TABLE I. Herpes Simplex Keratitis Treated with IDU.

Treatment with IDU begun	Total No. of eyes	48 hr after therapy: Corneal lesions	48 hr after therapy: No corneal lesions
24 hr before infection	7	0	7
2 " post "	12	0	12
12 " " "	10	0	10
24 " " "	12	0	12
48 " " "	6	0	6
72 " " "	4	0	4
5 days " "	3	0	3
Total treated	54	0	54
Saline control	22	22	0

cornea or lens, and the eyes showed no sign of inflammation. When therapy was stopped after 48 hours, there was no recurrence of the lesions.

Preliminary experiments suggest that similar improvement occurs in humans with herpetic keratitis treated with IDU, and that the drug is also effective against ocular vaccinia. These studies will be reported later. Experiments with 5-bromo-2'-deoxyuridine suggest that its effect may be similar to that of IDU, but 5-fluoro-2'-deoxyuridine has only a slight effect on prevention of infection by vaccinia and herpes simplex, and does not alter the course of established lesions.

Discussion. The eye presents a unique site for the trial of anti-viral agents since they can be applied topically in high concentration without systemic toxicity. Ocular and cutaneous infection thus appears ideal for screening of anti-viral agents.

Herpes simplex keratitis has been characterized as the most important specific keratitis in this country. If present impressions of the therapeutic efficacy in man of treatment with IDU are borne out, this may well provide a cure for this blinding and disabling disease.

As important, however, appears the clear-cut demonstration that it is possible to eliminate viral lesions by antimetabolite drugs that cause no apparent harm to the surrounding tissue. This indicates that, in fact, effective anti-viral agents can be found. Even established infection by viruses appears to be specifically treatable with agents that selectively inhibit the synthesis of virus.

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In vitro Alteration of Blood Group Phenotypes of Human Epithelial Cells Exposed to Heterologous Blood Group Substances.* (27170)

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A number of authors(1,2,3,4,5,6) have demonstrated mixed agglutination reactions between human erythrocytes and a variety of other human cells. Such reactions occurred only when the erythrocytes and the cells came from donors possessing a common blood group antigen A or B, indicating that the reactions involved in the agglutinations were specific and useful for identification of blood group antigens in human cells. Accordingly, mixed agglutination tests were applied by us to human amniotic cells cultured *in vitro*, and it was confirmed that these cells contain blood group substances corresponding to the donor's blood group. Identification of A and B

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substances in individual amniotic cells then prompted the use of these cells for experiments on possible transfer of genetic information among human cells, *e.g.*, transformations, involving ABO blood groups. In a preliminary experiment, amniotic cells of blood group A were cultured in the presence of relatively crude extract of DNA from human amnions and chorions from blood group B and O donors, and the mixed agglutination test for B antigen was applied to the treated cells. An unexpectedly large number of cells reacted positively to the test in cultures treated with the extract of B-type tissues, whereas cultures treated with the extract of O-type tissues were completely negative to the same test. The experiment was repeated with DNA purified from the B-tissue extract. This time all except a very small number of cells were negative for B, leading to the suspicion that the positive B-reaction of the cells in the first experiment may have been attributable to a coating of the cells with B substance present in the extract as a contaminant. If this were the case, amniotic cells would be expected to display similar alterations of blood group phenotype when cultured in the presence of blood group specific substances extracted from animal tissues, and likewise in the presence of heterologous human sera, for it has been shown(7,8) that human sera contain blood group antigens corresponding to those present in donors' tissues. Experiments undertaken to test these possibilities showed that new blood group phenotypes do appear in cells cultured under such conditions. This report concerns experiments undertaken to investigate the nature of this phenomenon.

Materials and methods. Fresh amniotic membranes from full-term births were cut into small pieces and agitated with a 0.25% solution of Difco trypsin (1:250) in Hanks' balanced saline (calcium and magnesium omitted) for 4 hours in a flask. At the end of each hour, cell suspension continuously produced from fragments of undissociated tissue was withdrawn from the flask and centrifuged. Sedimented cells were resuspended in culture medium and transferred to milk dilution bottles for cultivation in monolayer. Meanwhile, cell dissociation from tissue fragments was continued in the flask with fresh trypsin solution. The blood group of each tissue was determined with fetal cord blood. A number of stock cultures of 4 different blood groups was established in this way and maintained by serial passage. Experiments involving mixed agglutination tests were always carried out with dish cultures prepared by transferring cells from stock bottles as needed.

