrelationships.

From the foregoing it is evident that there may be a

wide range of variation in the behavior of physiologic races, especially in their pathogenicity. Environmental conditions affect the vigor of the pathogen, the resistance of the host, and the interaction between the two. Laboratory materials and procedures can be standardized; light, temperature, and humidity can be controlled only to a limited extent in the ordinary greenhouse; conditions in the field can be controlled still less; and the microflora of the soil defies standardization unless experiments are made in disinfected soil, and then the results have only limited application. But one of the most important reasons for variability is variation in the pathogen itself.

Mutation is extremely common in many fungi—probably even more common that it seems to be. Many mutants differ so slightly from their parents and other biotypes in morphologic or physiologic characters that there are difficulties of identification similar to those already discussed in connection with physiologic races. In fact, mutants often are new physiologic races, and, unless extensive comparative tests are made between closely related ones, differences do not become apparent. When effort is concentrated on the detection of certain important character differences only, others easily can be overlooked.

From long-time studies of mutation in *U. zeae* it is evident that a single biotype may produce hundreds of mutants in a short time. Single, haploid sporidia can be isolated, and since they multiply rapidly by asexual budding on artificial media, many different characters can easily be studied. Some haploid lines are extremely mutable and others are very stable, with many intervening degrees of mutability. There is a wide range in the magnitude of differences between parents and mutants, involving one or more characters. Mutation in color illustrates the point. From a line whose colonies produce a deep tan color mutants can be obtained that differ only in almost imperceptible tints. Similarly, a series differing only in faint vinaceous shades and tints can be obtained. There are similar differences in sex, enzyme activity, pathogenicity, and even mutability. So, unless it is known where and how to look for differences, many will never be observed. Without taking extraordinary precautions to maintain the purity of certain monosporidial lines of *U. zeae*, they may soon comprise many biotypes, some of which differ in appearance and some of which are alike in appearance but different in certain important physiologic characters that are not apparent in the mixed population of biotypes. If this is true of the haplophase of *U. zeae* and other smut fungi—and it is—what is the composition of monosporous lines of *Fusarium*, *Helminthosporium*, or *Alternaria*, all of which produce multicellular spores, in some of which the individual cells are multinucleate and possibly heterocaryotic? It may be considered surprising that isolates behave as consistently as they sometimes do.

Questions regarding the importance of heterocaryosis in the production of new biotypes and the degree of their stability cannot be answered categorically—at least not by the writer. The dicaryophase of smuts and rusts is, of course, heterocaryotic. It has been shown repeatedly that degree of pathogenicity depends on the haploid lines that were combined, just as the characters of hybrid lines of higher plants depend on the genes contributed by the parents. In the smuts and rusts this is equally true of dicaryotic hybrids, in which the two haploid nuclei of each dicaryon have different genes that are not recombined until after the termination of the dicaryophase. Such special cases of heterocaryosis furnish at least a basis for the assumption that hyphal anastomoses in some fungi may result in association of nuclei with different genes. Dickinson showed that some excised hyphal tips resulting from hyphal fusions between a red and white *Fusarium* produced pink colonies, which, however, soon dissociated into the original red and white types. Possibly the association is more permanent in some fungi. From the results of Hanson and others this seems to be true; consequently, additional doubts are created regarding the degree of genetic purity of monosporous lines of some fungi.

Physiologic races may be variable also because of semipermanent modifications induced by certain environmental factors. It has long been the practice of the writer and his associates to grow smut lines on the same medium and under the same conditions of light and temperature before comparing them, because of an observed "hangover" effect. Various attempts were made to find out whether monosporidial haploid lines of *U. zeae* could adapt themselves to arsenic and other poisons other than by mutation. It is now certain that the tolerance to arsenic and malachite green can be increased by successive transfers, as Jollos showed for *Paramecium*. Monosporidial lines, both haploid and diploid, differ in their ability to develop tolerance; and there appears to be no correlation between the mutability of lines and their ability to develop it. Miss Hirschhorn made an intensive study of mutation in relation to this adaptation but found no evidence that mutation accounted for the results. Moreover, the "adapted" lines reverted to normal in appearance and lost their acquired ability after several transfer generations on arsenic-free media. The ability was acquired gradually and lost gradually, just as Jollos showed for *Paramecium*. On the other hand, J. J. Christensen and associates could find no evidence that *F. graminearum* adapted itself to malachite green, ethyl mercury phosphate, and certain other substances except as a result of mutation. In his experiments the parental line did not change, but many mutants—physiologic races—appeared in the cultures. Some grew about equally well as the parents on the poisons, some very much more poorly, and some very much better. The methods and objectives in the experiments with *U. zeae* and *Fusarium* were essentially the same, but the

phenomena encountered appeared to be quite different. Easy generalization is about as dangerous here as in many of the other phases of physiologic specialization.

