Selective Occurrence of Glutathione Instability in Red Blood Corpuscles of the Various Jewish Tribes

By A. Szeinberg, Ch. Sheba and A. Adam

We embarked on the study of hematologic differences between Ashkenazic and non-Ashkenazic Jews when it was certain that only the latter were victims of hemolysis due to known (Vicia faba, sulpha drugs) or unknown (bacterial, viral?) noxious agents. We assumed the existence of a familial inherited trait of the red blood corpuscle, in addition to which a known or unknown trigger mechanism is required to produce hemolysis.1 We did not possess any laboratory tool for confirmation or elucidation of this difference between erythrocytes of the two branches of exiles, those from "Judea capta" 70 A.D. and those from Judea, first destroyed in 586 B.C. by the Babylonians. The enzymatic abnormalities found in Negroes susceptible to hemolysis following administration of primaquine2,3 directed us to the search of these chemical deviations in our patient material. Our search was rewarded by the confirmation of glutathione deficiency and glutathione instability in the erythrocytes of all cases of favism and drug-induced hemolytic anemia.4,6 We also found proof of familial occurrence of these abnormalities, but the material was not large enough to form a final opinion on the genetic pattern of transmission. Meanwhile, important progress has been achieved abroad in the study of the erythrocyte abnormality and its connection with susceptibility to hemolysis in other population groups.

Carson et al. and Schrier et al. discovered two enzymatic abnormalities in the erythrocytes of primaquine-sensitive American Negroes, namely, a deficient glucose-6-phosphate dehydrogenase activity and increased glutathione reductase activity.7,9 Kimbro et al. described cases of hemolytic anemia induced by nitrofurantoin in subjects with instable GSH and proved that, in vitro, this drug has a similar effect to acetyl phenyl hydrazine upon glutathione stability. They reported that random screening of a large group of Negro subjects revealed 7.8 per cent incidence of the intrinsic RBC abnormality with a predominant occurrence in males.10,11 Browne studied the inheritance pattern of this abnormality in American Negroes and suggested transmission by a sex-linked gene of intermediate dominance. Males with the abnormal gene and homozygous females were postulated to be drug sensitive.12

Zinkham and Childs have shown that hemolytic anemia caused by naphthalene occurs foremost in individuals with the same erythrocyte abnormality. They also found that though naphthalene does not influence in vitro GSH stability, its metabolites such as alpha and beta naphthol have an effect similar to acetyl phenyl hydrazine. An additional finding of prime importance

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*Ashkenazic: Western, non-"Oriental"; non-Ashkenazic: Eastern, "Oriental" (includes Spanish, Portuguese, Moroccan, Turkish, Iraqi, etc. groups).
was that glutathione in the erythrocytes of newborns is almost uniformly unstable to acetyl phenyl hydrazine, and that a connection exists between this instability and their susceptibility to hemolysis by vitamin K substitutes in the newborn. The same authors have shown an in vitro effect of vitamin K on the GSH stability in the erythrocytes similar to that of acetyl phenyl hydrazine. A similar activity in vitro of phenyl hydrazine hydrochloride, ascorbic acid, primaquine diphosphate, aniline and hydroxylamine has been described by Beutler.

In Italy, interest has been centered mainly around subjects of Sardinian origin sensitive to Vicia faba. Sansone and Segni found low GSH values and its instability in subjects sensitive to fava beans, with intermediate degrees of instability in the families of affected cases. Panizon and Pujatti described the appearance of Heinz bodies in cases of favism and thus found an additional link between this condition and drug-induced hemolytic anemia. A peculiar and characteristic microscopic appearance of erythrocytes during the acute stage of favism has been described by Sansone, and is reminiscent of the changes seen during hemolysis caused by Naphtalene or PAS—ascorbic acid and vitamin K—induced hemolysis in newborns.

Simultaneously with these developments elsewhere, we decided on a survey of our own population with the hope of obtaining enough information on:

1. The selective occurrence of the hemolytic reactions of the type concerned, along with enzymatic abnormality in the red blood cells in the non-Ashkenazic population only.
2. The tribal subdivision of the non-Ashkenazic group in relation to glutathione instability.
3. The usefulness of these methods of investigation as a tool for anthropologic screening of migratory populations of the eastern Mediterranean shore and the Mediterranean at large.
4. The genetic pattern of transmission of the enzymatic abnormalities in the erythrocytes.

The present communication is a report on the results of this study.

