HEINZ BODY PHENOMENON IN ERYTHROCYTES

A Review

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Among the early investigators of hemolytic substances were Casper and Hoppe who in 1859 observed the brown coloration of blood due to nitrobenzene. Since then there has been a continued interest in the action of such toxic materials on the formed elements of the blood. The accelerated development of synthetic organic chemistry during the latter half of the 19th Century created numerous industrial hazards and poisons, at the same time providing the toxicologists with many new compounds with which to work. Among the coal tar products and derivatives of aniline thus produced, phenylhydrazine was of great importance because of its marked physiological action. Prepared in 1875 by Emil Fischer, its behavior in rabbits was studied ten years later by Hoppe-Seyler, the discoverer of methemoglobin.

Early Observations on Morphologic Changes in the Erythrocytes

The marked action of chlorates had attracted the attention of several workers. As early as 1881, Riess described the presence of one or more small, generally round, globules and granules in the erythrocytes of a person poisoned by potassium chlorate. Drawings were included which showed the appearance of these particles. Somewhat later, Marchand, who had worked on chlorate intoxication previous to this discovery of Riess, observed similar changes in a dog poisoned with the sodium compound. Finally, Lewin had observed the formation of granules within red cells treated in vitro with hydroxylamine.

One year later, Robert Heinz (1865–1924), in 1890, studying the action of phenylhydrazine and its derivatives on the blood, observed the changes seen earlier by the above workers, described them in detail, in regard to their appearance, behavior and ultimate fate, and devised a method for staining them, using a wet preparation for this purpose. Drawings were also given showing both the stained and the unstained granules in the blood of several species of animals.

These bodies were depicted by Heinz as round, oval or serrated granules which are very refractile and hence can easily be seen. There may be one or more within the cell wall and they may move around (Brownian motion) or remain fixed in one position. Ordinarily they are eccentrically placed, being located near the margin. Sometimes they appear to protrude from a cell, as if hanging by a stalk, and frequently they can be observed outside the cells in the plasma (schistocytes of Ehrlich). The sizes of the particles vary greatly, being 1-2 microns in diameter in rabbits, guinea pigs and dogs and much larger in cats, often amounting to a third or half of the cell diameter. Heinz recommended supravital examination of the blood, using a dilute solution of methyl violet in isotonic saline solution for staining these

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particles blue; hence, the term blue particles (blaukörner). This staining solution had been used earlier, in 1881, by Bizzozero in demonstrating the presence of blood platelets.

Initially, Heinz regarded the presence of these granules as pathognomonic of poisoning by phenylhydrazine or its derivatives. However, further researches indicated a large number of substances capable of inducing similar changes. As observed by Heinz, these characteristic changes in the erythrocytes were found in one or more species of animals following administration of many organic compounds such as aniline, toluidine, toluylene diamine, nitrobenzene, dinitrobenzene, p-aminophenol, ethyl aminobenzoate (p), phenylhydrazine, acetylphenylhydrazine and phenylhydroxylamine. However, no such action was observed with benzene, phenol, phenacetin, acetonilid, antipyrine or benzyl amine, and the aliphatic amines. Among inorganic compounds, chlorates and hydroxylamine produced Heinz bodies but not hydrazine or sodium nitrite, although all four substances were very active in producing methemoglobin in vivo.

Ehrlich, who at that time was an authority both on anemia and on stain technology, referred to the frequent presence of hemoglobinemic inclusion bodies (hämoglobinämische Innenkörper) in erythrocytes of toxic animals as well as in the blood of certain anemic persons. Dustin pointed out that the term inclusion body is unsatisfactory since the particles often appear to be extruded from the cell. The term inner body is likewise objectionable since the particles sometimes appear to be on the surface of the erythrocyte.

Ehlich and Lindenthal, who found similar bodies in the blood of a person with chronic nitrobenzene poisoning, assigned priority for the discovery of the so-called inner bodies to Ehrlich. This, together with the fact that the senior author's name has been repeatedly misspelled 'Ehrlich' in the literature, presumably has much to do with the confusion which exists even today as evidenced by the use of such numerous terms as Ehrlich-Heinz or Heinz-Ehrlich bodies, Heinz' blue granules (Heinzsche Blaukörner), inclusion bodies (Innenkörper or Innenkörperchen), hemoglobinemic inner bodies, hemoglobinemic inclusion bodies, substantia metachromatica granularis, β-substance and Polkörperchen.

