such a marked improvement in our material facilities as the past four years. A review of the major items is instructive. They include the completion of the rebuilding of the main display conservatory; the fencing of the garden; extensive rearrangement and construction in the museum and administration building, including new wash-rooms, a members' room and the bricking up of the end walls; the construction of a new root cellar and coldframes, the extension of the Boulder Bridge; the construction of a new roadway and numerous paths, the rehabilitation of the Lorillard stables as a service building, the installation of a heating plant in the propagating houses; reconstruction of the interior of House No. 1 in Range 1; the building of a walled experimental garden and of a series of cold pits, and the resurfacing of the paths around Range 2. Some of these items were included in the agreement with the city made on the exchange of land. For all of them the garden is indebted, through the cooperation of the Park Department of the City of New York, to the City of New York and to the Works Progress Administration or its successor, the Work Projects Administration.

Not only have marked improvements been made in our buildings, roads and fences, but notable horticultural accomplishments for this period should be recorded. Exhibits were resumed at the International Flower Show, resulting in striking displays of begonias in 1939, of ferns in 1940 and of the plants of the Bible in 1941. We took part in the New York World's Fair. We have replanted and rearranged in attractive naturalistic form much of the display material in the main conservatories. We reinstituted the annual flower shows in the conservatories from November to May. We have more than doubled the number of our accessioned species and varieties of hardy trees and shrubs, and have increased the species and varieties of plants in cultivation at The New York Botanical Garden to more than 12,000. We have initiated and developed a program of education in gardening which now includes more than 500 adults annually.

Public interest in the garden is a difficult thing to measure. We have no means of recording our attendance, which might be used as one standard of public interest. We have not kept a statistical record of the inquiries from individuals, groups and business firms, which could be expressed in figures. From other sources, however, on which figures can be cited, it would appear that public interest has increased, and that the garden is performing more and better service than ever before. For example, subscriptions to the *Journal* were 75 in 1938, and 665 in 1941. This is in addition to the distribution made to members as part of their membership fee. Attendance at the Saturday afternoon lectures was 3,040 in 1937, and 7,000 in 1941, and attendance in the gardening courses increased fourfold between 1938 and 1941.

During this period in which the material facilities and the activities of the garden were increasing, it is particularly gratifying to report that our capital funds were so managed that they suffered no decrease. This is a noteworthy accomplishment on the part of our finance committee. Few institutions which depend in whole or in part on endowment can point, as the New York Botanical Garden can, to an endowment unimpaired from the original receipt of the gifts and bequests, and to a budget which is balanced annually and shows no deficit.

One of the outstanding events during the period under review was the completion of arrangements for a new base plan for the future development of the garden. Changes in the limits of the garden and its fencing in 1940 made necessary a reconsideration of traffic and landscaping. Major Gilmore Clarke has been retained by the board of managers to prepare such a plan and considerable progress has already been made. The preparation of this plan was made possible by the generosity of Mrs. Harold I. Pratt.

On the debit side must be recorded the loss of the services of a number of members of the staff: by retirement Mr. Robert S. Williams, Mr. Percy Wilson and Dr. J. H. Barnhart, and by resignation Dr. A. C. Smith. With the exception of Dr. Barnhart, none of these men has been replaced with new appointments. Furthermore, the garden has lost many of its most loyal friends by death.

SPECIAL ARTICLES

SULFHEMOGLOBIN FORMATION AND LABILE IRON IN VITRO AND IN VIVO

THE indispensability of oxygen in the formation of sulfhemoglobin from hemoglobin in the presence of sulfide ion has been explained[1] by postulating hydrogen peroxide from the autoxidation of hydrogen sulfide[2, 3] as the reactant. Other authors have concluded independently, either on the same basis[4] or from different premises,[5] that hydrogen peroxide has a significant function in sulfhemoglobin formation.

[1] G. Barkan and O. Schales, *Z. physiol. Chem.*, 253: 83–104, 1938.

[2] O. Schales, *Ber. chem.*, 71: 447–460, 1938.

[3] C. Henze, *Klin. Woch.*, 17: 24, 1938.

[4] R. Lemberg, *The Australian Chemistry Institute Journal and Proceedings*, 6: 170–180, 1939.

[5] H. O. Michel, *Jour. Biol. Chem.*, 126: 323–348, 1938.

On the basis of its manner of formation, its green color and its absorption spectrum, sulfhemoglobin was considered by Barkan and Schales[1] as possibly belonging in the class of open-ring derivatives of hemoglobin. Lemberg[4] considered it ". . . probable that the porphyrin ring of sulphaemoglobin is opened, but in such a way that by the action of alkali it is closed again; . . ." Jung[6] and Heubner[7] recently classified sulfhemoglobin as belonging to the group of iron-containing bile pigment compounds for which they use the general term "verdohemochromogen," a name previously introduced by Lemberg[8] for a well-defined open-ring heme derivative. Since in such open-ring derivatives the iron is "easily split off" or labile by the action of dilute acid,[9] an increase in the labile iron might be expected to accompany the conversion of hemoglobin to sulfhemoglobin; such an increase has been observed in experiments *in vitro*.[10] In those experiments the hemoglobin solutions had been treated with H_2S and oxygen for several hours, the minimum period being two hours. Secondary alterations and side reactions may have occurred during such length of time, giving rise to the formation of labile iron quite independent of sulfhemoglobin itself.