How important are mutation and hybridization in plant pathology? No categorical answer can be given. It is known that new races may appear—unless the supposedly new races are in reality old ones that are reappearing—and that some become established locally or regionally and attack hitherto resistant varieties of crop plants. How many of these new races were introduced from other regions and how many resulted from mutation or hybridization is not known. It is known, however, that occasional mutants—those of *U. zeae* and *H. sativum*, for example—may be more virulent than their parents. It is also known that new races, some of which are very virulent on certain varieties of crop plants, can result from hybridization, as is clearly true of certain rusts and smuts. The investigations of Keitt and his associates indicate that this is also true of the apple scab fungus.

In the smut fungi it has been shown at Minnesota and elsewhere that there may be extensive hybridization between biotypes within species, between different species, and between different genera. There is a wide range in virulence in the races resulting from recombinations. Some appear to have only slight survival ability; others are potentially dangerous. There is no information, however, regarding the origin of the new races that have appeared to attack previously resistant varieties. Investigations of the genetics of smut fungi have shown how new races can arise but not how they have arisen. The evidence is clearer with *P. graminis*.

It is known from the results of investigations in Australia, Canada, and the United States that hybridization between varieties or races of *P. graminis* on *Berberis* spp. can result in the production of many physiologic races, old and new—at least new to science. Of course, this is to be expected, as there are more than 200 known races of the tritici variety alone. A dozen or more races can even be isolated from aecia resulting from selfing certain races. Here it is possible to find out not only what can happen but also what actually does happen in nature, principally because the sexual process is localized on barberries and there are relatively quick and easy methods of identifying the resulting races.

The fact that new or unusual races of *P. graminis tritici* are produced abundantly on barberries in nature is evident from the fact that in large numbers of identifications of rusted material collected near barberries, a different race is found in about every four or fewer collections. When collections are made at random away from barberries, however, a different race is found in about every 60 collections, the exact ratio in each case depending on the year and locality or region from which the collections are obtained. As an example, in a small barberry-infested area in the eastern part of the state of Washington 32 races or biotypes were found near barberries from 1943 to 1946, and only 5 of these races were found in the entire United States away from barberries. Several of these isolates proved to be new races. In 1946, from 47 collections of rusted wheat in the same local area in Washington, 16 races were identified; in Virginia, 12 races were isolated from 51 similar collections. In the United States as a whole, on the other hand, 5 races made up 95 per cent of all isolates, and 4 made up 93 per cent. Many factors determine whether these new or rare races produced on barberries will become widely established. Many of them have not and may never become established; but, based on past experience, some of them may become prevalent and widespread.

Two outstanding examples of the establishment of new races of *P. graminis* illustrate what may happen. Ceres wheat, first distributed in 1926, was so resistant to stem rust that it soon became the most popular variety of spring wheat and occupied most of the acreage in the Dakotas and Minnesota and considerable acreage in Canada. In 1928 a new race of stem rust (race 56) was found in barberry areas in Iowa and Nebraska. This increased slowly in prevalence for several years. By 1934 it had become the most prevalent race, and it continued to increase in prevalence until 1938, when it comprised 66 per cent of all uredial isolates obtained from wheat. Ceres wheat was very susceptible to this race and was so severely injured in the epidemics of 1935 and 1937 that it was soon supplanted by other varieties. From 1934 to 1946, inclusive, race 56 has ranked first in prevalence in the United States every year except 1941, when it ranked second. Within 10 years this race spread over almost all of North America. The history of race 56 has been recited many times. It is, however, the outstanding example of the way in which a new race can appear and become widely destructive in a short time.

