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I hereby recommend that the thesis prepared under my supervision by James P. Hughes, M.D.

entitled The Significance of Erythrocytic Inclusion Bodies

be accepted as fulfilling this part of the requirements for the degree of Doctor of Industrial Medicine

Approved by: Robert V. Kelso

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THE SIGNIFICANCE OF ERYTHROCYTIC INCLUSION BODIES

A dissertation submitted to the
Graduate School of Arts and Sciences
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF INDUSTRIAL MEDICINE

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by

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B.S. University of Pittsburgh 1944
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INTRODUCTION

In cases of aniline intoxication in man, characteristic inclusion bodies in the erythrocytes have been described. Should this be of constant occurrence, particularly in incipient stages of intoxication, it may be of some value as a hematologic tool in surveying groups of workmen for evidence of significant aniline absorption. Determination of methemoglobin conversion has been used as such a tool, but this is a difficult and time-consuming procedure which requires the use of elaborate equipment.

* * * *

The problems concern: (1) staining methods; (2) morphologic differentiation of erythrocytic inclusion bodies; (3) the quantitative relationship, if any, between the degree of exposure to aniline and the numbers of erythrocytes containing inclusion bodies; (4) relationship to methemoglobin conversion; (5) presence of erythrocytic inclusion bodies in persons not exposed to aniline or related compounds; and (6) the mechanisms of inclusion body formation in and disappearance from the blood.

The present study consists of a report of four clinical cases of aniline intoxication, clinical and laboratory observations on a group of workmen exposed daily to aniline compounds, an industrial hygiene investigation of the work place of the exposed men, the occurrence of erythrocytic inclusion

bodies in a group of patients suffering from a variety of non-industrial hematologic disorders, and some experimental observations on laboratory animals following administration of an aniline compound.

REVIEW OF LITERATURE

1. Historical

A characteristic effect of aniline-like compounds upon erythrocytes was described by Heinz (1) in 1890. Phenylhydrazine administration to rabbits was found to give rise to the formation of multiple, rounded, highly refractile inclusions in large numbers of red cells. The inclusions occurred most frequently at the cell periphery, but occasionally were seen free in the plasma. In some erythrocytes stalk-like processes had formed, and the inclusions appeared to be extruding from the cells. Similar changes were produced in the blood of dogs and cats by exposure to phenylhydrazine derivatives and some related compounds.

Erythrocytic inclusion bodies were observed by Reis (2) in sodium chlorate exposure, by Roehl (3) in dinitrobenzene poisoning in man, and by Ehrlich (4). Heinz referred to these structures as "Blaukörper". Other terms have been affixed: hemoglobinemic inclusion bodies, hemoglobinemic inner bodies, Ehrlich-Heinz or Heinz-Ehrlich bodies, Heinz' blue granules, Heinz Inner-Korpern, substantia

metachromatico granularis, β-substance, Polkörperchen, Innenkorper, Binnekorper, and Innenkorpchen. The term "Heinz bodies" has been preferred in most publications in English.

Buckell and Richardson (5) collected reports of some 24 chemical substances giving rise to the formation of Heinz bodies, Webster (6) found 14 additional in the literature, while Treon and his coworkers (7) have added others. Toxic exposure resulting in the appearance of Heinz bodies in man has occurred with many of these substances. Chemically, most are compounds of the aromatic series containing nitrogen, chiefly amino- and nitro-derivatives of benzene, but substances of such widely varying chemical nature as cobalt, arsine and stibine, paraminobenzoic acid, hydroxylamine and naphthalene have been included.

Many of the compounds described in connection with Heinz bodies also result in methemoglobinemia, but this relationship is not invariable. The chief clinical significance of Heinz bodies lies in the fact that they have been observed in cases of exogenous methemoglobinemia of industrial origin. The enumeration of erythrocytes containing Heinz bodies is a much simpler task than the determination of methemoglobin conversion in the blood. The blood of TNT workers was examined by Horecker (8) and by Sievers et

al (9) in this country, and an indirect determination made of Heinz bodies. In 1941, the German government (10) issued instructions for the microscopic examination of blood for Heinz bodies in munition workers, and a discussion of the value of these determinations was presented by Gross, Bock and Hellrung (11). Convincing evidence of a strict relationship between Heinz body counts and methemoglobin determinations has not been presented, so that these tests cannot be considered to be of equal value in the evaluation of incipient stages of chemical intoxication.

2. Morphology

As described by Heinz (1), the bodies in the erythrocytes of rabbits poisoned with phenylhydrazine were round, oval or serrated granules, highly refractile, and usually situated at the periphery of the cells, so that they appeared to be in the cell membrane. They occurred singly, and sometimes two, in an erythrocyte. In wet preparations they exhibited Brownian motion. They sometimes protruded from the cell by a fine stalk, and were frequently observed free in the plasma. They varied in size in the erythrocytes of different species, usually 1 - 2 microns in rabbits, guinea pigs and dogs, but considerably larger in cats.

Bratley et al (12) observed the formation of Heinz bodies in vitro in the blood of dogs treated with pyr line.

The bodies formed gradually, appearing first as minute single granules at the cell periphery, but sometimes as four or five adjacent granules. A significant observation was made by Jung (13) using the electron microscope. In rabbit blood treated with dinitrobenzene, the inclusions first appeared as multiple submicroscopic particles which coalesced with growth. In these studies Jung also pointed out fine morphologic differences in the bodies which appeared with different toxic agents.

The number of bodies appearing in a single cell was found by Dustin (14) to vary with the concentration of phenylhydrazine in vitro, increasing with higher concentrations or prolonged contact periods.

The rate of formation of Heinz bodies and the relative numbers of cells containing them are functions of the dose of the toxic agent, according to Lambrechts and Nizet (15), Dustin (14) and Buckell and Richardson (5).

There is a species difference in susceptibility to the formation of Heinz bodies. Treon (16) lists cats, man, dogs, rats, rabbits and monkeys in descending order of susceptibility.

3. Chemical Nature

It was held by Ehrlich (4) that hemoglobinemic inclusion bodies are derived from hemoglobin. Hartwich (17) obtained Heinz bodies by hemolysis of affected erythrocytes,

and reasoned from their solubility in pepsin and hydrochloric acid that they contained protein, lipoid material and iron. Kunkel (18) identified a phosphatide, protein, cholestrol, and "an iron-containing blood pigment derivative". Jung (13) believed that the bodies are derived from the erythrocytic membrane, and Kiese and Seipelt (19) were in agreement with this view. All agreed that Heinz bodies are a protein substance; this is the only point of unanimity of opinion.

Decreased erythrocytic fragility to saline and hemolytic agents was reported by Morawitz and Pratt (20) in the blood of animals exposed to phenylhydrazine. Suzuki (21) found the same effect from pyrodine and toluene diamine, and attributed to extremely high resistance of the Heinz bodies, rather than to a diffuse pachydermia of the erythrocytes.

4. Origin and Fate

Webster (6) classified the theories of the mechanism of the formation of Heinz bodies as protoplasmic nuclear, pre-existent, reticulo-filamentous, and denaturation.

Heinz was the original proponent of the theory of protoplasmic origin of these bodies. He considered them as degeneration products of the erythrocytic protoplasm. Ehrlich conceived of the particles as derived from hemoglobin, and containing this pigment in the form of either

methemoglobin or altered hemoglobin. It was shown by the chemical studies of Hartwich (17) and of Kunkel (18), however, that Heinz bodies contain neither hemoglobin nor methemoglobin.

In the period around 1900, some German investigators identified Heinz bodies with Howell-Jolly bodies. The intense basophilia of Howell-Jolly bodies after fixation distinguishes them from Heinz bodies, as pointed out by Zadek and Burg (22).

Schwalbe and Solley (23) identified Heinz bodies with platelets. They suggested that such a body in the erythrocyte becomes liberated in the plasma as a platelet. It was held by Schilling (24), by Gutstein and Wallbach (25), and by Deutsch (26), that normal erythrocytes have a complex structure which includes a "Kapsulkörper" (capsule body), and that the Heinz body is a pathologic form of this capsule body. Special staining techniques, beset with dangers of artifact formation, are required for the demonstration of these structures. Dustin (14) disagreed on their significance.

There is some similarity between the appearance of a reticulocyte and an erythrocyte the seat of multiple Heinz bodies. Dustin (14) pointed out differential staining characteristics. Furthermore, Freifeld, Schilova and

Ludvinowsky (27) found no correlation between the number of reticulocytes and the numbers of Heinz bodies present in the blood of poisoned animals.

Webster (6) concluded that the denaturation theory of origin is most widely held at present. Heinz bodies are considered to be newly formed particles appearing in mature erythrocytes and formed from them in the course of an irreversible reaction with the toxic agent or with some intermediate metabolite. Huebner (28) and Dustin (14) considered these particles to be derived from erythrocytic protoplasm. Jung (13), on the basis of electron microscope studies, believed the cell membrane to be the site of origin.

Moeschlin (29) offered the in vitro formation of Heinz bodies as evidence of their peripheral origin. Lambrechts and Nizet (15) observed Heinz bodies in 60 to 80 percent of erythrocytes of animals within two to three hours after injection of phenylhydrazine. Following the oral administration of monomethylaniline to rabbits, Heinz bodies first appeared in erythrocytes at 1.5 hours, and were present in 85 percent of the red cells at 3.8 hours, according to Treon et al (7). These observations, together with the demonstration by Cruz (30) of inclusion bodies in all of the mature erythrocytes of dogs and rabbits following pyridine injection, but absence of such bodies in reticulocytes,

support the view of peripheral origin.

