greater effect that may be reversed by at least three mechanisms known to be capable of reducing virus activity. These same mechanisms are without effect when tested against nonhepatitis serum, normal gamma globulin, or antiserum prepared with the test erythrocytes.

The sera from convalescents which were effective in reducing the effect of the hepatitis sera showed clear specificity, although we have not yet found a serum capable of reversing the IH serum effect.

It is also clear that at least the higher agglutination titers could not be accounted for by any of the major Rh blood types. This agrees with an early observation of Wiener (*3*) that human Rh negative sera capable of reacting with Rh human erythrocytes were ineffective against rhesus red cells.

It is possible, therefore, to account for the high hemagglutination titers as a virus effect and to remain consistent with the few known characteristics of the hepatitis viruses (*4*).

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References and Notes

1. R. E. Hoyt and L. M. Morrison, *Proc. Soc. Exptl. Biol. Med.* 93, 547 (1956).
2. F. W. Hartman, G. A. LoGrippo, A. R. Kelley, *Am. J. Clin. Pathol.* 24, 339 (1954).
3. A. Wiener, *Rh-Hr Blood Types* (Grune and Stratton, New York, 1954).
4. This research was supported in part by the U.S. Air Force under contract No. AF 41(657)-46, monitored by the School of Aviation Medicine, Randolph Field, Tex.

12 August 1957

Sulfa Compounds in Reversible Inhibition of Sperm Metabolism by Carbon Dioxide

A recent report from our laboratory has presented evidence of reversible inhibition at 37°C of motility and glycolysis of bovine spermatozoa by anaerobiosis and relatively high tensions of CO_2 (*1*). The potential for maintenance of fertility of spermatozoa inhibited by CO_2 during storage for 1 week under the rigors of normal room temperature has been demonstrated (*2*).

The Illini variable-temperature diluent (IVT) used in the studies of fertility contained 20.0 g of sodium citrate dihydrate, 2.1 g of sodium bicarbonate, 0.4 g of potassium chloride, 3.0 g of sulfanilamide, 1 million units of penicillin G, and 1.0 g of dihydrostreptomycin sulfate per liter. The sulfanilamide, penicillin, and streptomycin were first added to this diluent primarily as antibacterial agents to prevent the rapid bacterial growth that otherwise occurs during storage of bull semen at room temperature (*3*). In addition to its bacteriostatic effect in diluted semen, sulfanilamide has been known for more than 10 years to inhibit respiration of spermatozoa at 37°C and to inhibit aerobic utilization of carbohydrate by bull semen that is stored in yolk-citrate at 5°C (*4*). Sulfanilamide improved the fertility of semen used routinely for artificial insemination of cattle (*5*). Thus, sulfanilamide has been an ingredient of most diluents used for storage of semen for several years. However, only recently has it been found that sulfanilamide also has a supplementary effect on the inhibition of anaerobic glycolysis brought about by relatively high levels of CO_2 in N_2. Neither penicillin nor streptomycin exerts such a marked inhibitory effect.

Table 1. Carbon dioxide evolved per 10^8 spermatozoa from bicarbonate during 4 hours at 37°C in Illini variable temperature diluent (IVT) supplemented with 0.02*M* sulfanilamide or 0.001*M* Diamox in N_2 plus CO_2 and CO_2 only.

Carbon dioxide (%)	IVT only (μl)	IVT and sulfa (μl)	IVT and Diamox (μl)
5	97	50	49
50	30	13	13
100	14	8	6

The function of sulfanilamide as a competitive inhibitor of *p*-aminobenzoic acid is recognized (*6*), as is its inhibition of phosphatases (*7*) and of carbonic anhydrase of animal origin (*7*). Zinc (*8*), a known component of carbonic anhydrase, and phosphatases have been found in bull semen.

It is the purpose of this report to present the evidence that sulfanilamide supplements the inhibition of metabolism by CO_2. Another compound, Diamox, or 2-acetylamino-1,3,4-thiadiazole-sulfonamide (*9*), which is considered as a specific inhibitor of carbonic anhydrase (*7*), also inhibits glycolysis but does so at a lower concentration.

For these studies, 0.2 ml of freshly collected bull semen, containing from 200 million to approximately 450 million sperm cells, was added, in Warburg flasks, after temperature equilibration to 37°C, to 1.0 ml of the Illini variable-temperature diluent containing none of the antibacterial agents. The diluent served as the control when the flasks were gassed for approximately 10 minutes with CO_2 or with N_2 containing 5 or 50 percent CO_2. The diluent in other flasks contained sulfanilamide (0.02*M* or Diamox (0.001*M*). The results reported in Table 1 are the mean cumulative evolution of CO_2 from bicarbonate by 10^8 spermatozoa for three semen samples during a 4-hour incubation at 37°C.