Heubner, on the basis of Ehrlich's report of 1892, assigned priority to Heinz for this discovery. However, due to the prominence of Ehrlich, many of his ideas prevailed. For example, Ehrlich preferred fixed and stained blood preparations rather than supravital methods. His triacid stain, developed in 1880, was relatively difficult to use, and the results were uncertain with respect to finding inclusion bodies. Heinz preferred the supravital technic but pointed out that both wet and fixed preparations should be used, in order to secure the most information.

Shortly after the initial work of Heinz a number of investigators observed similar intracellular bodies during poisoning with various chemical agents. Thus the earlier findings were confirmed by A. Huber with dinitrobenzene, by Ehlich and Lindenthal with nitrobenzene, by Schmauch with pyridine, by Schwalbe and Solley with toluylene diamine, by Winogradow with chlorate and by von Domarus with phenylhydrazine. However, much confusion existed in the literature during this period. Thus, Heinz bodies were sometimes identified
with Howell-Jolly bodies; Schmauch found his "endoglobular" bodies in normal cats; Schwalbe and Solley confused the bodies they saw with blood platelets and found similar forms in normal blood after coagulation. As pointed out by Jürgens and Schürer, marginal bodies (Randkörper), discovered by Röhl in 1893 and subsequently rediscovered by Huber, by Schwalbe and Solley, and others, were similar to Heinz bodies in appearance and were often confused with the latter. There was also considerable discussion regarding the identity of Heinz blue granules of the wet preparations with the hemoglobinemic inner bodies seen in Ehrlich's fixed stained preparations. Finally, for many years some workers doubted the existence of Heinz bodies, regarding them as artifacts while others held them to be nuclear debris or nuclear derivatives.

Contributions of the Schools of Pappenheim, Schilling, Heubner and Others

A second period in the development of knowledge regarding the Heinz bodies started about 1911 when Pappenheim and his students were attracted to this field. Almost simultaneously Schilling and his co-workers began similar investigations. Two problems were of paramount importance in the work emanating from these two schools during the following years. The first was concerned with the chemical composition and properties of the Heinz bodies; the other had to do with the mechanism by which they were formed. These problems were so interrelated and so complex that much more data were needed than were then available. A third problem, involving the relationship between methemoglobin- and Heinz body formation, was investigated with a variety of substances by Heubner and his students.

Chemical Nature of Heinz Bodies

Morawitz and Pratt had found that the erythrocytes from animals poisoned by phenylhydrazine were more resistant than normal, as measured with salt solutions and various hemolytic agents. Itami and Pratt proposed the term 'pachydermia' to describe this change in resistance of the erythrocyte. They found also that the stroma sediment of anemic blood would not dissolve in water, the particles contained in the residue appearing like Heinz bodies. Sattler, Hirschfeld and Rosenthal all studied the behavior of erythrocytes displaying this increased resistance without arriving at the chemical nature of such a change.

Hartwich, working with Pappenheim, isolated Heinz bodies in large enough quantities to work with them in "pure culture." From their solubility in pepsin and hydrochloric acid and from their other reactions it was concluded that the bodies contained protein and lipoid material together with some iron. Pappenheim and Suzuki investigated the behavior of Heinz bodies with respect to their resistance toward certain hemolytic substances, as saponin. Suzuki found that an increase in resistance occurs in blood poisoned in vivo by a mixture of pyrodine and toluylene diamine, this increase being caused chiefly by the extremely resistant Heinz bodies rather than by a diffuse pachydermia.

Further investigations of the composition of these particles were carried out by
Kunkel,\textsuperscript{74} who was able to show that they contained no altered hemoglobin but considerable phosphatide, some protein and cholesterol and a colored iron compound which was not identified. Hess and Müller,\textsuperscript{47} by selective staining technic, demonstrated that the Heinz particles gave reactions for lipid material.

Heuer\textsuperscript{55} drew conclusions somewhat different in that he believed the Heinz bodies to contain neither phosphorus nor phosphoprotein. Later Warburg et al.\textsuperscript{129} showed that Heinz bodies produced by phenylhydrazine agreed in behavior and properties with that of denatured globin. More recently, Horecker\textsuperscript{59,60} confirmed this work, using xylidine as the substance for producing the Heinz bodies.\textsuperscript{127}

By means of the electron microscope, Jung\textsuperscript{66} was able to follow the growth of the Heinz bodies from submicroscopic particles to those attaining half the diameter of the red cells. In the case of dinitroglycol, Heinz bodies were recognizable\textsuperscript{66} after a few minutes. Evidence was also obtained that these bodies were at or in the outer surface of the erythrocyte. However, it was found that the structure of the Heinz bodies produced by various hemolytic agents was not identical in all cases. Based on these results and upon their own work, Kiese and Seipelt\textsuperscript{73} were led to believe that Heinz bodies contained denatured proteins derived from the erythrocyte membrane.