The question of the fate of Heinz bodies has received considerable attention. Heinz saw these particles being extruded from erythrocytes, and identified many free in the plasma of his phenylhydrazine-exposed animals. Hess and Müller (31) reported the presence of Heinz body phagocytes in the spleens of rats exposed to pyrodine, and found no Heinz bodies in blood from the splenic vein. Schilling (32) observed an increase in Heinz body counts following splenectomy in a dog exposed to antifebrin. Both Heinz (1) and Schilling (32) held the view that the normal spleen filters out erythrocytes which are the seat of Heinz bodies.

5. Staining Characteristics and Methods of Demonstration

Heinz utilized methyl violet in wet preparations as a means of demonstrating these bodies. This type of vital staining has had many advocates, and a variety of stains have been used. Friedstein (33) found Nile blue sulfate to be the best of a group of basic dyes which also included brilliant cresyl blue, methyl violet, toluidine blue, azur I, malachite green and neutral red. Buckell and Richardson (5) added Bismarck brown and azur II. They found it inadvisable to counterstain, because the basic dye is often washed out of the Heinz bodies by this process. Webster (6)

found that after fixation the bodies had practically no affinity for basic stains but showed an attraction for acid stains. Dustin (14) reported that Heinz bodies are stained by acid dyes buffered to a low pH, and that staining with these dyes may be carried out before or after fixation.

The Pappenheim-Schilling method of supravital staining preferred by Friedstein (33) consists of smearing out the blood on top of a dried film prepared by evaporation of an alcoholic solution of a basic dye. The slide is allowed to stand in a moist chamber for five to seven minutes before examining the cells. Webster (6) described a common modification of this method by placing a drop of blood directly on a dried film of Nile blue sulfate and covering with a cover slip. Webster, Liljegren and Zimmer (34) found methyl violet and gentian violet superior to Nile blue sulfate since these violet dyes were easily soluble in water. The method developed by Treon and Keenan (7) consists of staining of erythrocytes in suspension in plasma by a double oxalate - crystal violet reagent. Blood smears are then made from this suspension, affording a permanent preparation.

Gutstein and Wallbach (25) reported staining of both fixed and unfixed preparations by either basic or acid dyes. They mixed fresh blood with aqueous solutions of the stain

and observed it between slide and cover slip.

Webster, Liljegren and Zimmer (34) showed 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. They stated that, since the erythrocytes are subjected to the least trauma in the wet preparations, estimation of the number of Heinz bodies can be made most accurately in this way. These authors (35) also utilized a method of simultaneously fixing and staining a smear with a solution of methyl violet in ethyl alcohol. They pointed out that, although smears are desirable in that they provide permanent records, it is probable that the mechanical trauma to erythrocytes during the preparation of smears may lead to removal of many Heinz bodies from the cells.

Schilling (32) used thick drop preparations, hemolyzing the drops after drying, and staining with Giemsa solution. This method has the disadvantage of destroying many cells, so that quantitative estimation of the numbers of intraerythrocytic bodies is not possible.

Fertman and Doan (36) investigated the staining properties of Heinz bodies produced in cats by the oral administration of erythrol tetranitrate. They reported that inclusion bodies in erythrocytes were seen readily in unstained preparations and in supravital preparations stained

with brilliant cresyl blue, methylene blue, and Janus green with neutral red. They were also seen, but less readily, in fixed preparations with brilliant cresyl blue. They were not apparent in preparations stained with Wright, Wright-Giemsa or with the Prussian blue reaction. This property of not taking the Wright or Giemsa stain accounts for the fact that Heinz bodies are so infrequently observed in laboratories where these are the routine methods of blood smear preparation.

Fertman and Doan (36) demonstrated Heinz bodies in unstained living blood films under both light field and under dark field illumination, and quoted Figge " - - - these bodies are much more easily observed in plain dried blood films examined under the high dry objective."

Differentiation of Heinz bodies from reticulocytes has depended upon the size of the cell, the appearance of the network of punctations, the degree of staining, the more basophilic cytoplasm of the reticulocyte, and digestion with ribonuclease, according to Buckell and Richardson (5). Lambrechts and Nizet (15) recommended dark field examination of a dried blood film stained with brilliant cresyl blue for this purpose. The staining is carried out and the film prepared in the same way as for routine reticulocyte counts. Under these conditions the reticular substance shows

up a clear yellow, the Heinz bodies brick-red, and the cell outline a dull wine-red. Dustin (14) summarized the differential staining characteristics of basophilic particles, Howell-Jolly bodies, reticulocytes and Heinz bodies. Fertman and Doan (36), in showing that Heinz bodies did not give the reactions for iron, differentiated them from siderocytes.

Other methods have been devised for the estimation of Heinz bodies. Cruz (30) and others (8, 9, 37) have taken the turbidity of laked blood, measured spectrophotometrically, as an indication of the numbers of Heinz bodies present. Rigdon and Breslin (38), using the blood of monkeys following oral administration of phenylhydrazine hydrochloride, hemolyzed the erythrocytes with distilled water and a solution of phloxine dye in a red cell diluting pipette, and counted the number of Heinz bodies on a haemocytometer, relating this to the total red cell count.

CLINICAL OCCURRENCE OF HEINZ BODIES

1. Collected Reports

Clinical reports of the formation of Heinz bodies in human blood have not been numerous, and have appeared chiefly in German. Webster (6) collected twenty-three papers, and four more were added by Buckell and Richardson (5) up to 1950. According to Webster (6), the first report of these

specific erythrocytic inclusion bodies in man was by Riess in 1882. This was a case of potassium chlorate poisoning. Other reports of clinical cases have been tabulated as follows (5, 6):

Author	Date	Condition (Poisoning or Disease)
Heinz	1890	Pyridine
Roehl	1890	Dinitrobenzene
Huber	1891	Dinitrobenzene
Ehrlich	1892	Anemia
Ehlich & Lindenthal	1896	Nitrobenzene
Huber	1912	Potassiumchlorate
Schilling	1921	Malaria
Schilling	1927	Aniline
Schilling	1928	Antifebrin
Zadek & Burg	1930	Aniline
Genkin & Raschewskaja	1933	Aniline
Freifeld et al	1937	Dinitrotoluene
Ungricht	1938	Phenylsemicarbazide
Moeschlin	1940	Sulfapyridine
Rohr	1940	Nitrobenzene
Doering	1941	Dimethylenediaminodiphenyl-sulfone
Dustin	1941	Colchicine
Wilhelmi	1942	Nitroglycerine
Willi	1942	Guaiacol

Author	Date	Condition (Poisoning or Disease)
Rejsek	1944	Dinitrobenzene
Pimenta de Mello	1945	Pyridine
Sievers et al	1945	Trinitrotoluene
Fertman & Doan	1945	Erythrol tetranitrate
Houstek	1947	Sulfanilamide
Willi	1947	Pyrimidine
Zuelzer & Apt	1949	Naphthalene
Reimer	1950	Sulfathiazole

To these are added the following recent reports of Heinz bodies in man:

Jasinski (39)	1948	Aniline
Sinios (40)	1949	Aniline
Ghiringhelli & Molina(41)	1951	Aniline

The reports of Ehrlich and of Schilling described Heinz body erythrocytes in cases of disease not associated with chemical intoxication. The probable nature of the inclusion bodies in these cases will be discussed in a later section.

There have been no descriptions in the English language of the morphology of Heinz bodies in the erythrocytes of man following industrial exposure to harmful substances. The blood of TNT workers was examined by Horecker (8) and by Sievers et al (9). In both studies

the relative turbidity of hemolyzed blood was taken as an index of the formation of Heinz bodies, although the numbers of affected erythrocytes were considered too few to be evaluated by means of the examination of blood films.

We have observed four chemical workers with clinical methemoglobinemia of exogenous origin. Each had been exposed to an aniline derivative. Heinz bodies were observed in the erythrocytes of three of the four men. The staining method of Treon et al (7) using crystal violet was employed (Appendix 1).

2. Report of Cases

Case 1. J.R. was a 72 year old white sheet metal worker. While he was repairing the hood over a kettle of cooling aniline his lips became blue. The duration of his exposure to aniline fumes was estimated at at 30 minutes. Cyanosis increased and was accompanied by moderate weakness and slight dyspnea at rest. On admission to the hospital six hours after exposure, examination of the venous blood by a spectrophotometric method disclosed 8.83 grams methemoglobin and a total hemoglobin of 14.2 grams, a conversion of 58.1 percent. At this time, 8 percent of the erythrocytes contained Heinz bodies (Figure 1). Forty-eight hours later, while the patient was still

slightly cyanotic, 24.5 percent of the blood pigment was present as methemoglobin, and Heinz bodies were present in 28 percent of the erythrocytes. On the seventh, fourteenth and twenty-first days after exposure, the Heinz body counts were 12.5 and 0 percent, respectively. There was no anemia, or other sequela.

Case 2. R.G. was a 49 year old white chemical operator. In charging an autoclave he unknowingly spilled a small amount of meta-chloraniline on his trousers. About one hour later it was noted that his lips were blue, and he became weak and dizzy. Four hours after the exposure, when cyanosis was most marked, 5 percent of the erythrocytes contained Heinz bodies (Figure 2). On the following day, slight cyanosis persisted, and the percentage of Heinz bodies was 18. On the third day the count was 28 percent, and on the seventh day 20 percent. There was no anemia.

Case 3. J.B. was a 43 year old white chemical operator. After one hour of cleaning the inside of an aniline kettle, during which time he wore an airline respirator, he felt slightly dizzy. His lips, nose and ears became increasingly cyanotic.