The medium used in this study was prepared according to the formula recommended by Eagle(9) with addition of 1 mM each of alanine, proline, serine and glutamic acid per liter of the medium. For stock cultures of amniotic cells the medium was always supplemented with 20% individual human sera of homologous blood group. Prior to use each serum was heated at 56°C for 30 minutes. Agglutination tests were carried out routinely with anti-A and anti-B typing sera, and with A and B red cells supplied by the same donors.

In tests of cultures for blood group substance—A antigen, for example—the cultures were washed at least 4 times with phosphate-buffered normal saline (PNS) of pH 7.0, treated with anti-A serum for 45 minutes at room temperature, washed again 4 times with PNS, treated with a 2% suspension of Group A red cells in PNS for 45 minutes, and finally washed gently with PNS until all free red cells were removed from the dishes. In tests for B antigen, anti-B serum and Group B red cells were used. Positive reactions were indicated by clusters of erythrocytes around the cells.

Tests with homologous and heterologous sera. Replicate dish cultures representing 4 blood groups were prepared from stock bottles (second subcultures) and maintained in media containing human sera of different blood groups. Each culture was initiated with approximately 10^3 cells. After incubation for one week, during which time the number of cells remained stationary, the mixed agglutination tests for A and B antigens were applied. Cultures in which agglutinated cells were found were recorded as positive, others as negative. A total of 32

TABLE I. Results of Tests for B Antigen (above Horizontal Line) and for A Antigen (below Line) Applied to Human Amniotic Cells of 4 Blood Groups Cultured in Media Containing 4 Types of Human Sera.

Blood group of cells	Blood group of serum in medium			
	O	A	B	AB
O	−	−	⊕	⊕
A	−	−	⊕	⊕
B	+	+	+	+
AB	+	+	+	+
O	−	⊕	−	⊕
A	+	+	+	+
B	−	⊕	−	⊕
AB	+	+	+	+

stock cultures was used in the experiment. Results are summarized in Table I.

Results of tests for B antigen applied to cultures of O, A, B and AB cells maintained in 4 types of sera are shown in the upper half of the Table, those for A antigen in the lower half. As indicated by a + sign without circle, agglutination occurred as expected in certain cultures in conformance with the cells' known genetic background. In other cultures, agglutination occurred even though it was not expected on the basis of the conventional understanding of the immunogenetic properties of the cells. These exceptional cultures indicated in the tabulation by a circled + sign, contained sera that were heterologous to the cells. Since, as mentioned earlier, human sera are known to contain blood group substances corresponding to those present in the donors' tissues, it would seem that the cells with altered phenotype had bound blood group antigens present in the heterologous sera, and that these attached antigens reacted in the tests. This assumption suffices to explain all cases of unexpected agglutinations encountered in these experiments.

Tests with A-O and B-O serum mixtures. The foregoing experiments were repeated with mixtures of A and O, and B and O, sera using different proportions of the 2 sera. Unexpected types of agglutination did not occur in cultures of Group O cells exposed to B-O serum mixtures in which the O serum was more than 25%, but they did occur in cultures exposed to mixtures containing less than 25% type O serum; the smaller the proportion of the O serum, the higher was the percentage of cells with altered antigenic properties in the cultures. Similar results were obtained with A-O serum mixtures. Again, free blood group substances in the serum mixtures appear to be the inducing factors for the phenotypic alteration of blood group exhibited by the cells.

Tests with undiluted sera. In the foregoing experiments cultures were maintained in serum-containing media for one week before the cells were tested for agglutinability, since the described, unexpected agglutinations sometimes failed to occur if the cells were tested earlier; this long period of exposure was presumably necessary because of an insufficient concentration of blood group substances in media containing 20% sera. Subsequent tests revealed that in the presence of undiluted B or AB sera, or in the presence of extracts of porcine and equine gastric mucosa containing high concentrations of A and B specific substances,† Group A and O cells become reactive to tests for B antigen within 30 minutes. Similar tests were conducted with A-specific and B-specific substances, prepared from amniotic fluids of Group A and B fetuses, by precipitating the substances in ethanol. Group A and O cells exposed to this B substance dissolved in PNS became B-positive in 30 minutes, and Group B and O cells exposed to the A substance for 30 minutes exhibited A phenotype. In the previous experiments where B and O cells were exposed to A-type sera, or to the solution of A and B substances from animal tissues, only very small percentages of cells acquired A phenotype, whereas in the present experiment where the same cells were exposed to A substance from amniotic fluid for the same length of time, the percentages of similarly altered cells were very high. Consequently, in later experiments in which Group B and O cells were to be tested for the ability to take up A substance from media, a solution of A substance prepared from amniotic fluid was used routinely in addition to Group A sera.