Finally, as Dustin\textsuperscript{17} has pointed out, in the absence of recent comprehensive studies very little can be said with certainty regarding the chemical composition of these particles except that they undoubtedly contain protein material.

**Mechanism of Heinz Body Formation**

The formation of Heinz bodies has been explained in a variety of ways, most of which are included in five principal mechanisms.

1. **Protoplasmic theory.** Heinz conceived of the blue granules, which were later called by his name, as produced by partial necrosis of the erythrocytic protoplasm. Ehrlich believed these particles to be identical with his hemoglobinemic inclusion bodies, the latter being regarded as containing hemoglobin in a resistant form, either as methemoglobin or as altered hemoglobin. This hypothesis was in agreement with the observation that as the inclusion bodies became larger and more dense, the hemoglobin in the cells appeared to become paler and often disappeared. Since these abnormal structures were produced by substances which simultaneously produced methemoglobin, it was natural to assume that the bodies contained some form of blood pigment. This was supported by the observation that although they were basophilic before fixation, the particles became acidophilic after fixation occurred. However, the work of Kunkel\textsuperscript{74} and of Hartwich,\textsuperscript{10} in showing that Heinz bodies contain neither hemoglobin nor methemoglobin, appears to dispose of this protoplasmic theory. The observed acidophilic behavior is explained by Gutstein and Wallbach\textsuperscript{36} as due to the lipid content of these particles rather than to their hemoglobin content, as believed by Ehrlich.

2. **Nuclear theory.** The early attempt to relate Heinz bodies and nuclear remains, such as Howell-Jolly bodies, met with little success. The latter show intense basophilia after fixation while Heinz bodies do not. Zadek and Burg\textsuperscript{135} held that no confusion existed in identifying these particles although Schilling\textsuperscript{116} maintained that he had observed a transition between these two forms. Cats appear to be the
species giving the greatest difficulty in experimental work since the blood of young animals often contains Howell-Jolly bodies.\textsuperscript{24} According to Schilling,\textsuperscript{116} part of the Schmauch inner bodies of cats are Howell-Jolly bodies and part are Heinz bodies. Gross and associates\textsuperscript{29} have reported that the latter are found regularly in normal animals of this species.

3. Pre-existent theory. Two forms of this theory were advanced, the first of which was given by Schwalbe and Solley\textsuperscript{119} who identified Heinz bodies with platelets. They thought that there existed within a normal red blood cell a structure similar to that of the Heinz body, the latter being liberated and appearing as a platelet in the plasma. Aside from shape, refractile properties and occurrence in the plasma, these bodies have nothing in common.

The other form of the theory was derived from the work of Schilling\textsuperscript{13, 37, 109--111, 114} who conceived of the normal erythrocyte as having a very complex structure similar to a nucleated cell. He advanced the idea that the Heinz body is a pathogenically produced form of the capsule body normally present. These capsule bodies are not observable except by special histologic and staining technic, whereas the Heinz bodies are frequently visible even in unstained preparations. This theory was also supported by the experimental investigations of Deutsch.\textsuperscript{7}

Gutstein and Wallbach,\textsuperscript{34--37} although agreeing in general with the pre-existent theory of Schilling, differed somewhat in details. They held to the pre-existence in normal erythrocytes of both "Innenkörper" and "Innenkörperchen," the latter corresponding to the "Kapsulkörper" of Schilling and the former to the "Glaskörper" of Schilling. It is the Innenkörperchen which are the pre-existing forms of the Heinz bodies. Gutstein and Wallbach reached these conclusions also through special staining techniques which they regarded as superior to those of Schilling.

Since the special technic used to produce these structures in normal erythrocytes require somewhat severe and rough processes great artifacts may result. For this reason, Dustin\textsuperscript{17} rejected this pre-existent theory.