On his discharge from the hospital 48 hours later, there were Heinz bodies in 30 percent of the erythrocytes. On the fourth day the count was 35 percent, on the fifth 24 percent, on the eighth 18 percent (day of return to work), on the fourteenth 10 percent, and on the forty-fourth there were no Heinz bodies. There was no anemia.

Case 4. E.B. was a 42 year old white electrician. While wiring a chemical building, his clothing was probably contaminated with an aniline compound. Within a few hours he had developed very mild cyanosis of the lips, oral mucosa and finger tips. Methemoglobinemia to the extent of 26.3 percent was found, but there were no Heinz bodies in the erythrocytes of the peripheral blood. There was mild occipital headache during the night, but he returned to work on the following day. Cyanosis was absent, and there were no Heinz bodies in the blood on several subsequent examinations.

3. Comment

Each of the three men whose blood exhibited Heinz bodies was strikingly cyanotic, and had other symptoms of anemic anoxia. The clinical diagnosis of exogenous methemoglobinemia was quite apparent. Methemoglobin was determined

to verify the clinical impression, but the method is tedious (Appendix 2) and requires spectrophotometric equipment. The enumeration of Heinz bodies is the simpler technique by far, and if it could be correlated with the occurrence or extent of methemoglobinemia, might be a valuable diagnostic test. To be useful, however, Heinz bodies would have to be shown to occur at an early stage of aniline intoxication and in lesser degrees of methemoglobinemia than were present in the cases described. These cases were the result of accidental exposure, and did not occur in what might be considered normal operations.

INDUSTRIAL HEALTH SURVEY IN A CHEMICAL PLANT

1. Examination of Workmen

Exploratory clinical and hematological observations were carried out on a group of chemical workers who were exposed daily to aniline compounds under controlled operating conditions, in order to learn whether Heinz bodies were found under conditions involving little exposure.

a) Nature of the Exposure

A single building of a chemical plant was investigated. Large quantities of aniline and meta-chloraniline were used in several operations. In addition, processes employing certain aliphatic amino- substances were also carried on (Appendix 3). There were 36 white male workmen on three

shifts. All had some exposure to both types of compounds (Table I). Exposure was intermittent and occurred chiefly during charging, emptying and cleaning of equipment and during sampling. The processes were largely carried out in closed systems. General ventilation of the building was adequate. Personal hygiene of the men was excellent.

Most of the personnel had worked seven eight-hour shifts each week for several months. Nine of the 36 had been in this building for five years or longer; the mean exposure period was 41.2 months. The mean age of the group of 36 was 28.2 years, and the median age 30 years.

b) Results

The complete medical and occupational history of each man was taken (Table II). Each was questioned closely as to the occurrence of cyanosis, weakness and dizziness, and of symptoms referable to the skin and respiratory systems. Nine men gave a history of one or more episodes of "blue lip", or methemoglobinemia (Subjects 1, 3, 4, 15, 19, 28, 31, 32, 33); two of them had each had a single attack within the previous year (Subjects 3, 15).

The clinical and laboratory studies were conducted in the plant medical department and included physical examination, routine urinalysis, hemoglobin determination by the Haden-Hausser method, complete blood count, and enumeration

of erythrocytes with inclusion bodies by the method of Treon et al (7) (Tables III, IV, V). All of the men had received either a preplacement physical examination including chest roentgenogram or an annual periodic physical examination during the preceding eighteen months, and had been found to be free of significant disease.

There was no unusual incidence of any disorder. No diseases of occupational origin were found. Urinalyses revealed no abnormalities. No significant deviations from the normal were found by the routine blood examinations.

The percentages of erythrocytic inclusion bodies found are recorded in Table V. Of the 36 workmen, 28 (77.8 percent) were found to have one percent or less of their red cells containing such bodies. In no case was there more than 4 percent. The inclusion bodies were morphologically indistinguishable from the Heinz bodies described in our cases of clinical methemoglobinemia.

c) Comment

A number of specific types of erythrocytic inclusion bodies have been described, including siderocytes, Pappenheimer bodies, Howell-Jolly bodies, Röhl's bodies and Maragliano bodies (42). These differ little in appearance, and may be difficult to distinguish from Heinz bodies. Even occasional reticulocytes resemble cells containing

Heinz bodies in an early stage of formation. Moreover, it is not always possible to differentiate occasional artifacts from inclusion bodies. Even a platelet superimposed upon an erythrocyte may be mistaken for an inclusion body. On the other hand, the mechanical force required for smearing a blood film may detach some true inclusion bodies from their erythrocytes. For these reasons, enumeration of inclusion bodies in stained smears probably has an error of at least 5 percent. Therefore, the small numbers of affected erythrocytes encountered in the blood of the workmen in this survey cannot be considered to be significant.

2. Air Sampling

a) Method

Grab samples of air were collected in 500 cc. evacuated flasks. There were seven air samples taken at three locations on four different days. Samples were collected under average operating conditions so that usual exposures could be determined. Determination of aniline was made by the colorimetric method of Elvove (43) using calcium hypochlorite solution.

b) Results

The analytical results of atmospheric sampling are listed in Table VI. In Sample No. 1 all aromatic amines are calculated as monomethylaniline; in samples No. 2 and

3 all chlorinated aromatic amines are calculated as metachloraniline; in samples No. 4, 5, 6, and 7 all aromatic amines are calculated as aniline. All contaminants are calculated as parts per million of air.

c) Comment

The highest concentration of all contaminants, 1.3 p.p.m. of aniline, occurred during steaming of the cement floor to remove aniline compound spills. Large doors in the side of the building immediately adjacent to the area were always opened during the cleaning operation, and personnel stayed clear of the vapor clouds which were formed. Ten minutes after cleaning, a second sample taken at the same site contained only 0.27 p.p.m. of aniline. The mean concentration of aniline compound contaminant in the seven samples was 0.53 p.p.m. The maximum allowable concentration of aniline for prolonged exposure is usually given as 5 p.p.m. All of the samples contained considerably less than this concentration. Based on these results, there appeared to be little hazard from the inhalation of aniline compounds during normal operations. This conclusion is supported by the absence of significant clinical findings attributable to chemical exposure in the 36 workmen examined. In every case of clinical methemoglobinemia that had occurred in this group in the past, there was history of some unusual accidental

exposure to aniline compounds. This usually had consisted of contamination of the clothing.

Another factor of safety in regard to aniline in the air is the characteristic odor of these compounds. Perceptible odor occurs at concentrations of approximately 0.5 p.p.m., while the upper limit of safe exposure is considered to be 5 p.p.m. There is ample warning of contamination of the air in this range. In addition, experienced workmen are so familiar with the "blue lip" of aniline intoxication that they have a high respect for its warning odor.

HEINZ BODIES IN RABBITS

1. Effect of the Spleen on Heinz Bodies

Following splenectomy in man and experimental animals, large numbers of erythrocytes of the peripheral blood have been observed to contain Heinz bodies. These have been described as siderocytes (44), as Pappenheimer bodies (45), and occasionally as Heinz bodies (46). The spleen has been considered to be of considerable importance in the Heinz body phenomenon (32, 36). In order to further elucidate the nature of the splenic effect, monomethylaniline was administered intravenously to rabbits, splenectomy performed 24 hours later, and the tissue examined for the presence of Heinz bodies.

a) Method

Two white male adult rabbits were given intravenously 0.18 grams of monomethylaniline per kilogram of body weight. This dosage was determined by Treon et al (7) as giving rise to the formation of Heinz bodies in up to 98 percent of the erythrocytes. Heinz body counts were made at six hour intervals. Twenty-four hours after exposure both rabbits had 90 percent of the erythrocytes with Heinz bodies. They were anesthetized with sodium pentothal and splenectomized. The spleens were sectioned immediately, and scrapings made

of the splenic pulp. This material was stained vitally with crystal violet in the same manner as peripheral blood (Appendix 1), and also by the supravital technique using Janus green and neutral red as practiced in Doan's laboratory.

b) Results

In one rabbit there was marked increase in phagocytic clasmatocyte activity in the splenic pulp. Clasmatocytes were increased both in size and number, up to 12 per high power field. In the second animal clasmatocytic activity was only moderately increased. The preparations were similar in other respects. The phagocytosed elements consisted mostly of erythrocytes, but there were occasional polynuclear leukocytes and platelets. In many of the phagocytosed erythrocytes Heinz bodies were identified in the crystal violet stained preparations. In many clasmatocytes hemosidern particles were seen with the supravital stain. There was a slight increase in plasma cells in the splenic pulp, and a scattering of basophils and eosinophils. Most of the erythrocytes contained inclusion bodies.

c) Comment

The demonstration of phagocytosed Heinz body erythrocytes in the spleens of these rabbits verifies the observations of Hess and Müller (31) on pyridine poisoned rats. This indicates one mechanism for the removal of affected

erythrocytes from the circulation.