The presence of sulfanilamide and Diamox depressed the glycolysis of the spermatozoa to a level much below that in the Illini variable-temperature diluent alone at all levels of CO_2. Most of the glycolytic activity in the presence of these two additives at the 100-percent-CO_2 level occurred during the first 15 minutes of incubation. When either sulfanilamide or Diamox was used, the inhibition of glycolysis was as effective in 50 percent N_2 and 50 percent CO_2 as that occurring in the diluent under an atmosphere of pure CO_2. The recovery of spermatozoan motility after the incubation and after aeration upon opening the flasks was optimum in the diluent alone and in that with sulfanilamide added but was depressed slightly by Diamox, the mean values being 55, 55, and 44 percent, respectively. Diamox levels higher and lower than the 0.001*M* level were not as effective in controlling glycolysis and did not improve the recovery of spermatozoan motility upon aeration after incubation.

The effect of sulfanilamide on the glycolytic activity of CO_2-inhibited spermatozoa has been repeatedly confirmed. In comparisons with 15 additional semen samples, the number of microliters of CO_2 produced per 10^8 sperm cells in 4 hours at 37°C in the absence and presence of sulfanilamide were 95 and 56, 24 and 12, and 11 and 8 under 5, 50, and 100 percent CO_2, respectively.

These supplementary inhibitory effects of sulfanilamide and Diamox were not due to differences in *p*H. The mean final *p*H's of the flask contents were 6.7, 7.0, and 6.7 in 5-percent CO_2 for the Illini variable-temperature diluent alone, with sulfa added, and with Diamox added, respectively. With 50-percent CO_2 the final values were 6.8, 6.6, and 6.7; with 100-percent CO_2, they were 6.7, 6.7, and 6.5, respectively.

The above results confirm the earlier report of CO_2 inhibition of glycolytic activity of spermatozoa and show that at least two sulfa compounds increase the inhibitory effect of CO_2. The mechanism of sulfa inhibition as well as CO_2 inhibition of spermatozoan glycolysis remains to be identified (*10*).

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References and Notes

1. G. W. Salisbury and N. L. VanDemark, *Nature,* in press.
2. N. L. VanDemark and U. D. Sharma, *J. Dairy Sci.* 40, 438 (1957).
3. ———, *J. Animal Sci.* 15, 1212 (1956).
4. C. B. Knodt and G. W. Salisbury, *J. Dairy Sci.* 29, 285 (1946).
5. G. W. Salisbury and C. B. Knodt, *ibid.* 30, 361 (1947).

6. D. D. Woods, *Brit. J. Exptl. Pathol.* 21, 74 (1940).
7. E. R. Waygood, *Methods in Enzymology* (Academic Press, New York, 1955), vol. 2, pp. 836–846.
8. I. Dyrendahl, *Nord, Veterinärmed.* 6, 873 (1954).
9. The Diamox was supplied by the Lederle Laboratories Division, American Cyanamid Co.
10. This work was supported in part by a grant from the Rockefeller Foundation.

12 August 1957

New "Fast" Hemoglobin Associated with Thalassemia

To date, through electrophoresis, four variants of human hemoglobin with higher anodic mobility than normal adult hemoglobin have been recognized (hemoglobins H, I, J, and K) (*1*). Recently we identified another "fast" hemoglobin in the cord blood of a full-term infant. Through paper electrophoresis of hemoglobin solutions in both barbitone (*p*H 8.8; $\Gamma/2$, 0.025) and phosphate (*p*H 6.5, 0.03*M*) buffer, two spots were obtained: a large one, corresponding to a mixture of hemoglobins F and A, and a smaller one which migrated toward the anode. Comparison of the latter with hemoglobin H (*2*) proved that H is of higher anodic mobility in both alkaline and acid buffers (Fig. 1); it was possible to separate the artificial mixtures in both buffers. The new hemoglobin also differs from hemoglobin I (*3*); at *p*H 6.5, hemoglobin I showed almost no separation from hemoglobin A, while the new hemoglobin migrated clearly away from A. Hemoglobin I, of all fast hemoglobins with the exception of H, takes a more anodic position in acid buffer (*4*). Consequently, the new hemoglobin differs from both hemoglobins J and K, which on paper at *p*H 6.5 resolve less than hemoglobin I (Fig. 2).

The new hemoglobin is not alkali-resistant. At the birth of the infant it amounted to 14 percent of the total (determined by elution), the content of hemoglobin F being 60 percent, and of A, 26 percent. The infant was neither anemic nor icteric. During the next 3 months there was a progressive reduction in the amount of the fast fraction present to 4 percent, and of hemoglobin F, to 20 percent.

An investigation of the infant's family showed that the mother has thalassemia major, while the father has thalassemia minor. Three of the grandparents, all having thalassemia minor, originated from the same village in Asia Minor (Fig. 3). Consanguinity is denied by them. The results of genotyping with eight antisera were consistent with the claimed parentage. Fast hemoglobin was found in neither the parents nor in the relatives who were examined. The hematologic and genetic data indicate that the infant definitely carries one, and possibly two, doses of the thalassemia gene (*5*).