4. Reticulo-filamentous theory. The basophilic nature of the Heinz bodies and of the reticulocytes suggested a relationship between them. However, it is known that on supravital treatment basic stains, such as brilliant cresyl blue, precipitate material existing normally in reticulocytes in a diffuse form so that it becomes visible. On the other hand, under similar conditions the stain does not alter Heinz bodies which, if large enough, can be seen in an unstained condition. Restaining of the supravital preparation, following fixation with methyl alcohol, will color the reticulocytes but not the Heinz bodies to any marked extent, according to Dustin.\textsuperscript{17}

The two kinds of bodies can be further differentiated by a study of their occurrence. Heinz bodies being found usually, though not invariably, in mature erythrocytes and rarely in the young cells (reticulocytes). Freifeld, Schilowa and Ludwinowsky\textsuperscript{48} have demonstrated the lack of correlation between reticulocytosis and the number of Heinz bodies present in the blood stream of poisoned animals.

5. Denaturation theory. The theory most widely held at the present time favors the view that Heinz bodies are newly formed particles generally found in mature erythrocytes and formed from them in the course of an irreversible reaction with the toxic agent or with some intermediate metabolite formed therefrom. Heub-
ncr4952 and Dustin'7 held that these particles are derived from the erythrocytic protoplasm which suffers injury. However, investigations by Jung68,59 with the electron microscope support the contention22,72 that the particles are denatured proteins derived from the cell membrane. It is difficult to reconcile the latter view with the frequently observed phenomenon of Brownian movement of the Heinz particles in wet blood preparations.15,131 It is quite possible that during the preparation of the blood cells for photography with the electron microscope, marked changes occur in the structure of the Heinz bodies.

Finally, it is not known for certainty where the Heinz bodies are formed. However, the fact that they can be produced in vitro is regarded by Moeschlin89 as strong evidence of their peripheral origin.

Methemoglobin Formation and Heinz Bodies

Most of the early workers assumed that there was a close connection between the formation of Heinz bodies and the formation of methemoglobin. However, in 1911 Friedstein27 showed that no direct relation existed between the two. This was confirmed later by Heubner and his students4,19,46,49,50,70,75,97,117,132 with many different hemolytic substances. Moeschlin88 reported finding Heinz bodies in white mice treated subcutaneously or orally with sodium nitrite, although Pulina57 was unable to confirm this. Following oral administration of sodium nitrite in food, Richardson99 found in white Swiss mice Heinz bodies in most of the erythrocytes and also cyanosis and methemoglobinemia. On the contrary, Gutstein and Wallbach58 were able to produce Heinz bodies in mice by injection of nile blue sulfate without the formation of methemoglobin. Webster, Liljegren and Zimmer131 have demonstrated in similar fashion that Heinz bodies can be produced in 75–100 per cent of the erythrocytes of mice by administration of sulfanilamide without the formation of appreciable amounts of this same blood pigment.

Goodman and Gilman92 offered as a probable explanation of the action of phenylhydrazine on the erythrocyte the splitting of the hemoglobin into hemin and globin, at least a portion of the remaining hemoglobin being catalyzed by the globin to form methemoglobin and perhaps other unidentified substances.

Heubner,49 however, regarded the action as an opening of the tetrapyrrrol ring of hemoglobin, the toxic material or some derivative thereof being able to modify the globin which was precipitated as a Heinz body and acquired an affinity for basic stains.

The presence of other blood pigment derivatives, formed in many cases along with Heinz bodies and methemoglobin, has been studied by Heubner,19,40,52,54 by Jung,67,85 and by Kiess72 and associates. These pigments, known as verdoglobin, appear to have different spectral characteristics according to the substances producing them.72 The constitution of one of the most important of these blood pigments, sulfhemoglobin (called also verdoglobin S) is unknown. Heubner and his school49 believe that it contains no sulfur but this view is not widely held.99 Lemberg and associates80 remarked about the confused state of knowledge concerning the chemical nature of sulfhemoglobin.

Heubner55,52 and Kiese and Seipelt73 held that the occurrence of Heinz bodies,
methemoglobin and verdoglobin were three separate and independent phenomena, although they frequently occurred simultaneously. Kiese and Seipelt,7' studying the effect of certain hemolytic substances on the blood of dogs and rats, found that Heinz bodies occurred whenever verdoglobin was present. This was explained by assuming that those substances which were toxic to the hemoglobin and converted it into verdoglobin, were also toxic to the membrane of the erythrocyte, thus denaturing it more or less completely and forming one or more Heinz bodies. Simultaneous formation of Heinz bodies and methemoglobin was explained in the same way. Heubner52 attributed the nonuniform behavior of certain substances, with respect to these three phenomena, to different compounds produced during the course of intermediate metabolism of these substances.