2. The Bone Marrow of Monomethylaniline Poisoned Rabbits

The concensus of opinion is that Heinz bodies are to be found only in mature erythrocytes. The two chief arguments in favor of this view are that Heinz bodies are never seen in reticulocytes (30), and that they have appeared in large numbers in the erythrocytes of the peripheral blood within as short a period of time as 10 minutes following administration of toxic agents (11). Support for this hypothesis was sought in examination of the bone marrow of rabbits poisoned with monomethylaniline.

a) Method

The two white male rabbits described in the previous section were used. Twenty-four hours after administration of monomethylaniline, when 90 percent of the erythrocytes of the peripheral blood contained Heinz bodies, marrow from the femur was aspirated through a large calibre needle, stained by the crystal violet method, and by supravital technique.

b) Results

In the supravital preparation the bone marrow was moderately well preserved, with a moderate increase in fat, although cellularity was of normal concentration with a normal erythrocyte-leukocyte ratio. Immature erythrocytes

were mostly at the normoblastic level of maturation, although some late and a few early erythrocytes were seen. Leukocytes were mostly at the polynuclear level. There were occasional mature and active megakaryocytes. A few scattered lymphocytes and plasma cells were seen. The marrow was interpreted as showing slight toxic change, but as having nearly normal characteristics. In the crystal violet preparation approximately 90 percent of the mature erythrocytes contained inclusion bodies. Fifty isolated normoblasts in the preparation were enumerated, and one had a questionable Heinz body; the others were free of inclusion bodies. A similar observation was made in reticulocytes.

c) Comment

It was our impression that Heinz bodies are not found in immature erythrocytes.

3. Methods of Identifying Heinz Bodies

In the rabbits given monomethylaniline intravenously, specimens of the peripheral blood were taken when Heinz body count reached 90 percent. A comparison of staining and microscopic techniques for demonstration of Heinz bodies was made. Preparations were examined wet and unstained, wet and stained by vital and supravital methods, dry and unstained, and dry and stained.

a) Methods and Results:

I. Wet - Unstained

A. Light field microscopy-Heinz bodies seen poorly

B. Dark field - seen moderately well, refractile

C. Phase contrast - seen well, highly refractile

D. Polarized light - seen well, refractile

II. Wet - Stained (Light field)

A. Basic dyes: Crystal violet - seen well; brilliant cresyl blue - seen moderately well

B. Janus green with neutral red (supravital): could not be distinguished from reticulocytes

III. Dry - Unstained

A. Heinz bodies could not be seen by light field microscopy

IV. Dry - Stained

A. Wright-Giemsa - not seen

B. Prussian blue - not seen

C. Prussian blue (after formalin fixation)-not seen

D. Basic dyes (smears made from wet preparations without counterstaining) - seen well

E. Basic dyes (counterstained with phloxine after formalin fixation) - seen moderately well.

b) Comment

Ordinary hematologic staining methods as used in most laboratories are not satisfactory for the demonstration of Heinz bodies. Wet staining methods with basic dyes are highly satisfactory; use of phase contrast microscopy increases their usefulness. The most convenient method, however, is

the preparation of dried smears from wet stained material.

OTHER ERYTHROCYTIC INCLUSION BODIES

Inclusion bodies of the erythrocyte other than Heinz bodies have been discussed by Mills and Lucia (42) and others (47). So numerous have been the descriptions of other forms that there is a great deal of confusion over eponymic terms in the literature. It is apparent that what one author describes as Heinz bodies, another may term Howell-Jolly bodies; what are siderocytes to some, are Pappenheimer bodies to others. It is further apparent that certain normal physiologic phenomena of erythrocytes are sometimes interpreted as toxic manifestations. Where in vitro studies are concerned, all manner of abnormal erythrocytes have been seen, often the result of cell senesence and degeneration. Thus, the significance of any abnormal form of the erythrocyte must be considered with caution. These may be classified as follows:

1. "False" Inclusion Bodies

   a) Parasites

      Plasmodium - differentiated by staining reaction.

      Bartonella - an uncommon disease of South America.

   b) Röhl's Marginal Bodies

      A degenerative change consisting of peripheral punctations in erythrocytes stored in vitro.

c) Maragliano Bodies

Another degenerative form consisting of round or elliptical vacuolated bodies.

d) Artifacts

Those due to precipitated stain or foreign bodies.

Platelets superimposed upon erythrocytes

Cabot rings

Hemoconia of Müller

2. Nuclear or Cytoplasmic Remains

a) Howell-Jolly Bodies

b) Reticulocytes

c) Stipple cells

All of these forms should be distinguished from Heinz bodies because of tinctorial differences

3. Iron Containing Bodies

a) Siderocytes

b) Pappenheimer bodies

These are discussed below, and are characterized by staining with the Prussian blue reaction

4. Schmauch Bodies

These are normally present in the blood of cats, increasing in numbers with the age of the cat, and are sometimes considered to be identical with Heinz bodies. The staining reactions are the same.

Discussion

Of all these forms of the erythrocyte, the ones which are perhaps of the greatest clinical significance in relation to Heinz bodies are the iron-containing bodies. Grüneberg (48) described in the blood of normal rats, mice and human embryos, erythrocytes which contained some free stainable iron. These cells, called siderocytes, showed distinct siderotic granules, sometimes a dozen or more per cell. This author (44) later found siderocytes in the blood of seven adult human patients. Five of these had been splenectomized for various hematologic disorders, one had severe anemia, and another renal insufficiency. It is uncertain whether or not the siderocyte represents a senescent cell, or arises as the result of "depraved hematopoiesis" (47).

In three cases of severe anemia reported by Pappenheimer et al (45), iron-reacting structures occurred in the erythrocytes following splenectomy. Certain minor morphologic differences from the siderocytes of Grüneberg were pointed out.

Schilling (32) noted the appearance of Heinz bodies in the blood of splenectomized normal animals, and found an increase in Heinz bodies in a pyrodine poisoned dog following splenectomy. Zadek and Burg (22) confirmed the presence of Heinz bodies in human blood after splenectomy.

Some relationship between siderocytes, Pappenheimer bodies and Heinz bodies might be postulated.

The tinctorial differences between these forms may be tabulated thus (6, 45, 48):

Stain	Heinz Body	Siderocyte	Pappenheimer Body	Reticulocyte
Basic	+	+	+	+
Wright-Giemsa	-	+	+	+
Iron	-	+	+	-

Despite these reported staining characteristics, there undoubtedly has been some overlapping of nomenclature in the cases of erythrocytic inclusion bodies appearing after splenectomy. In discussing a case of "inclusion body anemia" following prolonged erythrol tetranitrate administration in a man, Doan (36) posed this question: "Should it be considered, then, that minimal inclusion body formation may occur, in association with subclinical endogenous toxins, in otherwise normal individuals, but in numbers so small that they are ordinarily withdrawn by a physiologically functioning spleen?" If this hypothesis be correct, then erythrocytic inclusion bodies might be expected to appear in the peripheral blood following splenectomy, regardless of

the basic condition for which the operation was performed.

Hematologic observations were made on a group of 23 splenectomized patients with this concept in mind.

Hematologic Observations on Splenectomized Patients and Control Groups

1. Material and Methods

Clinical histories were obtained on 23 persons whose spleens had been removed, chiefly because of hematologic disorders. In one case splenectomy had been performed because of traumatic rupture of the spleen. The pre-splenectomy diagnoses are listed in Table VII. Complete hematologic data were obtained on each patient on the occasion of his regular post-splenectomy visit to the clinic of Doctor Charles A. Doan, Ohio State University, Columbus. At the same time, a specimen of blood was stained for inclusion bodies by the crystal violet method (Appendix I), and the erythrocytes containing these bodies enumerated. Specimens were obtained by finger puncture.

The blood of 19 persons with intact spleens was examined by the same methods. These fell into two groups: 9 were under observation for the same general types of hematologic disorders as occurred in the splenectomized persons, and in some of these splenectomy was being considered; 10 were under active treatment by one of the therapeutic agents, aminopterin, urethane, or nitrogen mustard.

2. Results

Hematologic data of the 23 splenectomized persons are

recorded in Table VIII. There were inclusion bodies in significant numbers of erythrocytes in all of the 23. The counts ranged from 3 percent of erythrocytes affected to 50 percent, with a mean of 21 percent.

The inclusion bodies were morphologically similar to those observed in the erythrocytes of the 3 aniline poisoned chemical workers previously described. They stained dark blue with crystal violet. They sometimes occurred singly, but more commonly two or three were situated at the periphery of a cell. They were differentiated from reticulocytes by these characteristics of number and site of location within the cell. An erythrocyte containing more than three or four inclusion bodies, and especially if these were not adjacent to the cell membrane, was not included in the inclusion body count.

Inclusion body counts were considered in relation to the diagnosis of the basic hematologic disorder for which splenectomy was performed, to age of the patient, erythrocyte count, leukocyte count, reticulocyte count, and the interval of time since splenectomy.

a) Diagnosis - see Table IX

Mean numbers of erythrocytes with inclusion bodies ranged from 10 percent with neutropenic leukopenia and with traumatic rupture of the spleen (one case of each), to 36 percent with chronic lymphoid leukemia (three cases). The mode count was 26 percent with congenital hemolytic anemia (eight cases).

b) Age of Patient - see Table X

Mean inclusion body counts had a range of from 3 percent at age 53 (one case) to 32 percent in the seventh decade (six cases).

c) Erythrocyte Counts - see Table XI

Mean inclusion body counts had a range of from 10 percent with a total erythrocyte count of 5½ to 6 million (one case) to 40 percent with a total erythrocyte count of under 2½ million. In the group of 10 patients with erythrocyte counts below 4 million and therefore considered to have some degree of anemia, the mean inclusion body count was 26 percent. In the other 13 patients without anemia the mean inclusion body count was 18 percent.

d) Leukocyte Counts - see Table XII

Mean inclusion body counts ranged from 17 percent with leukocyte counts of 8,000 to 10,000 (5 cases) up to 50 percent with leukocyte counts of 4,000 to 6,000 (2 cases). In

the 9 cases with leukocyte counts below 10,000, mean inclusion body counts for the group averaged 21 percent. In the remaining 14 cases with leukocyte counts over 10,000 considered to have leukocytes, mean inclusion body counts averaged the same - 21 percent.

e) Reticulocyte Counts - see Table XIII

In the 11 cases where reticulocytes were enumerated, the inclusion body counts had a range of from 16 percent where the reticulocyte count was normal, or under 2 per 100 erythrocytes (5 cases), up to 34 percent where the reticulocyte count was over 20 per 100 erythrocytes (2 cases).

f) Interval Since Splenectomy - see Table XIV

Mean inclusion body counts ranged from 10 percent in 3 cases with an interval of less than one month since splenectomy, up to 28 percent in 2 cases with an interval of 13 to 18 months. The median inclusion body counts occurred between the intervals of 12 and 13 months.