Formation of sulfhemoglobin and an increase in the labile iron can now be demonstrated after much shorter periods of treatment. Table I shows typical results as obtained with blood of different sources. It can be seen that admission of H_2S for only a few minutes results in a definite increase in the labile iron fraction.

TABLE I

EFFECT OF SHORT TREATMENT WITH H_2S UPON SULFHEMOGLOBIN FORMATION* AND LABILE IRON

No.	Source of blood	Duration of H_2S-flow	Labile iron†	
			Before	After
		minutes	mg per 100 ml of blood	
1	man	1	1.28	2.19
2	man	2	1.05	4.26
3	calf	3	1.68	5.32
4	rabbit	5	1.12	4.46
5	rabbit	5	1.54	3.43

* Sulfhemoglobin was spectroscopically identified in all experiments after H_2S-treatment.

† For the iron determinations in this paper either Barkan's (*Klin. Woch.*, 16: 300, 1937) or Barkan and Walker's (*Jour. Biol. Chem.*, 135: 37, 1940) method was used; care was taken that the preliminary digestion with diluted hydrochloric acid took place under identical conditions in the normal and in the sulfhemoglobin containing blood solutions.

An inhibitory action of carbon monoxide on the lability of the additional iron had been missed in the previous experiments of much longer duration,[10] although the characteristic spectroscopic change occurred, described long ago by Clarke and Hurtley.[11] In the short-time experiments, such an inhibition could occasionally be found.

In a series of experiments with human blood, the amount of sulfhemoglobin formed by a few minutes' treatment with hydrogen sulfide (in presence of oxygen) *in vitro* was compared with the increase in labile iron (see Table II). Only a third or less of the iron calculated from sulfhemoglobin present was found to be labile. It was assumed for the purpose of calculation that the iron content of sulfhemoglobin is the same as that of hemoglobin. This assumption agrees with Haurowitz's[12] analytical figures.

TABLE II

RELATION OF INCREASE IN LABILE IRON TO SULFHEMOGLOBIN CONTENT ON SHORT TREATMENT WITH H_2S OF HUMAN BLOOD *in vitro*

No.	SHb*	Increase in labile iron		Found/ calculated
		Calculated from SHb	Found	
	grams per 100 ml of blood	mg per 100 ml of blood		per cent.
1	1.34	4.56	0.9	19.7
2	4.75	16.15	3.37	20.9
3	4.27	14.52	3.21	22.1
4	4.66	15.85	3.64	23.0
5	0.26	0.88	0.29	32.8
6	0.68	2.31	0.34	14.7
7	3.33	11.32	2.82	24.9
8	0.64	2.18	0.42	19.3
			Average:	22.2

* Quantitative sulfhemoglobin determinations were made photoelectrometrically according to K. A. Evelyn and H. T. Malloy (*Jour. Biol. Chem.*, 126: 655, 1938).

Sulfhemoglobinemia in rabbits was induced, following the procedure of Hijmans van den Bergh and Engelkes,[13] by the daily administration of two grams of precipitated sulfur dispersed in water with gum arabic, using a stomach tube. The dose was repeated three to seven times. After a total administration of six or eight grams of sulfur, sulfhemoglobin was spectroscopically demonstrable in the blood, increasing with continuation of the feeding of sulfur (quantitative SHb determination as in Table II, footnote). The labile iron was determined both before the sulfur administration had been started and after sulfhemoglobinemia was established. In four rabbits studied, demonstrable enterogenous sulfhemoglobinemia was not accompanied by an increase in the labile iron. This fact sets it off sharply as an entity quite different from the "sulfhemoglobinemia" produced by exposure of blood *in vitro* to hydrogen sulfide and oxygen. The amount of sulfhemoglobin usually found in such experiments was in the neighborhood of 0.3 grams per ml of blood. Assuming an iron content of sulfhemoglobin of 0.34 per cent., the 0.3 grams of sulfhemo-

[6] F. Jung, *Arch. exp. Path. und Pharmakol.*, 194: 16–30, 1939.

[7] W. Heubner, *Ergebn. d. Physiol.*, 43: 9–56, 1940.

[8] R. Lemberg, *Biochem. Jour.*, 29: 1322–1336, 1935.

[9] G. Barkan and O. Schales, *Z. physiol. Chem.*, 248: 96–116, 1937.

[10] G. Barkan and O. Schales, *Z. physiol. Chem.*, 254: 241–249, 1938.

[11] T. W. Clarke and W. H. Hurtley, *Jour. Physiol.*, 36: 62–67, 1907.

[12] F. Haurowitz, *Z. physiol. Chem.*, 151: 130–144, 1926.