In the United States, race 8 of *P. graminis avenae*, the oat stem rust, has had a career similar to that of race 56. In 1937 it was found in barberry-infested areas of Iowa, Wisconsin, and Pennsylvania, and comprised only a very small percentage of the total number of isolates of oat stem rust in the United States. In 1938 it was found only near infected barberries in southwestern Virginia, but since that time it has extended its geographic range and increased in prevalence. In 1945 it comprised almost 50 per cent of all the isolates of oat stem rust in the United States, and in 1946 more than 50 per cent. It now is known to extend from Canada to south-central Mexico and at least from the Rocky Mountains to the Atlantic Seaboard. This race attacks heavily certain recently produced varieties of oats that were well on their way to monopolizing the oat acreage in many of the heaviest oat-producing areas of northern United States because of their resistance to stem rust and other diseases. These varieties were resistant to the races of stem rust that predominated while they were in the making, but

the increase in race 8 has exposed them to a new and dangerous biotic environment. Fortunately, still other varieties have been produced that are resistant to race 8. But race 7, which can attack some of these varieties, has been appearing in barberry areas. Whether it will ever become prevalent, as race 8 has, cannot be predicted. In the meantime, varieties are being produced which seem to be resistant to all known races, although there is some evidence that they are susceptible to certain of them at high temperatures.

Some of the best stem rust-resistant commercial varieties of spring wheats now grown in the United States are resistant to race 56 and the other races now prevalent but are susceptible to certain as yet unprevalent races that are found only near barberries. Crosses now are being made in the attempt to produce varieties resistant to these races, should they duplicate the history of race 56. Moreover, it is known that there are rust races in certain other countries that are far more virulent than anything yet found in North America. Whether these will ever be produced naturally in North America, whether they will be introduced, or whether they will be carried into North America by the wind are questions that only the future can answer. Plant breeders and plant pathologists proceed on the assumption that the sort of thing that has happened in the past may happen in the future. But to prepare for the future it is necessary not only to know the physiologic races of the present but also to learn the principles basic to predictions regarding those which are potential.

The National Academy of Sciences:

Abstracts of Papers Presented at 1947 Meeting

The Sun a Regular Variable Star

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The variation of values of the solar constant of radiation for the years 1924–44 reveals a regular periodicity of 6.6456 days. Statistical studies of temperature departures at Washington, D. C., St. Louis, Missouri, and Helena, Montana, show that this solar periodicity is attended by fluctuations of temperature of identical average period and an average range of 5°F. Apparently these temperature fluctuations have not hitherto been recognized as periodic because, while the solar period is invariable, its terrestrial effects are subject to phase displacements of ± 1, 2, and occasionally 3 days, and the amplitudes of the temperature effects range from 2° to 20°F.

Blood Protein Studies With Labeled Elements

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In mammals, absorption of the most essential foodstuffs from the gastrointestinal tract is indiscriminate; it does not depend on whether or not the body is already liberally supplied with this substance. The disposal of surplus amounts is through degradation and excretion.

The metabolism of iron has been found not to follow this general rule. Hemoglobin, the iron-containing red protein of the blood erythrocyte, is the primary means of oxygen transport in mammals. Iron is thus essential to mammalian life. Early studies with radioactive iron show that in this case the body excretes surplus iron only in negligible amounts. Instead, normal dogs and humans absorb iron from the gastrointestinal tract when needed and, when the need is satisfied, allow the iron to pass unabsorbed into the feces. Further experiment with radioactive iron, carried out by research groups a Rochester and at Berkeley, indicate that even sterile infections, such as abscesses induced by turpentine injections, prevent iron absorption in animals anemic through blood loss and needing iron. Also, such infections prevent hemoglobin formation even from injected iron.

An average life span of 120 days for the dog erythrocyte is indicated from studies on excretion of porphyrin breakdown products by Hawkins and Whipple. Studies with radioactive iron aimed at confirming this life-span figure failed to give adequate data for this purpose for an unexpected reason: it was found that the dog utilizes almost exclusively the iron liberated from his own worn-out red cells for building new ones, even with very large stores of other reserve iron in his body.

Later, Shemin and Rittenberg showed, by the use of the distinctive heavy isotope of nitrogen, that glycine nitrogen is the source of the porphyrin nitrogen of the hemoglobin. In subsequent experiments, in which such a labeled glycine was fed to a human subject and then the rate of disappearance of the resulting labeled hemoglobin followed, they have shown that the average life span of the human red cell is also about 120 days.

Distinctive isotopes, radioactive and stable, are also of proven value in investigations of blood-plasma proteins. Originally, it was supposed that in addition to forming a blood clot following injury, their function was principally that of maintaining the osmotic pressure of the blood and thus preventing plasma loss into the intercellular tissue spaces. Later, vital immunological functions were definitely attributed to certain of these proteins.

In 1938, Howland and Hawkins showed that injected plasma protein can be used by dogs for other nutritional purposes,