* * * *

In 19 patients with intact spleens, none had significant numbers (over 2 percent) of erythrocytes containing inclusion bodies similar to those observed in the splenectomized group.

* * * *

Three patients were observed for periods of up to three weeks following splenectomy, and inclusion body counts made

at frequent intervals:

Case 1.

J.W., a white male aged 14, was under treatment for thrombocytopenic purpura. On the first day following splenectomy, 2 to 3 percent of the erythrocytes had inclusion bodies; on the second day, 5 percent; on the tenth day, 14 percent; and on the twentieth day, 22 per cent.

Case 2.

H.S., a white male aged 60, diagnosis chronic lymphoid leukemia, had inclusion bodies in less than 1 percent of the erythrocytes before splenectomy. On the second postoperative day there were inclusion bodies in 1 to 2 percent of the erythrocytes, and on the fifth day in 2 to 3 percent.

Case 3.

S.E., a white female aged 23, diagnosis thrombocytopenic purpura, had inclusion bodies in less than 1 percent of the erythrocytes before splenectomy, but in 3 to 5 percent on the second postoperative day.

* * * *

In one of the splenectomized patients, W.T., a white male aged 20, whose spleen had been removed following traumatic rupture nine months prior to this study, differential

staining was carried out on sternal bone marrow and on peripheral blood.

The bone marrow was of normal cellularity with a normal erythrocytic-leukocytic ratio as observed on supravitally stained preparations using Janus green and neutral red. With the crystal violet wet stain used for Heinz body identification, peripheral inclusion bodies were observed in approximately 10 percent of the mature erythrocytes. In smears of the marrow stained by the Prussian blue method occasional erythrocytes contained marginal blue bodies. These erythrocytes were much less numerous than those with inclusion bodies in the crystal violet preparations. In bone marrow smears stained with Wright's, morphologically similar bodies were observed in 3 to 4 percent of the erythrocytes, and in occasional normoblasts.

The staining characteristics of erythrocytic inclusion bodies occurring in the peripheral blood in up to 10 percent of the cells were similar to those observed in the bone marrow preparations. No normoblasts were seen.

3. Comment

The presence of erythrocytic inclusion bodies in all 23 splenectomized persons suggests that these bodies occur in significant numbers due to the physiologic effect of removal of the spleen. There was no relationship of the inclusion body counts to diagnosis, age of patient, eruthrocyte count, leukocyte count or interval since splenectomy. There was a possible relationship to the reticulocyte count.

There was no apparent relationship to diagnosis because 19 patients with similar hematologic disorders but with intact spleens had no erythrocytic inclusion bodies. Of these, 10 were under treatment with hematologically active drugs. These agents had no effect on the erythrocytes insofar as the formation of inclusion bodies was concerned.

In the 23 splenectomized patients there was a normal spread of mean inclusion body counts in each decade.

There was a slight degree of apparent increase in inclusion body counts in splenectomized persons with anemia. Considered on the basis of total numbers of affected erythrocytes, this apparent increase resolves itself.

Mean inclusion body counts were the same in persons with leukocytosis as in those with normal leukocyte counts.

Except for a period of several days following the operation, the interval of time since splenectomy did not affect

the mean inclusion body counts.

There was a slight increase in mean inclusion body counts in cases of reticulocytosis over those with normal reticulocyte levels. There was sufficient variation in inclusion body counts at different severities of reticulocytosis that inclusion body levels could not be directly related to reticulocyte levels. It is suspected that inclusion body erythrocytes have the same staining characteristics as reticulocytes with the Dameshek supra-vital technique employed in this clinic, so that some inclusion body erythrocytes may have been enumerated as reticulocytes. That the reverse error may have been committed to a small degree with the crystal violet technique cannot be denied, although it is unlikely that this error was significant since reticulocytes commonly have many more punctations than do inclusion body erythrocytes.

The rate of appearance of inclusion body erythrocytes following splenectomy was demonstrated to rise in a straight line beginning on the first postoperative day and leveling off by the twentieth day. It is interesting to note that this is quite similar to the rate of disappearance of Heinz body erythrocytes from the blood after aniline poisoning.

By differential staining methods it was demonstrated that some but not all of the affected erythrocytes after

splenectomy contain iron-bearing inclusion bodies similar to those described in siderocytes. Siderocytes, therefore, account for only some of the inclusion body erythrocytes seen in these cases. The iron-negative particles may be Heinz bodies.

DISCUSSION

It is apparent from a review of published observations on inclusion bodies in erythrocytes that the red cell is affected in specific and morphologically demonstrable ways by the action of certain chemicals and by the physiologic alterations resulting from splenectomy. In the search for simple hematologic tools that may be applied to the clinical evaluation of the degree of industrial exposure to these chemicals, chiefly compounds related to aniline, some application of the inclusion body phenomenon might be considered. While inclusion bodies, or Heinz bodies, have been described in cases of frank clinical intoxication by these substances, no studies of the effects of lesser degrees of exposure have been presented until this time.

Of the four cases of chemical intoxication due to absorption of aniline compounds which are described, three had moderately severe degrees of methemoglobinemia. Heinz body erythrocytes in significant numbers were observed in the blood of these patients. In a fourth case of methemoglobinemia of milder degree, there were no Heinz body erythrocytes. The conclusion that formation of Heinz bodies does not occur in significant numbers in human blood except in cases of clinically apparent intoxication was verified by the absence of these characteristic erythrocytes from the blood

of exposed but healthy workmen. The demonstration of inclusion body red cells in splenectomized persons marks an exception to this rule.

Determination of methemoglobin levels, although tedious, would seem to be a much more reliable index of dangerous absorption of aniline compounds. In cases of incipient or mild intoxication, the appearance of cyanosis of the nose, lips, ears and nailbeds, the classical "blue lip" of chemical workers, provides a valuable diagnostic sign. In Negro workmen, where cyanosis would not be readily detectable, the Heinz body count may be of value in the diagnosis of aniline intoxication.

In three of our cases of aniline poisoning, absorption of the toxic agent had been through the skin, and arose from accidental contamination of the clothing. This has also been the mode of exposure in most of the reported cases of aniline intoxication in man. It is suggested that provision of light-colored work clothes, upon which the presence of aniline would be readily apparent, would be an effective measure in the prevention of serious exposure to these compounds.

The prompt appearance of Heinz body erythrocytes in these cases of aniline intoxication suggests that they are formed in the peripheral blood. This impression is strengthened by

the absence of Heinz bodies from the immature forms of the erythrocytic series which were observed in the bone marrow of rabbits given monomethylaniline. The slow rate of disappearance of human Heinz body erythrocytes following reconversion of methemoglobin leads to the conclusion that these cells are removed by the spleen. This observation is supported by the demonstration of Heinz body erythrocytes in large numbers engulfed in splenic phagocytic clasmatocytes in the rabbits mentioned.

With the present method of examination of the blood, Heinz body enumeration is not considered to be a survey procedure of value in industrial medical practice. This conclusion is based on: a) the absence of Heinz bodies in cases of mild aniline intoxication; b) the difficulty of making a specific differentiation of individual inclusion body cells, especially when these are present in the blood in small numbers; and c) the low relative sensitivity of human erythrocytes to the formation of Heinz bodies, possibly due to the efficiency of the normal human spleen in removing affected cells from the circulation.

Other possible clinical uses to which the Heinz body phenomenon might be applied include the determination of blood volume, the survival rate of erythrocytes, and evaluation of the phagocytic functions of the spleen.

The occurrence of inclusion bodies in significant numbers of the erythrocytes of all 23 splenectomized persons suggests that this is an ubiquitous effect of removal of the spleen. These inclusion bodies have been variously described as Heinz bodies, Howell-Jolly bodies and siderocytes. Their morphologic similarity to the erythrocytic inclusion bodies of chemical workers with clinical aniline intoxication, together with their tinctorial characteristics, suggest that they are closely related to Heinz bodies. These observations support the hypothesis that minimal inclusion body formation may occur in erythrocytes as a sign of senesence or degradation, but in numbers so small that they are ordinarily withdrawn by a physiologically functioning spleen. Following splenectomy, large numbers of affected erythrocytes remain in the blood. The striking formation of Heinz bodies in the erythrocytes in cases of certain chemical intoxications lead to the conclusion that the degradation process may be accelerated by exposure to these compounds.