**METHODS**

1. GSH estimation was performed by the method of Grunert and Philips modified by Beutler.
2. The glutathione stability test was carried out with 5 mg. of APH and an incubation period of two hours at 37 C. Fresh blood (not later than two hours after its collection) was always used.
3. GSH stability test with additional glucose was performed on samples to which 0.1 cc. 4 per cent glucose per 1 cc. blood was added a short time after its collection.
4. The examination of the glucose 6 phosphate dehydrogenase activity was performed using the method of Carson et al. The details of the technic were published by us elsewhere.

*The following abbreviations are used in this communication: GSH—reduced glutathione; APH—acetylphenylhydrazine; G6P—glucose 6 phosphate; 6PG—6 phospho-gluconate.
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MATERIAL

Blood of 288 Ashkenazic and 279 non-Ashkenazic subjects, selected at random, forms the material for the present investigation. (The Ashkenazic is the light-complexioned Jew originating from Eastern, Central or Western Europe, and the non-Ashkenazic usually is the dark-complexioned Jew of Oriental or Mediterranean origin.) The chances of misplacement of subjects were negligible, as until now the distinction between the two groups is very clear. The birth place of the subjects was considered as the country of origin, except for those born in Israel, in whose case the parents (or sometimes an earlier generation) served as an index of the origin. All age groups were represented; the subjects were either hospital patients (blood and liver diseases completely excluded) or healthy blood donors. For the genetic part of the study we examined families of accidentally-discovered cases of glutathione instability as well as families of patients with hemolysis associated with GSH abnormalities. Altogether 25 families with 58 offsprings were examined.

Survey of GSH Stability in Erythrocytes of a Random Sample of Population

Results of the GSH stability tests are presented in figures 1 and 2 and summarized in table 1. In this table (and throughout this communication) the final GSH level after incubation with APH served as the criterion of GSH stability, 30 mg./100 cc. being considered the dividing line between the stable and unstable sample. Only cases in which the GSH level fell below 30 mg./100 cc. RBC in the stability test performed on blood to which glucose was added were considered sensitive.

We summarize the results as follows: NO case with instable GSH was found among the Ashkenazic subjects investigated. In the non-Ashkenazic group 36 cases (11.7 per cent) with sensitive GSH were found. Males and females showed a similar frequency of the abnormal finding. When the non-Ashkenazic group was subdivided according to the country of origin it became evident that it was not homogenous. A very high frequency (about 20 per cent) of the abnormality was encountered among persons originating from Iraq (Babylonia), while Yemenite and North African communities showed a considerably lower percentage of sensitive persons (about 5 per cent). Cases with unstable GSH were also found among non-Ashkenazic subjects originating from other regions, but the small numbers precluded even preliminary conclusions about the relative frequency of the abnormality among them. The same applies to Arabs in Israel. Twenty-seven were so far investigated and one woman with unstable GSH was found.

Genetic Investigation

Evidence of familial occurrence of the GSH abnormality based on the study of families of persons with a clinical history of hemolysis were presented in an earlier publication.4

The material for this part of the investigation consists of 11 families (with a total of 30 children) of subjects in which the GSH abnormality was detected by chance during the present survey and of 14 families with 28 chil-

*Throughout this communication subjects with GSH sensitive to destruction by APH are referred to as "sensitive persons" or "persons with unstable glutathione."
In preparation of the material for genetic analysis the necessity of a rigid definition of normal and abnormal levels became obvious at once. Such definition could not be based on low GSH level in fresh erythrocytes, since...
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TABLE 1.—GSH Stability in Various Population Groups Studied

<table>
<thead>
<tr>
<th></th>
<th>Number examined</th>
<th>% of sensitive</th>
<th></th>
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</thead>
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<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Ashkenazic</td>
<td>203</td>
<td>85</td>
<td>288</td>
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<tr>
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<td>173</td>
<td>133</td>
<td>308</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Subgroups of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Ashkenazic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iraq</td>
<td>72</td>
<td>52</td>
<td>124</td>
<td>15</td>
<td>12</td>
</tr>
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<td>42</td>
<td>30</td>
<td>72</td>
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</tr>
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<td>1</td>
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<tr>
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<td>2</td>
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<td>3</td>
<td>—</td>
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<td>2</td>
<td>—</td>
<td>—</td>
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<td>3</td>
<td>9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
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<td>3</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Greece/Buchara</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Unknown</td>
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<td>1</td>
<td>3</td>
<td>—</td>
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</tr>
</tbody>
</table>

such levels were also encountered among Ashkenazic subjects which do not belong to the drug or favism-sensitive population. (No case of hemolytic anemia, due to drugs or fava bean ingestion has ever been brought to our attention during 20 years of search for its occurrence.) On the other hand the GSH stability test (performed with addition of glucose) was well suited for classification purposes if the dividing line between the normal and sensitive subjects was set at 30 mg./100 cc. RBC. Not a single Ashkenazic was found to have GSH level below 30 mg per cent following incubation with APH.