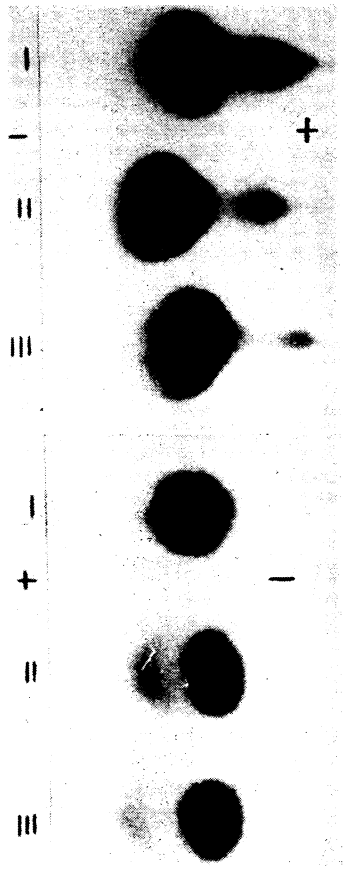

Fig. 1. Electrophoresis of (I) hemoglobin I + A, (II) new hemoglobin and A + F, (III) hemoglobin H + A. (Top) Electrophoresis in barbitone buffer (*p*H 8.8; $\Gamma/2$, 0.025; 10 hr, 0.3 ma/cm; Whatman No. 3). (Bottom) Electrophoresis in phosphate buffer (*p*H 6.5; 0.03 *M*; 5 hr, 1 ma/cm, Whatman No. 3).

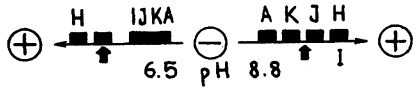

Fig. 2. Schematic representation of relative positions of "fast" hemoglobins as found through paper electrophoresis. Arrows indicate the position of the new fraction.

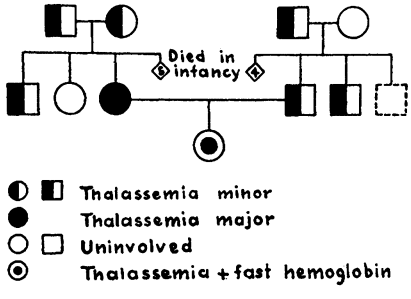

Fig. 3. Family tree showing occurrence of thalassemia and new "fast" hemoglobin.

The presence of the abnormal hemoglobins S, C, D, E, I, J, and K is genetically determined, and the abnormal component is always found in at least one parent of affected persons. Hemoglobin H differs from these in that it appears in the phenotype only in association with the thalassemia gene (*2*, *6*). The appearance of the hemoglobin under study could be compared with the genetic behavior of H; this hemoglobin differs from H, however, since neither the father, who has one dose of the thalassemia gene, nor the mother, who has a double dose, shows the abnormal component. The data on hand are suggestive that this may be an abnormal form of fetal hemoglobin, hitherto not recognized, which found expression because of its association with the thalassemia gene. An alternative explanation could be that we are in the presence of a mutation.

The thalassemia gene, although it is not responsible for the synthesis of a specific abnormal hemoglobin, is considered to be the causative factor of such alterations of the hemoglobin pattern as (i) the persistence of a high percentage of fetal hemoglobin beyond infancy; (ii) the increase of hemoglobin A_2 several times above normal (*7*, *8*); (iii) the increased production of hemoglobins S, C, E, and possibly G (*9*) when associated with the respective genes thereof; and (iv) the "phanerosis" of hemoglobin H. In the present case, a further alteration seems to have been caused by the thalassemia gene (or genes), the abnormal component being already present at birth. Further investigations on new-born infants likely to be affected by thalassemia are necessary (*10*).

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References and Notes

1. H. Lehmann, *Blood* 12, 90 (1957).
2. This hemoglobin was obtained from patients on whom a report was made previously [A. Gouttas *et al.*, *Le Sang* 26, 911 (1955)]. It has been compared with and found identical with hemoglobin H [described by D. A. Rigas, R. D. Koler, E. E. Osgood, *Science* 121, 372 (1955)]. We are indebted to Dr. Rigas for the comparison.
3. Kindly supplied by Dr. R. Cabannes, Algiers, Algeria.
4. A. R. Robinson *et al.*, *Blood* 11, 902 (1596).
5. A detailed report is in preparation.
6. A. G. Motulsky, *Nature* 178, 1055 (1956).
7. H. G. Kunkel and G. Wallenius, *Science* 122, 288 (1955).
8. Unpublished observations.
9. H. C. Schwartz *et al.*, *Blood* 12, 238 (1957).
10. In order to avoid confusion, we postpone assigning a letter to this hemoglobin until all workers in the field are in agreement.

5 September 1957