Species differences may play an important role. For example, it is well known that it is difficult to produce methemoglobin in rabbits but not in cats,48 51 this pigment regularly occurring in the blood of normal cats.63 Again, Richardson98 has shown that it is difficult to produce methemoglobin in mice using sulfanilamide, sulfhemoglobin being formed much more easily. However, the reverse is true for chickens. Similar species differences have been shown by the inability of Kunz75 to produce Heinz bodies in guinea pigs with m-dinitrobenzene, whereas they were readily produced by Bredow and Jung,4 using cats as experimental subjects. Likewise, negative results were obtained with sulfapyridine by Moeschlin and Horschler90 when rabbits were used but mice gave positive findings. It is evident, therefore, that negative results with rabbits or guinea pigs, for example, should not be interpreted as meaning that a given substance cannot produce Heinz bodies in other species or in man. Since Heinz bodies and anemia have been shown to occur with little or no production of methemoglobin, care should therefore be taken in drawing conclusions either from the presence or absence of granules and/or altered blood pigment.

Role of the Spleen in Heinz Body Phenomenon

During the existence of Heinz bodies in experimental animals there frequently occur also secondary signs of hemolytic anemia, such as anisocytosis, polychromatophilia, and reticulocytosis. In addition, there is often evidence of splenomegaly.17

The role of the spleen in Heinz body phenomenon has been of interest ever since the primary observations of Heinz.43 Hess and Müller37 showed that in the macrophages of the spleen of rats poisoned by pyrodine were found large numbers of Heinz body phagocytes. However, the blood leaving the spleen by the splenic vein contained no Heinz bodies and they concluded that the particles collected within the sinuses were the cause of the observed swelling. Schilling112 had noted the increase in Heinz bodies of an antifebrin poisoned dog following splenectomy and Zadek and Burg39 confirmed these experimental observations on several patients. Schilling112 likewise observed the presence of Heinz bodies in splenectomized normal animals, i.e., those not made toxic by any Heinz body-producing material. It therefore appears as if splenectomy were a predisposing factor in the formation of Heinz bodies.86

It is difficult to reconcile this filtering action of the spleen with the experimental
observations on the persistence of Heinz bodies in the blood stream following dis-
continuance of the toxic material. Thus, with pyrodine, Cruz found that the
length of time required for Heinz bodies to disappear ranged from 8–9 days for
rabbits to 9–18 days for dogs. Webster, Liljegren and Zimmer observed Heinz
bodies in a guinea pig for 11 days following a single exposure to stibine, SbH₃,
in a rat for 33 days and in mice for 55 days after similar exposures.

Further work on the relationship between Heinz body occurrence and the action
of the spleen, using various species of normal and splenectomized animals and
various hemolytic substances, appears to be necessary in order to extend our knowl-
edge of the role played by the spleen in hemolytic anemias produced by chemical
agents.

**Staining Characteristics of Heinz Bodies**

Enough has been mentioned to indicate that while Heinz preferred wet prepara-
tions stained with methyl violet, Ehrlich preferred fixed and stained smears. Advocates of both methods are well represented in the literature.

**Supravital Staining**

Friedstein, working with Pappenheim, investigated the vital staining proper-
ties of Heinz bodies with a considerable number of basic dyes, such as brilliant
cresyl blue, methyl violet, toluidine blue, azur I, malachite green, neutral red
and nile blue sulfate. The latter was regarded as being the quickest, most intense
and easiest to use of those studied. The author found that after fixation the Heinz
bodies had practically no affinity for basic stains but showed an attraction for
acid stains.

Since nile blue sulfate is but slightly soluble in water an alcoholic solution is
usually used, allowing a thin film to form on a slide by evaporation. The blood is
then smeared out on top of the dried film, the slide being allowed to stand in a
moist chamber for 5–7 minutes before examining the cells. This is the usual Papp-
enheim-Schilling technic of supravital examination. A common modification of
this method consists of placing a drop of blood directly on the dried film of nile
blue sulfate and covering with a cover slip.

Gutstein and Wallbach claimed to be able to stain both fixed and unfixed prep-
arations by either basic or acid dyes. In their supravital staining technic, they
mixed the fresh blood with aqueous solutions (0.1 per cent) of the stain and ob-
served it between slide and cover slip.

Webster, Liljegren and Zimmer, investigating the staining of Heinz bodies,
found methyl violet and gentian violet superior to nile blue sulfate since these
violet dyes were easily soluble in water and in Locke’s solution, the latter being
modified so as to be more nearly isotonic with the blood to be examined.