The frequency of the GSH instability tabulated according to sex of the subjects is summarized in table 2. Some affected families are presented in figures 3 and 4.

Although the material is still limited it suggests the following: (a) In the affected families about 75 per cent of the children show GSH instability even though in the majority of them only one of the parents was shown to be affected. This finding suggests a dominant character of heredity. Supporting evidence can be seen in two cases of sensitive children in families where only one of the parents was non-Ashkenazic, the other being Ashkenazic (fig. 3, families 1 and 3). (b) In some families with affected children both parents had normal GSH stability (fig. 3, families 4 and 5, and fig. 4, families 3 and 4), indicating an incomplete penetrance of the gene in these cases. It is noteworthy that up to now all sensitive children found in such families have been males. (c) When the sex incidence of the GSH instability was compared without regard to its degree (i.e., all cases under 30 mg./100 cc. considered equally sensitive) no difference was apparent between the two sexes (table 2). When the sensitive cases were subdivided into three groups, according to the final level of GSH after incubation with APH, a difference between sexes became evident (table 3). The majority of the males belonged
to the group with the lowest GSH values, while the females were equally divided among the three groups. This finding considered in conjunction with the fact that up to now no sensitive males were found in families, where the abnormality was found in fathers only (fig. 3, family 6, and fig. 4 families 1, 6), leads to the following preliminary hypothesis: *The abnormal trait is sex-
linked with incomplete dominance, the gene being located on the X chromosome. The expressivity of this gene in males is stronger than in females, in which the whole spectrum from 100 per cent to no penetrance is being encountered. Our material does not show a greater incidence of abnormality in females, expected in the case of a sex-linked completely dominant trait (because of the occurrence of female cases with no penetrance of this incompletely dominant gene). This hypothesis is supported by the results of the glucose-6-phosphate dehydrogenase activity in the same patient material as reported by us elsewhere.\(^2^2\) We found that in all the males with instable GSH the glucose-6-phosphate dehydrogenase activity was markedly reduced, while in females various degrees of the enzyme activity could be found (from high expressivity to lack of penetrance). The material is still limited and the validity of this hypothesis is being investigated on a larger scale.

As to the ethnographic significance of our findings, suffice it to state at this point, the Iraqi Jews, among whom the highest rate of glutathione instability was found, are the only ones who have the right to claim direct descendence from those Judeans (an estimate of 30,000 families) who were driven by Nebuchadnezzar from Judea to Babylon and who remained there in spite of the restoration of Jerusalem by Cyrus in the year 538 B.C.\(^2^3\) They were an independent, flourishing community, anxious to avoid intermarriage with the indigenous population\(^2^4\) and even journeyed to Jerusalem (458 B.C.) to campaign against such intermarriage of the returning exiles with local non-Judean elements.\(^2^5\) The Yemenites and the North African Jews were not so sheltered and the Ashkenazy Jews emerged 700 years later as descendants chiefly of such Judeans who were deported by the Romans into Italy (following 70 A.D.) as prisoners of war, without a female population of their own. One will notice that 650 years, indeed very tumultuous ones, divided the two exile groups. This will be discussed in a future report.
DISCUSSION