[13] A. A. Hijmans van den Bergh and H. Engelkes, *Klin. Woch.*, 1: 1930–1933, 1922.

globin would correspond to about one milligram of iron. This amount of iron and even a fraction of it would be easily measurable by the method used, if it were in a labile, open-ring compound.

DISCUSSION

In 1939 Lemberg and his associates[14] confirmed "that the iron of sulphemoglobin can be easily detached," but they pointed out that sulfhemoglobin ". . . cannot be a bile pigment-haematin compound." From the present study it becomes perfectly clear that the iron in sulfhemoglobin as it occurs *in vivo,* at least during enterogeneous sulfhemoglobinemia in rabbits, can not be a part of an open-ring compound, since there is no increase whatsoever in labile iron coincident with the occurrence of sulfhemoglobin in the blood.

It is equally clear that the preparation of sulfhemoglobin *in vitro,* even in experiments of very short H_2S-treatment, is accompanied by an increase of the labile iron, in 8 experiments with human blood averaging about 22 per cent. of the calculated sulfhemoglobin iron.

The question which remains undecided is whether in short-time experiments the substance or substances with labile iron are independent compounds or rather intermediates in the formation of sulfhemoglobin. These may be formed by the well-known action upon heme of nascent hydrogen peroxide originating from oxidation of H_2S.[2, 3] The conversion of the presumptive precursor with labile iron into sulfhemoglobin with firm iron may well be by the entry of sulfur into the molecule.[5, 14, 15] The findings of the present study, together with the data in the literature[5, 12, 14, 15] make it most probable that sulfhemoglobin in its final stable form, as best exemplified when it is produced *in vivo,* is a substance with closed ring. If the ring be opened in a first step reaction, it seems highly reasonable that the reclosure is accomplished by a sulfur-containing group; no direct proof of this last suggestion is available so far.[16]

GEORG BARKAN[17]
BURNHAM S. WALKER

THE EVANS MEMORIAL, MASSACHUSETTS MEMORIAL HOSPITALS AND THE DEPARTMENT OF BIOCHEMISTRY, BOSTON UNIVERSITY SCHOOL OF MEDICINE

[14] R. Lemberg, J. W. Legge and W. H. Lockwood, *Biochem. Jour.,* 33: 754–758, 1939.

[15] F. Haurowitz, *Jour. Biol. Chem.,* 137: 771–781, 1941.

[16] This work, assisted by a grant from the *Fonds d'Etudes "Roche,"* was begun in the Institut de Physiologie de l'Université de Lausanne, Switzerland. One of the authors (G.B.) wishes to express his gratitude to Professor A. Fleisch, head of the named institute, for his friendly hospitality and helpful cooperation during the summer of 1938.

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SKIN SENSITIVITY TO HUMAN PLASMA

A RECENT report of a reaction to the transfusion of human plasma[1] contradicts the published opinion[2] that the intravenous administration of plasma is entirely innocuous. Our own experience with group-specific plasma indicates that reactions do occur. We have employed a number of experimental methods to ascertain the cause of these reactions. One of these, skin testing with plasma, has yielded some new and interesting information.

The plasma used in these experiments was syphoned off freshly drawn blood. The erythrocytes were allowed to settle by sedimentation for from 24 to 72 hours at 4–8° C. before the plasma was removed. The tests were made by injecting 0.05 cc of undiluted plasma intradermally on the volar surface of the forearm. The sites of injection were observed after 10, 30 and 60 minutes.

Positive reactions were indicated by the formation of a wheal 0.8–3 cm in diameter, plus a surrounding zone of erythema. Erythema alone was found to be of no significance. The wheal usually appeared in 10 minutes, reached a maximum in 30 minutes and began to fade at the end of one hour. Citrate and sulfanilamide as used in the preservative and anticoagulant solution yielded uniformly negative skin tests. Of 109 patients tested with the plasma from all blood groups, 20 per cent. were positive to one or more plasmas. Sensitivity to the various plasmas was not confined to individuals of any particular blood group. The positive reactions were not associated with any specific plasma, since the same specimen gave negative and positive results in different individuals. We were able to transfer passively the plasma sensitivity to non-sensitive individuals.

It was possible to transfuse nine of the sensitive patients. Seven of these gave a reaction to the intravenous administration of the same plasma giving a positive skin test. Reactions encountered in the sensitive individuals included headache, dyspnea, epigastric distress, chills, fever and urticaria. No fatal reactions occurred. Plasmas giving negative skin tests have not produced any transfusion reactions.

There are three likely causes for the positive skin tests: allergins, iso-antibodies or A and B substance in the plasma. Schiff[3] has demonstrated the presence of the A factor in serum. We have confirmed the work of Aubert and his coworkers[4] in establishing the presence of A and B substance in plasma. Of the three causes of positive skin tests, it is our belief that

[1] *Jour. Am. Med. Asn.,* 118: 1050, 1942.

[2] *Ann. of Surgery,* 111: 623, 1940.

[3] *Klin. Wschr.,* 3: 679, 1924.

[4] *Jour. Path. and Bact.,* 54: 89, 1942.