Friedstein confirmed Heinz’s observations that Heinz bodies could be de-
tected in supravital preparations much earlier in the course of an intoxication than
they could be detected in stained smears. Observations in this laboratory have
shown that the initial formation of Heinz bodies, when these particles are very
small, can best be recognized in wet preparations, where the cells are moving and
rolling over. Since the cells are subjected to the least trauma in the wet preparations, estimation of the number of Heinz particles can be made most accurately in this way.

Staining of Fixed Smears

Ehrlich's triacid stain did not prove to be very satisfactory for use in staining Heinz bodies and many other technics were devised. Panoptic staining (May-Grunwald-Giemsa) after fixation is not very satisfactory since the Heinz particles are not markedly stained. Recently, a method has been developed utilizing the scheme of simultaneously fixing and staining the smear with a solution of methyl violet in ethyl alcohol.

Smears have the advantage of giving permanent records but the mechanical trauma during preparation of the slides frequently lead to removal of many of the Heinz bodies from the cells, as can be readily seen on inspection of the thinnest portions of the slide.

Heinz Bodies in Thick Drops

Basing his work upon a technic of Ross, Schilling made extensive use of thick drop preparations, the drops after drying being hemolyzed and stained with Giemsa solution. This process has the disadvantage that the cells are largely removed so that estimation of the number of Heinz bodies in the cells is not possible. However, Schilling regarded this method as demonstrating the presence of Heinz bodies with certainty.

Differential Methods

Zadek and Burg and Dustin summarized the differential staining characteristics of basophilic particles, Howell-Jolly bodies, reticulocytes and Heinz bodies. The latter author cautioned also against confusion between Heinz bodies and other types of granules. Friefeld, Schilowa and Ludwinowsky warned against confusing the marginal bodies (Randkorper) of Rohl with Heinz bodies and Jurgens and Schuer showed how these could be distinguished. Nizet advocated use of dark field examination as well as special staining technics for distinguishing between Heinz bodies, reticulocytes and basophilic particles. Fertman and Doan showed that Heinz bodies did not give the reactions for iron shown by siderocytes so that the two kinds of particles could be differentiated.

Experimental Production of Heinz Bodies

Production in Vitro

Friedstein held that Heinz bodies are formed only in vivo, the toxic material and the blood in vitro forming only methemoglobin. This was the opinion of most investigators during the first quarter of this Century. In vitro formation of these particles was not found by Strampelli with pyrodine nor by Lambrechts, Nizet and Khady with sulfonamides. The observation by Lewin in 1889 of the formation of granules within erythrocytes by the in vitro action of hydroxylamine appears to have gone unnoticed. However, beginning in 1930, it was shown
by Waddell, Wolff and Lanou,\textsuperscript{128} by Bratley, Burroughs, Hamilton and Kern\textsuperscript{3} and by Cruz\textsuperscript{2} that Heinz bodies can be produced in erythrocytes in vitro by means of acetylphenylhydrazine. Moeschlin,\textsuperscript{59} Nizet,\textsuperscript{93} Lambrechts, Nizet and Khady\textsuperscript{76} and Gajdos and Típerez\textsuperscript{55} likewise obtained positive results with phenylhydrazine. In vitro production of Heinz bodies by certain sulfonamides was reported by Moeschlin\textsuperscript{59} and by Jürgens and Schürer,\textsuperscript{71} although this could not be confirmed by Lambrechts and associates.\textsuperscript{74,75} Willi\textsuperscript{133} observed Heinz body formation in vitro with a preparation of guaiacol and Gross and associates\textsuperscript{29} found similar action with dinitroglycol. More recently, Webster, Liljegren and Zimmer\textsuperscript{131} have demonstrated this action with mouse blood for a number of hemolytic agents, using supravital staining technic.

Therefore, it can no longer be held that these morphologic changes within erythrocytes are restricted to action taking place in the living body. However, it appears that the conditions necessary for the development of Heinz bodies are much more favorable in vivo than in vitro.

\textit{Production in Vivo}

Among the series of aromatic compounds which were found to produce Heinz bodies when administered to animals, the best known examples are phenylhydrazine and the acetyl derivative, pyrodine. These two drugs remained favorites, either used alone or in connection with toluenediamine, in which the acetyl compound is quite soluble, as a means of producing and studying Heinz bodies in experimental animals. However, the extremely toxic nature of these substances caused systemic effects which were undesirable.