SUMMARY

Inclusion bodies in the mature erythrocytes of experimental animals were observed by several German investigators in the latter part of the last century. In most cases the animals had been poisoned by aromatic nitro- or amino- compounds. Robert Heinz gave the first complete description of the phenomenon in 1890. Studying the action of phenylhydrazine, he produced highly refractile round, oval or serrated granules, usually single but occasionally multiple, in the erythrocytes of a number of animals. A variety of names has been appended to these bodies, but they are most frequently referred to as "Heinz bodies" in English language publications. Identical inclusion bodies have been observed in man in cases of exposure to aniline derivatives and to nitrobenzenes, and more recently in cases of sulfonamide overdosage. There have been few reports of erythrocytic inclusion bodies demonstrated morphologically in man after toxic industrial exposures. Heinz bodies are not seen well with the usual Wright or Giemsa stains of dried blood films, but require either a modification of a vital staining technique, or examination of wet preparations by dark field or phase contrast microscopy. We have used a vital stain of crystal violet in double oxalate solution. Smears of the stained

blood are made. Heinz bodies appear as small light blue to deep purple inclusions in the erythrocytes, occurring most frequently at the cell periphery.

Four cases of methemoglobinemia in aniline workers were studied. In three of these, up to 35 percent of the erythrocytes had Heinz bodies. In one case of milder intoxication, Heinz body formation did not occur. Maximum numbers occurred 2 to 3 days following exposure, after methemoglobin reduction was nearly complete. The maximum time for complete disappearance of Heinz bodies from the peripheral blood was three to six weeks. Anemia did not occur. There was complete recovery without sequelae in all cases.

To evaluate the Heinz body count as an indication of incipient intoxication, exploratory tests were made on 36 chemical workers exposed daily to aniline compounds. Air sampling in the exposure area was performed in three cycles. The maximum concentration of aniline found was 1.3 parts per million of air, and the mean concentration of aniline in seven samples was 0.53 p.p.m. No significant physical or hematological abnormalities were found in the group of men. In 28 of the 36, 1 percent or less of the erythrocytes had inclusion bodies, and in two others, 4 percent. The latter was the highest count obtained, and is not considered to be significant.

Following splenectomy in man and experimental animals, large numbers of the erythrocytes of the peripheral blood have been observed to contain inclusion bodies. These have been described by various investigators as siderocytes, as Pappenheimer bodies, and occasionally as Heinz bodies. The spleen seems to play a significant role in the erythrocytic inclusion body phenomenon. In order to further elucidate the nature of the splenic effect, rabbits given monomethylaniline intravenously were splenectomized when 90 percent of the erythrocytes contained Heinz bodies. In stained scrapings of the splenic pulp, there was an increase in activity of phagocytic clasmatocytes. These phagocytes contained numerous erythrocytes, most of which were the seat of Heinz bodies.

To clarify the effect of splenectomy in man, the blood of 23 splenectomized patients was examined. The erythrocytes of each contained inclusion bodies morphologically indistinguishable from Heinz bodies. The mean number of affected erythrocytes was 21 percent for the group. The inclusion body counts bore no relation to the presence or degree of anemia, to the leukocyte counts or to the reticulocyte counts. The inclusion bodies began to appear in increasing numbers on the second postoperative day, reached a peak within three weeks, and then maintained a

constant level. Age of the patient was not a factor, nor was the basic hematologic condition for which splenectomy was performed. A control group of 19 similar hematologic patients with intact spleens showed no formation of erythrocytic inclusion bodies.

Evidence presented suggests that Heinz body formation may be a normal phenomenon, but that the human spleen removes affected cells from the circulation so rapidly that they are not observed in the peripheral blood except under conditions of severe intoxication. With the present methods of examination, Heinz body enumeration is not considered to be a survey procedure of value in industrial medical practice.

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TABLE I

CHEMICAL WORKERS - WORK HISTORY

Subject No.	Age Years	Months Exposure	Job Classification	Operation
1	35	120	Foreman	(A)
2	28	6	Foreman	(A)
3	50	60	Operator	(A)
4	27	48	Operator	(A)
5	25	36	"	(A)
6	48	7	Helper	(A)
7	26	5	Helper	(A)
8	21	10	"	(A)
9	33	5	"	(A)
10	46	6	"	(A)
11	24	48	Operator	(B)
12	28	48	Operator	(B)
13	29	40	"	(B)
14	21	12	"	(B)
15	28	48	"	(B)
16	26	48	"	(B)
17	30	60	"	(B)
18	33	60	"	(B)
19	24	36	"	(B)
20	43	6	Helper	(C)
21	22	5	Helper	(C)
22	18	7	"	(C)
23	43	2	"	(C)
24	26	2	"	(C)
25	36	3	"	(C)
26	37	36	Operator	(D)
27	22	49	Operator	(D)
28	30	108	"	(D)
29	34	60	Maintainence	(C)
30	24	2	Laboratory	(C)
31	43	120	Foreman	(C)
32	35	120	Foreman	(C)
33	32	120	Foreman	(C)
34	37	108	Supervisor	(C)
35	30	24	Asst. Supervisor	(C)
36	21	7	Helper	(C)

OPERATION A - "7 - Isomer" manufacture (see Appendix)

OPERATION B - Handling all amines
OPERATION C - Handling all substances in the building
OPERATION D - "Roccal" manufacture (see Appendix)

TABLE II

CHEMICAL WORKERS - HISTORY AND PHYSICAL EXAMINATION

Subject No.	Interval since last "blue lip" (months)	Significant Clinical Diagnoses
1	24	Peptic ulcer, dental caries
2	-	Peptic ulcer, tension headaches
3	6	None
4	48	Dental caries
5	-	Obesity
6	-	None
7	-	Dental caries
8	-	None
9	-	Peptic ulcer
10	-	Partial nerve deafness
11	-	Dental caries
12	-	Gingivitis
13	-	Pyorrhea alveolaris, tension headaches
14	-	None
15	1	None
16	-	Chronic maxillary sinusitis
17	-	Bronchial asthma, sinusitis
18	-	None
19	12	Rheumatic heart disease, inactive; pyorrhea
20	-	None
21	-	None
22	-	None
23	-	Chronic alcoholism
24	-	None
25	-	Dental caries, pyorrhea
26	-	Dental caries
27	-	None
28	36	None
29	-	Pyorrhea alveolaris
30	-	Hay fever
31	72	None
32	36	None
33	96	Hypertension, dental caries
34	-	None
35	-	None
36	-	None

TABLE III

CHEMICAL WORKERS - URINALYSIS

Subject No.	Specific Gravity	Albumin	Sugar	Microscopic, Sediment
1	1.018	0	0	0
2	qns	0	0	Rare finely granular cast
3	1.012	0	0	0
4	1.016	0	0	0
5	1.028	0	0	Occasional WBC
6	1.018	0	0	0
7	1.020	0	0	0
8	1.016	0	0	Occasional WBC
9	1.028	0	0	Rare coarsely granular cast
10	1.024	0	0	0
11	1.020	0	0	WBC clumps
12	qns	0	0	0
13	1.026	0	0	0
14	1.008	0	0	0
15	qns	0	0	Rare coarsely granular cast
16	1.018	0	0	Occasional WBC
17	1.018	0	0	0
18	1.006	0	0	0
19	1.006	0	0	Rare coarsely granular cast
20	qns	0	0	Rare WBC
21	1.014	0	0	Rare WBC
22	1.016	0	0	Occasional WBC
23	1.022	0	0	Occasional WBC
24	1.018	0	0	0
25	1.028	0	0	0
26	1.016	0	0	0
27	1.028	0	0	0
28	1.024	0	0	Rare coarsely granular cast
29	1.024	0	0	Occasional WBC
30	1.014	0	0	Occasional WBC
31	1.020	0	0	Rare WBC
32	qns	0	0	0
33	1.022	0	0	Occasional finely granular cast
34	1.016	0	0	Occasional WBC
35	1.026	0	0	0
36	1.010	0	0	Occasional WBC

TABLE IV

CHEMICAL WORKERS - HEMATOLOGY

Sub. No.	WBC per mm^3	Neutrophils	Bands	Lymphocytes	Monocytes	Eosinophils	Basophils
1	13,250	76	1	22	1	0	0
2	8,200	67	0	28	2	2	1
3	5,350	58	1	32	4	4	1
4	12,100	68	0	27	3	1	1
5	10,200	63	1	32	3	0	1
6	9,600	64	0	28	6	1	1
7	7,750	58	2	36	1	1	2
8	7,350	45	1	43	4	3	4
9	9,200	70	1	22	4	2	1
10	7,650	55	1	33	7	3	1
11	7,700	64	0	31	2	0	3
12	9,000	62	1	34	0	1	0
13	6,200	47	1	44	4	2	2
14	10,250	51	0	43	4	2	0
15	4,800	72	0	22	3	2	1
16	11,650	62	0	33	4	1	0
17	12,350	64	1	30	3	1	1
18	14,050	71	1	22	4	2	0
19	9,500	55	0	38	4	3	0
20	9,450	54	0	39	3	3	1
21	7,050	62	1	26	2	3	6
22	12,550	59	0	24	0	15	2
23	9,450	69	2	24	2	1	2
24	12,500	72	0	25	0	3	0
25	11,600	60	0	35	2	0	3
26	9,300	40	2	53	3	1	1
27	8,100	69	1	24	2	2	2
28	7,650	55	0	42	2	0	1
29	13,000	67	0	28	1	3	1
30	7,400	51	0	45	4	0	0
31	12,000	54	0	41	4	1	0
32	11,800	83	0	7	4	5	1
33	9,000	66	1	30	3	0	0
34	13,200	62	0	29	8	0	1
35	9,950	60	0	35	5	0	0
36	9,850	50	1	45	1	1	2

TABLE V

CHEMICAL WORKERS - HEMATOLOGY (CONT.)