The significance of glutathione abnormalities in the pathogenesis of hemolytic anemias in certain communities have been proved up to now in the American Negro population, in Sardinians and other Islanders (of possibly Phoenician stock as against the non-Phoenician peninsula inhabitants) in Italy and in the non-Ashkenazic Jewish population of Israel. Cases of hemolysis due to primaquine, sulpha drugs, paramino-salicylic acid, naphtalene, furadantin and fava beans have been described in persons with glutathione instability. Moreover there are indications that additional agents are implicated in precipitating a hemolytic crisis in such subjects. Cases of hemolysis following infectious disease (typhoid, viral infections, even the present "Asian Flu" epidemic) were encountered in this country in the non-Ashkenazic population. The information on the occurrence of these hemolytic episodes is still very incomplete. The mechanism of hemolysis associated with glutathione instability and glucose-6-phosphate dehydrogenase deficiency awaits final elucidation, as no hemolysis could be produced on incubation of sensitive erythrocytes with any of the precipitating agents. It is also not even certain that the mechanism is identical in all cases. Beutler and al. observed hemolysis in all sensitive subjects treated with primaquine. Vullo and Panizon, who transfused normal subjects with Cr\textsuperscript{51}-tagged blood of sensitive ones and who a week later fed the recipients with fava beans and studied the destruction of the transfused erythrocytes, reported the following results: in 6 out of 10 transfused subjects no increased destruction was observed. In two recipients destruction was evident already before ingestion of the beans, while in the remaining two hemolysis followed the ingestion of beans. Some subjects in whom we established GSH instability claimed to have eaten fava beans on some occasions without visible untoward effect. We have on record a woman with unstable GSH and a proved abnormality of glucose-6-phosphate dehydrogenase activity, who developed an extremely severe hemolytic anemia following sulpha drugs and who claimed to have eaten fava beans without any visible untoward effect.

The racial distribution of the abnormality seems of interest from the genetic point of view. In the U.S.A. it is evidently encountered only (or predominantly) among Negroes; in Italy, mainly in Sardinians, while in Sicilians, while in the Jewish population of Israel, only in persons of oriental or Mediterranean origin. Even in this non-Ashkenazic population it is not evenly distributed, and is especially prevalent among persons of Iraqi (Babylonian) origin. This special distribution raises, as we pointed out already, interesting ethnographic problems (Babylonians in relation to ancient inhabitants of Malta, Sardinia and Sicily). The mechanism of the hereditary transmission seems to be similar in all the investigated groups.

The similarity of the chemical defect in the different groups seems to be evident too. GSH instability has been reported in all the national groups studied. Our findings of glucose-6-phosphate dehydrogenase deficiency are in line with those of Carson et al. and Beutler et al. There seems to be a parallel between the biochemical abnormality and the severity of the
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Sartori reported a preponderance of males among the favism patients hospitalized in Sardinia, but found that this sex difference was caused by the fact that among females many cases appeared with only mild symptoms not requiring admission to a hospital. A similar possibility may exist in Israel where, during the last 7 years, 28 male patients and only 12 females were admitted to our hospital on account of favism or drug-induced hemolytic anemia.

SUMMARY
1. Experience with GSH stability test performed on blood samples to which glucose has been added is summarized and the procedure is recommended for routine use for the detection of sensitive subjects.
2. Glutathione stability of erythrocytes has been studied in various population groups of Israel. No case with instable GSH has been found among Jewish subjects originating from Eastern, Central or Western Europe. Instability of GSH was however found in about 20% of subjects originating from Iraq and about 5% of subjects originating from Yemen or North Africa. Isolated cases of this abnormality were also discovered among a small number of persons from other Oriental or Mediterranean countries as well as among the Arab inhabitants of Israel.
3. The genetic analysis points to a transmission of glutathione instability by a sex-linked, incompletely dominant gene with variable expressivity.
4. Variable expressivity of glucose-6-phosphate dehydrogenase activity has also been detected in the defective erythrocytes.

SUMMARIO IN INTERLINGUA
1. Es summarisate experientias con tests del stabilitate de glutathionia reducite, effectuate in specimens de sanguine a que glucosa habeva essite addite. Le technica es recommendate a! uso routinari pro le detection de individuos sensibile.
2. Le stabilitate de glutathionia del erythrocytos esseva studiate in varie gruppus del population de Israel. Esseva trovate nulle caso de instabilitate de glutathionia reducite in subjectos judee de origines in Europa occidental, central, o oriental. Del altere latere, instabilitate de glutathionia esseva constatate in circa 20 pro cento del subjectos originari de Irak e in circa 5 pro cento del subjectos originari de Yemen o Nord-Africa. Casos isolate de iste anormalitate esseva etiam discoperite in personas originari de paises oriental o mediterranee e in plus inter le habitantes arabe de Israel.
3. Le analyse genetic pare indicar que le transmission de instabilitate glutathionic se face per un gen que es sexualmente specific, incompletemente dominante, e de expressivitate variabile.
4. Expressivitate variabile de activitate de dishydrogenase glucosa-6-phosphatic esseva etiam detegite in le defective erythrocytos.

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