chambers were opened. The experiments were carried out at room temperature which ranged from 14° to 20° C., with the exception of two experiments on the induced eggs, where the temperature varied between 16° and 25° C.

The following results were obtained for naturally deposited eggs:

(1) Pressures of oxygen added as above have no apparent gross morphological effect upon development through the blastular stage.

(2) Increased pressures of added oxygen have an accelerative effect upon gastrulative and neuralative processes.

(3) Embryos subjected to treatment as above, removed from the chambers, and placed in finger bowls of shallow fresh water continued to develop as follows: (a) Up to 1½–2 atm. of added oxygen no apparent deleterious effects were observed in the tadpole condition. They may show acceleration in development compared with controls; (b) embryos experiencing 3 atm. of added oxygen develop various conditions of abnormal development in the tadpole stage.

(4) Compared with the controls, oxygen added under pressure approximating 1–1½ atm. appears beneficial to early frog development.

Comparison of development of pituitary-induced eggs of *R. pipiens* with that in normally deposited and normally ovulated eggs is under investigation. Thus far the results of experiments with eggs induced during the postbreeding season indicate a greater sensitivity to oxygen administered as above. The following general statements may be made:

(1) Pressures approximating 1 atm. of oxygen added to the normal air in the chamber accelerate development.

(2) Similar pressures produce a recovery effect in some of these pituitary-induced eggs. Comparison with controls demonstrates a greater number of normal-appearing neural fold conditions, with subsequent development into vigorous tadpoles in the oxygen-treated group.

(3) Pressures of added oxygen of 3 atm. suppress development at an advanced blastular condition. A few of these embryos may make abortive gastrulation attempts.

Red Blood Cell Sensitivity to the Blood-Group-Enzyme

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The blood-group-enzyme, termed BGE, was first described by Schiff and co-workers (*4*), who considered it a physiological component of certain human secretions and excretions, being notably present in saliva and normal feces. The designation was adopted because these investigators found that the enzyme, independently of its source from an A, B, or O individual, acted on the blood group antigens of all the blood groups by destroying them. More recently, Schiff and Boyd (*3*) proposed the more inclusive designation "blood-group-property-destroying-factor."

Significant in relation to the present report is the statement by Schiff and Akune (*2*) that this enzyme was not found in the brain or the blood serum.

The method of production of the BGE by the early workers consisted in inoculating a nutrient broth either with a saline extraction of normal nondiarrheal feces or with saliva (Sievers, *5*) and then incubating the broth at 37° C. for 24 hours. By this procedure, the enzyme, which in the natural state is attached to either cellular debris or certain anaerobic bacteria, becomes enriched.

There was no evidence that BGE played any role in human pathology until 1941, when Neuda, in work done at the Pneumonia Laboratory of the Harlem Hospital, New York City, showed that the red cells of negroes suffering from sickle-cell disease developed sickle cells in from 10 to 30 minutes (at times much more rapidly) when suspended in a fluid medium prepared by the Schiff method and presumably containing the BGE. The preparation of the test fluid and the test itself were described by Neuda and Rosen (*1*) in 1945. In this paper, reasons were enumerated which suggest that Schiff's blood-group-enzyme might be identical with, or closely similar to, the sickle-accelerating substance. Whether or not this assumption is correct can be established only by future investigation. At the present stage of the work, however, this preliminary assumption seems appropriate as a working hypothesis. There are already further evidences at hand which point in the same direction. There remains the outstanding fact that a fluid produced by the Schiff method will rapidly reveal the presence or absence of the sickling quality if red blood cells obtained from a negro are suspended in this fluid.

Observations made since the previous paper appeared, which will be published shortly, have demonstrated that the sensitivity of human red cells to the BGE is not restricted to sickling negro cells. Essentially similar intermediate changes as were found to precede the final sickle-cell configuration in the red blood cells of negroes were found to occur also in red cells derived from Caucasians. The final twisted cell silhouette, however, remained a characteristic of the negro red cell.

This observation led to the conclusion that the possible damage to the red cells by this enzyme fluid might be more widespread than in sickle-cell disease alone. We have therefore coined the phrase "hemolysis of sickle-cell type" to signify this peculiar kind of blood destruction, and the expression "red cell sensitivity to the blood-group-enzyme" to indicate the liability of any red cell to react in characteristic fashion to the substance produced by the Schiff method.

The Schiff method has the disadvantage of using a material which is greatly contaminated by bacteria, so that the resultant changes in the red cells could be attributed to bacterial action. Schiff and Akune, however, have disproven this possibility regarding the BGE action by demonstrating that rigid filtration with removal of the bacteria weakened, but did not completely abolish, the action of the BGE.

A first attempt was therefore made to apply the Schiff method of enrichment by culture to plasma (or serum) of a sickle-cell case. This was done by pipetting about 2 cc. of sterile plasma (or serum) into 25 cc. of sterile nutrient broth, the mixture being corked and incubated at 37° C. for 24 hours.