Richardson\textsuperscript{98-100} demonstrated in 1940-41 that Heinz bodies could be produced in white Swiss mice following oral administration of sulfanilamide, sulfapyridine, sulfathiazole, sulfanilylguanidine, and sodium nitrite. Using mice, positive results with nitrite were reported by Moeschlin\textsuperscript{88} but this was not confirmed by Pulina.\textsuperscript{97}

Renewed interest in Heinz bodies began in 1940 following the discovery by Moeschlin\textsuperscript{97} of these particles in the blood of a number of persons treated with sulfapyridine. Comparison of this drug, one of the first of the sulfonamides to be used clinically, was carried out with sulfathiazole\textsuperscript{88} and other derivatives.\textsuperscript{99} Moeschlin and his associates found that sulfanilamide produced the greatest number of Heinz bodies and sulfathiazole the least. Since the response of rabbits to these compounds was very slight, white mice were used as experimental animals. Similar work was carried out by Hurschler\textsuperscript{61} and by Lambrechts and associates.\textsuperscript{77,78} The occasional failure to produce Heinz bodies may have been partly due to differences in modes of administration of the toxic substances and also to species differences.

Following the work of Moeschlin, Heubner and his students investigated the formation of Heinz bodies in experimental animals after the administration of a number of industrially important aromatic compounds and nitro compounds, such as nitroaniline, nitrobenzenes, nitrotoluenes, nitroglycerin and nitroglycols.\textsuperscript{1,3,4,16-20,21,24,29,30,31,38,39,41,60,69,70,73,75,97,117,132}
Recently, Figge\textsuperscript{23} showed that these altered erythrocytes could be easily and quickly produced in mice by the administration of sulfanilamide in their drinking water. Such a technic has been used\textsuperscript{121} as a means of studying Heinz body formation for many months without the severe systemic effects produced by hydrazine derivatives.

The chemical constitution of the substances capable of producing these marked changes in erythrocytes has long been of interest. With the exceptions of chlorates and hydroxylamine, only compounds belonging to the aromatic series and containing nitrogen have been included until recently. Dustin\textsuperscript{17} believes that the action by chlorates is quite different from that of other substances producing Heinz bodies. If this be accepted, it would seem that nitrogen in the form of amino or nitro groups associated with a benzene nucleus was potentially capable of inducing changes in the erythrocytes. That this is true for a variety of substituted hydrazine compounds can be seen from the work of Hueper\textsuperscript{61} and of Von Oettingen and Deichmann-Gruebler.\textsuperscript{126} Von Oettingen’s summary\textsuperscript{125} of the action of aromatic amino and nitro compounds likewise suggests the possibility of similar action with many of these industrially important substances.

In the original work by Heinz, this investigator examined the blood of the experimental animals for Heinz bodies about 2.4 hours following the administration of the toxic material. Recently, Gross, Bock and Hellrung,\textsuperscript{32} working with cats, have shown the rapid formation of these particles. Within 10 minutes after subcutaneous injection of dinitroglycol, 100 per cent of the erythrocytes in the peripheral blood were found to contain one or more refractile particles.

Jung\textsuperscript{64} has mentioned that arsine is capable of forming Heinz bodies, and Figge\textsuperscript{24} has indicated that they can be formed by such varied substances as cobalt, paramino benzoic acid and acetalilide.

Work in this laboratory\textsuperscript{131} has shown that a number of other substances are also capable of inducing Heinz body formation. Among those investigated, positive results were obtained with arsine, stibine (antimony hydride), sodium nitrate and sodium nitrite besides several others used in earlier studies.

From these results it is evident that our knowledge of the relationship between chemical constitution and Heinz body formation is quite fragmentary and there exists a great need for fundamental investigations in this field.

Estimation of Number of Heinz Bodies in Erythrocytes

Since the Heinz bodies are not hemolyzed by water or saponin solution, these particles remain suspended in the solution or they can be thrown down in a centrifuge. When suspended they cause a turbidity which frequently interferes with hemoglobin determinations or counting of white cells, since in the latter case they are insoluble in acetic acid. Cruz\textsuperscript{6} made use of this turbidity in quantitatively estimating the amount of Heinz bodies present. This method was also followed by Horecker\textsuperscript{59,60} and by Pimenta de Mello.\textsuperscript{64} Such measurements, however, do not give the actual number of particles or the percentage of erythrocytes containing such particles. For such determinations counting may be done on fixed stained...
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smears or on supravital preparations. The latter method has the advantage that even the smaller Heinz bodies can be seen and thus the beginning stages of the phenomenon can be detected and followed.