Subject No.	Hemoglobin Gm.	Erythrocytes per cu.mm. Millions	Percent Erythrocytes with Inclusion Bodies
1	18	5.60	less than 1
2	15	4.82	less than 1
3	12	4.02	2
4	16	5.10	1
5	14	4.86	1
6	15	5.94	1
7	13	4.27	4
8	13	4.66	less than 1
9	15	4.91	3
10	15	4.78	less than 1
11	15	4.81	3
12	17	5.96	1
13	13	4.44	2
14	16	5.06	1
15	14	5.37	less than 1
16	15	5.60	1
17	15	5.08	less than 1
18	14	4.94	1
19	15	5.45	less than 1
20	14	5.29	2
21	16	5.31	1
22	15	5.42	less than 1
23	15	5.62	less than 1
24	15	5.70	1
25	16	5.52	less than 1
26	16	6.03	less than 1
27	15	5.63	4
28	15	5.57	less than 1
29	14	4.74	2
30	17	6.01	1
31	13	5.17	1
32	14	5.06	less than 1
33	16	5.22	less than 1
34	14	4.84	1
35	18	5.33	1
36	15	5.58	less than 1

TABLE VI

ANALYSIS OF AIR GRAB SAMPLES - ANILINE BUILDING OF A CHEMICAL PLANT

Sample No.	Date	Time	Contaminant	Concentration Parts per Million
1	4-6-51	-	Monomethylaniline	1.25
2	4-6-51	-	Metachloraniline	0.13
3	4-18-51	-	Metachloraniline	0.29
4	4-13-51	-	Aniline	0.34
5	4-14-51	10:05am	Aniline	0.06
6	4-14-51	10:10am	Aniline	1.3
7	4-14-51	10:20am	Aniline	0.27

Note - The mean concentration of the samples is 0.53 p.p.m.

COLLECTION SITES:

SAMPLE No. 1 - At dimethylaniline receivers

SAMPLE No. 2 - In basement near methachloraniline separator

SAMPLE No. 3 - Same as No. 2, but during filling of separator tank.

SAMPLE No. 4 - Same as No. 1, but during process of manufacture of diethylaniline at adjacent aniline still.

SAMPLE No. 5 - At dimethylaniline claves

SAMPLE No. 6 - Same as No. 5, but while steam flushing basement floor below

SAMPLE No. 7 - Same as No. 5, but 10 minutes after flushing

TABLE VII

Splenectomized Patients - Diagnoses

Subject No.	Sex	Age Years	Diagnosis
1	F	63	Monocytic Leukemia
2	F	62	Lymphoid Leukemia
3	F	69	Lymphoid Leukemia
4	M	65	Hemolytic Anemia
5	F	69	Hemolytic Anemia
6	M	31	" "
7	M	4	" "
8	F	3	" "
9	F	6	" "
10	F	34	" "
11	M	3	" "
12	F	40	Thrombocytopenic Purpura
13	F	67	Hemolytic Anemia
14	F	36	Thrombocytopenic Purpura
15	F	23	Neutropenic Leukopenia
16	M	53	Lymphoid Leukemia
17	F	22	Thrombocytopenic Purpura
18	M	20	Traumatic Rupture of Spleen
19	M	1/4	Hemolytic Anemia
20	M	13	Thrombocytopenic Purpura
21	F	14	Thrombocytopenic Purpura
22	M	29	Banti's Disease
23	M	36	Banti's Disease

TABLE VIII

Splenectomized Patients - Hematologic Data

Subject No.	Post-Splenectomy Interval Months	RBC Mill.	Reticulocytes Per cent of RBC's	WBC per mm^3	% Erythrocytes with Inclusion Bodies
1	18	2.98	3.0	263,000	28
2	10	4.27	1.4	6,750	21
3	3	2.60	18.6	5,700	50
4	3	1.89	68	17,950	40
5	216	4.25	2.0	10,200	26
6	108	3.06	15.0	19,500	17
7	8	4.58	-	11,100	35
8	8	4.59	-	10,050	32
9	9	5.05	-	12,600	20
10	63	-	-	-	34
11	13	3.88	-	8,000	27
12	28	4.48	-	10,200	11
13	8	2.55	-	8,500	25
14	1½	3.09	20.2	13,000	28
15	20	3.65	1.8	4,350	10
16	1/3	4.42	-	26,300	3
17	1/6	4.26	-	8,550	5
18	9	5.52	1.2	9,300	10
19	2	3.79	-	19,800	15
20	2/3	3.35	-	21,400	20
21	2	4.62	5.4	9,700	20
22	108	4.52	-	8,700	25
23	4	4.52	-	12,050	12

TABLE IX

Diagnosis - 23 Splenectomized Patients

Diagnosis	No. Cases	Mean % of RBCs with Inclusion Bodies
Primary hypersplenism:		
Congenital hemolytic anemia	8	26
Thrombocytopenic purpura	5	13
Neutropenic leukopenia (splenic)	1	10
Secondary hypersplenism:		
Acquired hemolytic anemia	2	33
Banti's syndrome	2	19
Chronic lymphoid leukemia	3	36
Monocytic leukemia	1	28
Traumatic rupture of spleen	1	10

TABLE X

Age - 23 Splenectomized Patients

Age - Years	No. Cases	Mean % of RBCs with Inclusion Bodies
Under 1	1	15
1 - 10	4	29
11 - 20	3	17
21 - 30	3	13
31 - 40	5	16
41 - 50	0	-
51 - 60	1	3
61 - 70	6	32

TABLE XI

Erythrocyte Counts - 23 Splenectomized Patients

RBC - Millions	No. Cases	Mean % of RBCs with Inclusion Bodies
Under 2.50	1	40
2.51-3.00	3	34
3.01-3.50	3	22
3.51-4.00	3	17
4.01-4.50	5	13
4.51-5.00	6	23
5.01-5.50	1	20
5.51-6.00	1	10

TABLE XII

Leukocyte Counts - 23 Splenectomized Patients

WBC	No. Cases	Mean % of RBCs with Inclusion Bodies
4,000 - 6,000	2	30
6,000 - 8,000	2	24
8,000 - 10,000	5	17
10,000 - 12,000	4	26
12,000 - 14,000	3	20
Over 14,000	7	19

TABLE XIII

Reticulocyte Counts - 23 Splenectomized Patients

Reticulocytes Per 100 RBCs	No. Cases	Mean % of RBCs with Inclusion Bodies
Under 2	5	16
2 - 4	1	28
5 - 10	1	20
11 - 20	2	33
Over 20	2	34

TABLE XIV

Interval Since Splenectomy - 23 Patients

Post Splenectomy Interval - Months	No. Cases	Mean % of RBCs with Inclusion Bodies
Under 1	3	10
1 - 6	7	25
7 - 12	6	24
13 - 18	2	28
19 - 24	1	10
Over 24	4	20

APPENDIX I

METHOD OF STAIN FOR HEINZ BODIES:

The reagents used for the determination of the proportion of erythrocytes containing Heinz bodies are (1) Quick's double oxalate and (2) Crystal violet. The first is made by dissolving 0.75 gram of reagent grade potassium oxalate and 1.25 grams of reagent grade ammonium oxalate in 100 ml. of distilled water. A half gram of crystal violet is dissolved in 5 ml. of 100% methyl alcohol. This solution is poured into a 3 or 4 inch smooth glass mortar and swirled along the sides until the alcohol has completely evaporated. The double oxalate (100ml.) is then added and mixed well with a glass pestle. The solution of oxalate and stain is filtered before each use. There is deterioration of the stain after two weeks.

To 0.1 ml. of blood contained in the cavity of a hanging drop slide a single drop of oxalate-stain reagent is added and mixed thoroughly with a small glass rod. This is covered to prevent evaporation and allowed to stand 10 minutes in order that the cells may be stained while still in suspension in plasma. From this mixture a blood smear is prepared on a clean glass slide. It is allowed to dry, then examined with a microscope, using an oil immersion lens (900 X). Staining is adequate when platelets and the nuclei

of leukocytes appear pale blue. The number of deeply stained granular structures within the cell (Heinz bodies) in 300 red cells are counted. To facilitate counting, a glass disc, divided into about 25 sections, may be placed into each eyepiece of the microscope.

APPENDIX 2

Method of Methemoglobin Determination:

Methemoglobin was determined in laked oxalated blood by spectrophotometric measurement of the change in specific extinction at 634 mμ that follows the addition of potassium cyanide. The total pigment was determined spectrophotometrically at the same wave length after any unaltered hemoglobin in the blood had been converted to methemoglobin by the action of potassium ferricyanide.

The amount of methemoglobin in 0.5 milliliter of blood laked with phosphate and diluted to 25 milliliters was determined by measuring the amount of light transmitted at 634 mμ before and after conversion of the methemoglobin to cyanmethemoglobin.

To determine the total pigment in 0.25 milliliter of blood laked with phosphate and diluted to 25 milliliters, the oxyhemoglobin was first converted to methemoglobin by means of potassium ferricyanide and then to cyanmethemoglobin by the action of potassium cyanide. The absorption of light by the cyanmethemoglobin was measured at 2 wave-lengths, 560 and 575 mμ.

APPENDIX 3

Description of Chemical Plant Building #20 and Processes:

In this building five major manufacturing processes were carried on:

1. Dimethylaniline and diethylaniline.

   The charge consisted of aniline, methanol (or ethanol) and sulfuric acid. After mixing and weighing the charge, it was autoclaved, delivered to separators, steam distilled and drummed. The major methemoglobin-formers were aniline and the two end-products.