This plasma(serum)-broth mixture developed the same sickle-accelerating substance as was originally produced from feces. The same procedure was successful in four more cases suffering from the following diseases: hemolytic jaundice,

ulcerative colitis, pernicious anemia, and cancer of the breast (postoperative).

The observations were carried further, and it was found that occasionally fresh plasma of certain individuals would produce the sickling effect on red cells directly, without the process of enrichment by culture. This was observed in two cases of cirrhosis of the liver, two cases of toxemia of pregnancy, and one case of myelogenous leukemia.

TABLE 1

Schiff's enzyme	Diagnosis of cases	No.
In plasma (serum)-broth	Sickle-cell disease	1
	Hemolytic jaundice	1
	Ulcerative colitis	1
	Pernicious anemia	1
	Cancer of the breast	1
In natural plasma	Cirrhosis of the liver	2
	Toxemia of pregnancy	2
	Myelogenous leukemia	1
Total		10

A total of 10 cases have revealed, by two different methods, the presence of the sickle-accelerating substance in blood plasma, a substance which, using Schiff's method, was found originally only in feces and saliva (Table 1).

It appears therefore, that the sickle hemolysis-inducing substance, presumably BGE, or a substance closely related to it, is occasionally present in human blood under conditions still to be studied, and that it exists in two forms (demonstrable by the sickling test on the slide): one, less active but enrichable by culture procedure; the other, highly active as shown by the direct test.

The appearance of the red cells in the test is clean cut. The successive changes in shape from the normal round form of the red cell to the fine and delicately bent figures occur rapidly, and their occurrence is indicative that "red cell sensitivity to the blood-group-enzyme (Schiff's substance)" is present.

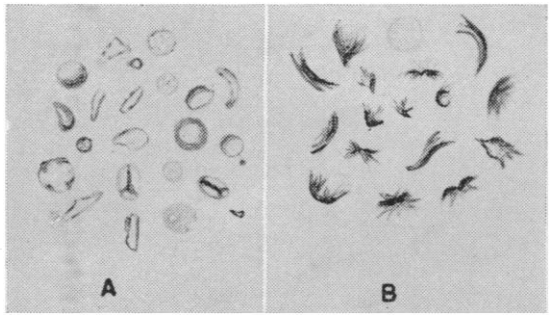

FIG. 1. Jackson washed red cells: A in saline, B in an enzyme broth prepared from feces. The first characteristic figures in B were seen in 8 minutes. The drawings were made after 1 hour.

The necessity for additional studies in this field is emphasized. Further investigation of the significance of the occurrence of this sickle-accelerating substance in human disease is in progress.

The rapid sickling of the red cells obtained from two negroes, in the enzyme-containing media, is shown in Figs. 1–3. Jackson suffered from sickle-cell anemia, while Eddy had the sickling condition without anemia. Both were chosen because of their O blood group, to avoid any interference with the group antibodies when plasma was added.

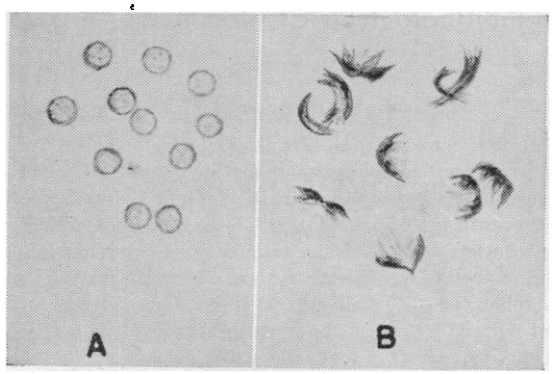

FIG. 2. Eddy washed red cells: A in saline, B in an enzyme-containing natural plasma (from a case of cirrhosis of the liver), The first characteristic figures in B were observed after 20 minutes. The picture was drawn after 1 hour.

The development of the successive changes in enzyme-sensitive sickling red cells, leading to the so-called "sickle," is characterized by many distinctive features which will be described in detail later.

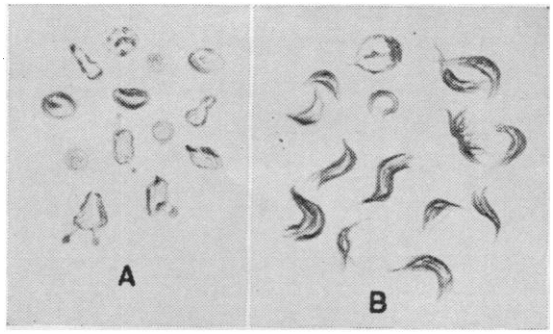

FIG. 3. Jackson washed red cells: A in saline, B in an enzyme-containing plasma (from a case of toxemia of pregnancy). The first characteristic figures appeared after 30 minutes. The drawing was made after 6 hours.

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