**Clinical Observations and Use of Hemolytic Substances**

It should be recalled that the first observations of refractile bodies in erythrocytes were made on persons poisoned by chlorates. Ehrlich likewise observed them in certain cases of anemia and Ehlich and Lindenthal found them in a person suffering from chronic nitrobenzene poisoning.

Following Hoppe-Seyler’s work in 1885, phenylhydrazine was extensively used as a chemical for producing experimental anemia in animals but it was not until 1918 that it was introduced by Eppinger and Kloss for the treatment of polycythemia rubra vera. Since then it has received extensive clinical application. However, the use of this drug was attended by some danger and less toxic derivatives were sought. The acetyl compound, prepared by Liebreich, was introduced in a somewhat impure form in England in 1887 by Dreschfeld under the name pyrodine, not for reducing the red cell count but as an antipyretic agent. Its high toxicity, which in rabbits was shown to produce jaundice and hemoglobinemia, led to its disuse until it was introduced in 1926 by Stone and co-workers and by Bassett and co-workers for the symptomatic treatment of polycythemia vera.

Little interest in Heinz bodies has been shown in this country, particularly with reference to clinical studies. This was pointed out by Cruz and by Pimenta de Mello following a search of the literature on phenylhydrazine and pyrodine, and more recently by Fertman and Doan. Almost all references to Heinz body occurrence in human blood are found in the German literature.

Schilling, who reported finding Heinz bodies in children poisoned from aniline, coined the term ‘Innenkörperanämien’ to designate those illnesses in which the presence of inner bodies was regularly found in the erythrocytes of the circulating blood.

The discovery of Moeschlin in 1940 that certain sulfonamides were capable of producing Heinz bodies in human beings stimulated other investigators to look for morphologic changes in the erythrocytes in their hematologic examinations. From the work of Heubner and his students, who have shown that a large number of substances are capable of producing Heinz bodies in animals, it is evident that more attention should be paid to this phenomenon in persons exposed to these substances in industry. As early as 1941, directions were given for the microscopic examination of blood for Heinz bodies in German munition workers. In this country examination of TNT workers by Sievers et al. indicated the need for further investigations of this phenomenon. Moreover, Gross, Bock and Hellrung recommended that examination for Heinz bodies be made routinely in munitions plants, at least in cases of suspected poisoning, since such examinations require only a few moments and the simplest equipment. The supravital method of examination, requiring only a drop of fresh blood, can quickly reveal even minute Heinz bodies, if present. This is in contrast to the determination of methemo-
Table 1.—Summary of Chief Clinical Reports of Heinz Bodies in Man

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globin, which requires expensive special equipment for accurate evaluation. Moreover, indications of methemoglobin cannot be used as a criterion of the presence of Heinz bodies.

Finally, table 1 lists the chief clinical reports of Heinz bodies found in man.
HEINZ BODY PHENOMENON IN ERYTHROCYTES

**SUMMARY**

Certain morphologic changes in the erythrocytes, first described accurately by Heinz in 1890, have been noted by many investigators both in experimental animals and in man. These Heinz bodies, called by various names, appear to be newly formed particles originating either from the protoplasm or the membrane of the red blood cells in the course of irreversible injury by a toxic agent. The chemical nature of these particles is uncertain but they appear to consist largely of denatured proteins. They may occur in the blood in the absence of methemoglobin or sulfhemoglobin and without anemia, these phenomena being independent of each other. Removal of these bodies from the blood stream is frequently accomplished by their destruction in the spleen, often with resulting increase in size of this organ.

From the staining characteristics of Heinz bodies it is usually possible to distinguish them from other similar particles and to measure them quantitatively. Little is known of the relationship of chemical constitution of toxic substances to Heinz body formation. The indications are that some inorganic substances are capable of inducing this action as well as many aromatic nitro and amino compounds.

The presence in the blood stream of significant amounts of Heinz bodies is evidence of some injury to the erythrocytes. If this injury is severe it may lead to marked hemolysis and anemia.

Clinical cases of Heinz body occurrence in man, due either to drugs or to industrial poisoning, are cited and the need for further work and especially for Heinz body evaluation in routine hematologic examinations is pointed out. A bibliography is included in this review of the literature covering the chief contributions to work on Heinz bodies.

**REFERENCES**

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24 FISCHER F. H.: (Personal communication) Quoted by Fertman, M. H. and Doan, C. A. 1948.
37 ———: (Personal communication) Quoted by Fertman, M. H. and Doan, C. A. 1948.
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