2. "7 - Isomer"

   This is a complex chlorinated aromatic compound. The charge consisted of metachloraniline, sodium ethyl oxalacetate, hydrochloric acid and "Dowtherm" (diphenyl and diphenyl oxide). After mixing, the charge was steamed, cooled, passed through a filter press and the products drummed. The major methemoglobin-former was metachloraniline, principally in the form of vapor rising from an incompletely covered separator. The other chemical hazards were the sodium ethyl oxalacetate, a respiratory irritant which required the use of dust respirators in its normal handling with shovels, and Dowtherm, a skin

irritant.

3. Ethyl Benzyl Aniline

The charge consisted of monoethylaniline, diethylaniline and benzyl chloride. After steaming in a Pfaudler it was passed to a separator, then distilled and the end-product drummed. The major methemoglobin-formers were the three aniline compounds. In addition, benzyl chloride had an irritating effect on the nasal mucosa.

4. "Roccal"

This is a complex chlorinated nitrogen compound with a long aliphatic side-chain and a tolyl group. The charge consisted of "Lorol" dimethylamine and benzyl chloride. After steaming in a Pfaudler, it was diluted with water, passed through a filter press and drummed. None of these compounds had been proven to form methemoglobin, but benzyl chloride irritated the nasal mucosa.

5. "Lorol" Dimethylamine

This is an amine with a long aliphatic side-chain. The charge consisted of dimethylamine, "Lorol" chloride (a chlorinated C_{12} compound), and sodium hydroxide. After autoclaving it was passed to a separator, then to a vacuum still, and to storage for use in Process #4 above. None of these

compounds were known to form methemoglobin.

The building was of sheet iron construction and had two floors with a basement at one end. There was a cement base. Other flooring consisted of iron grating. Most of the processes, inclusing all of the potentially hazardous ones, were carried out in closed systems. Ventilation was through windows on three sides of the building and large doors at one end. In addition, there were air vents in the roof. Spills and leaks were promptly attended to. The odor of mixed amines and Dowtherm was quite apparent at all times. Housekeeping and the personal hygiene of the workmen were excellent. There was a satisfactory awareness of the necessity for safe handling practices.

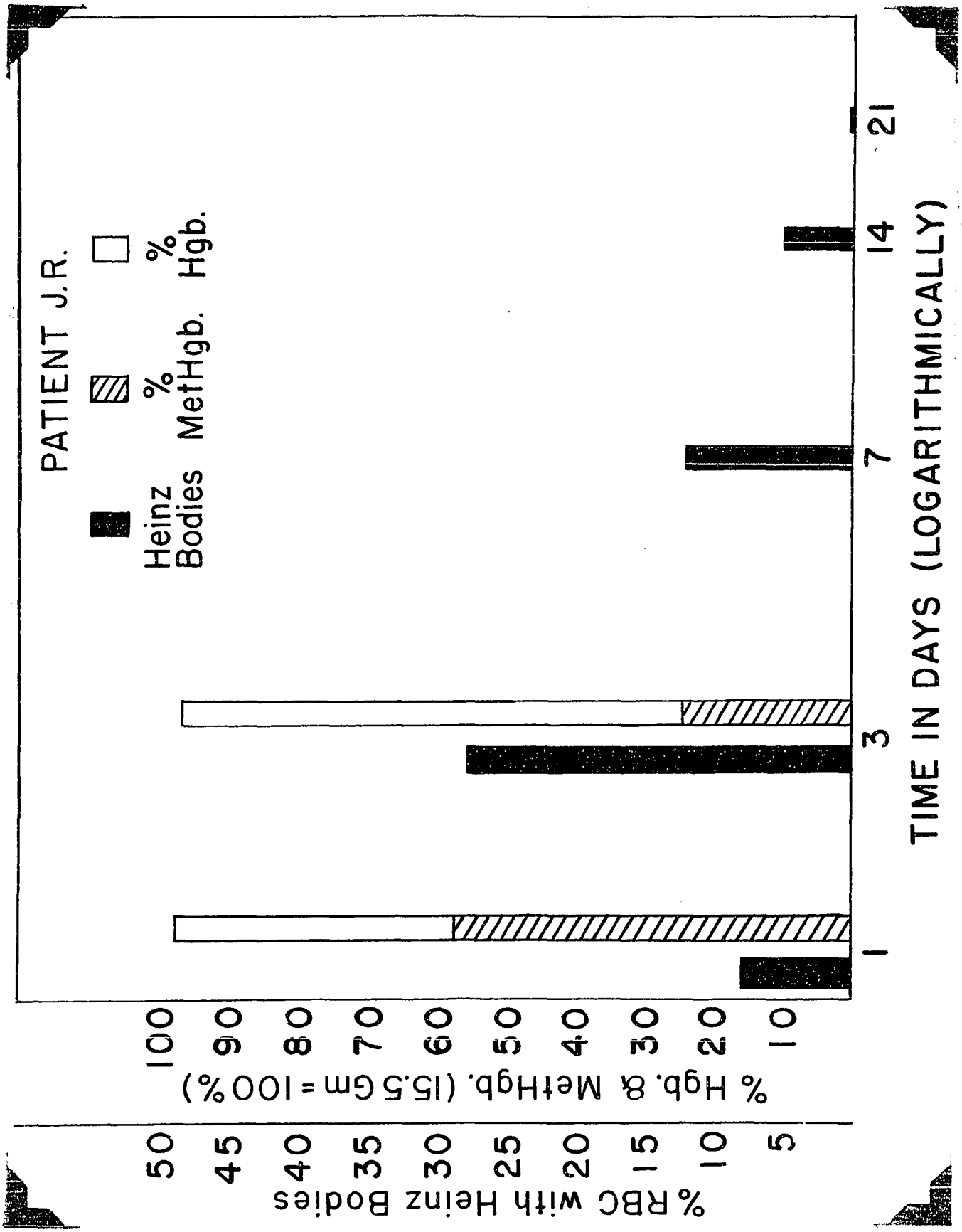

Figure I

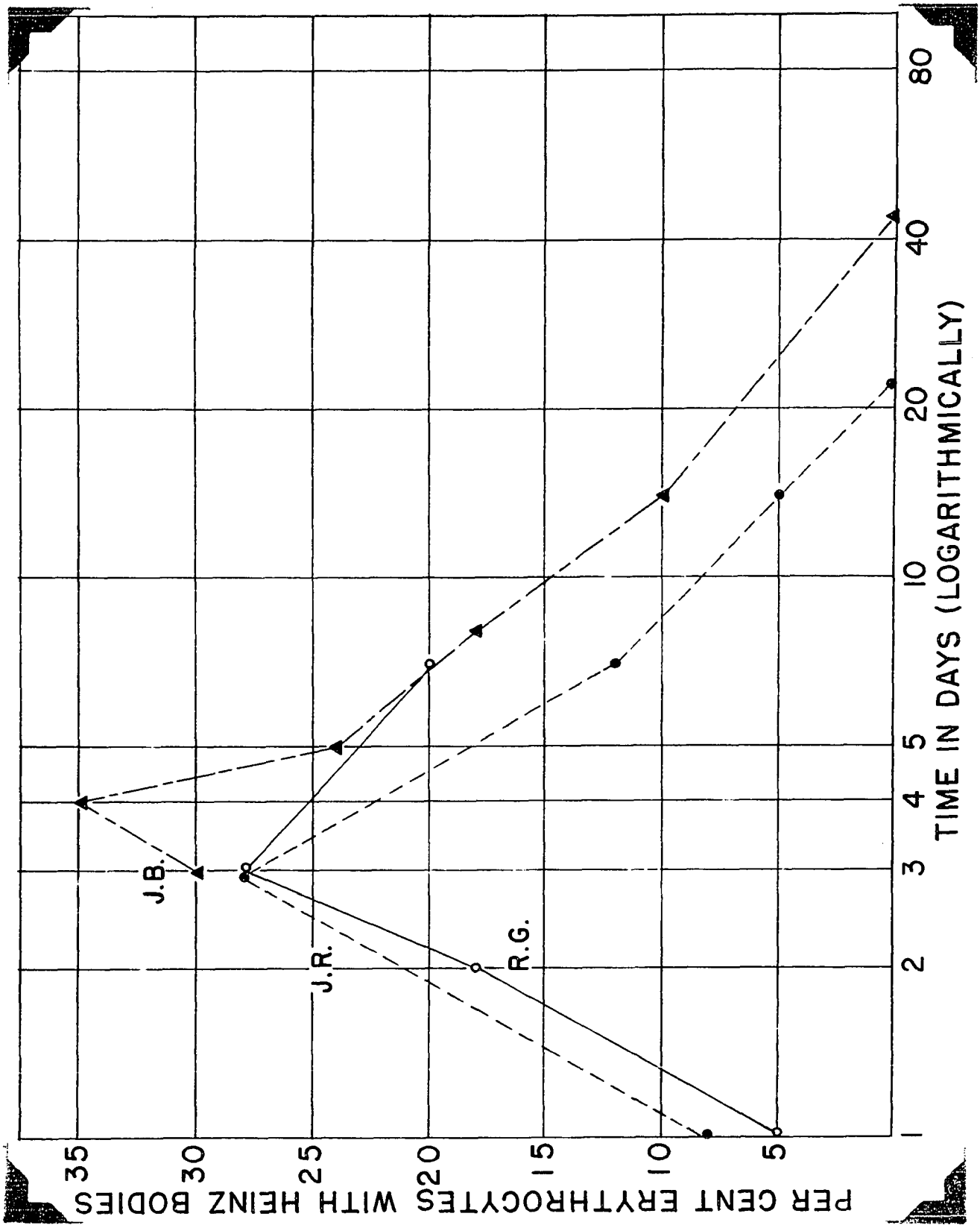

Figure II