Effects of trypsin. Group A and O cells

† Obtained from Merck Sharp and Dohme.

exposed to an undiluted B serum, and thereafter proved to be coated with B antigen, were treated with a 0.1% solution of Difco trypsin in PNS for 15 minutes at 37°C. Red cells were completely dissociated from the cells during the treatment. Cells detached from the dishes were transferred to new dishes with fresh medium containing an O serum. Two hours later, when most of the cells adhered to the glass, the cultures were tested again for B antigen. The results of this second test were completely negative, indicating that the B antigen was removed from the cells by the trypsin treatment. The cultures were then returned to fresh medium containing the O serum. Twenty-four hours later the cultures were exposed to an undiluted B serum for 30 minutes and tested for B antigen. All cultures were negative, indicating that the cells were now unable to bind the B antigen of the serum. The cultures were maintained in the same medium once more for 24 hours, and reexposed to the B serum for 30 minutes. The test for B antigen was positive in every dish, although the percentage of agglutinated cells was very small. Evidently, when treated with trypsin, the cells not only lost the heterologous blood group substance which they previously had picked up from the serum, but also suffered a temporary loss in their capacity to bind such antigen. Upon recultivation they regained this capacity in 48 hours.

While these experiments were under way the same Group A and O cells were exposed to the undiluted B serum for 30 minutes, and after thorough washing with PNS they were removed from the glass by treatment with a versene solution or by scraping with a rubber policeman, and transferred to new dishes with the medium containing O serum. As soon as the cells adhered to the glass they were tested for B antigen, and the results were positive in all cultures. Evidently the cells did not lose B antigen which they previously picked up from the serum.

Development of antigen-binding capacity. A culture of Group A cells which were previously exposed to an undiluted B serum and proved to have bound B substance was treated with a 0.1% trypsin solution for 15 minutes at 37°C, and a series of cultures was established with the detached cells. Each culture contained Group A serum (20%) and approximately 700 cells. Owing to the low initial cell number, the cultures remained stationary during the entire period of the experiment. After a given number of days of incubation, individual cultures were exposed to an undiluted B serum for 2 hours, and then tested for B antigen. The 2-hour period was chosen to assure an optimal opportunity for binding of the antigen. Agglutinated and unagglutinated cells were then counted in each culture, and the percentages of the agglutinated cells were plotted against time of cultivation (Fig. 1). The Figure shows that the cells were unable to bind B antigen for at least 24 hours after trypsin treatment, but thereafter they gradually regained the capacity to bind the antigen in the serum. Since the cultures were all stationary, the increase of positive cells obtained with time could not be due to a selection of such positive cells. That individual cells in these cultures did actually undergo a gradual restoration of the capacity to bind the antigen is also attested by the finding that they agglutinated with an increasing number of erythrocytes as the cultures aged.

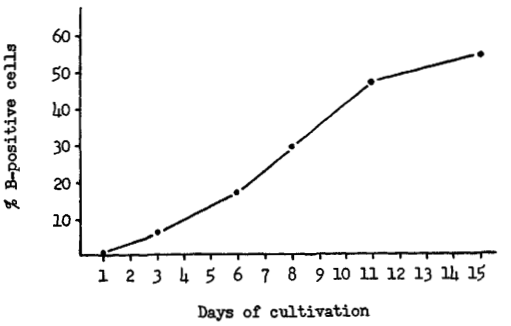

FIG. 1. Increase of B-antigen-binding capacity in cultured human amniotic cells of A blood group.

Tests of non-secretors' sera. The human sera used in all of the foregoing experiments were obtained from secretors. Two serum samples from non-secretors of B and AB‡ blood groups were tested by culturing Group O cells in the presence of these sera in culture

‡ Kindly supplied by Dr. Hans Hager, Ortho Research Foundation.

media and subsequently testing them for B antigen. The cells became B-positive, indicating that B substance was present in the sera. The secretor gene determines the presence in secretions and organs of only the water-soluble form of blood group antigens, but not the alcohol-soluble form which is present in nearly all organs and red cells but not in secretions(8). Since the serum is a tissue and not a secretion, it should contain the alcohol-soluble form of the antigens regardless of whether it comes from secretors or non-secretors.

Tests with trypsin-inhibitors. Cells of A and O blood groups were first treated with a 0.1% trypsin solution for 15 minutes at 37°C. and then with soy bean trypsin-inhibitor in PNS or with a 50% egg albumin solution in PNS(10) for 12 hours. The cells were then exposed to an undiluted B serum for 2 hours and tested for B antigen. The results were completely negative, indicating that these trypsin-inhibiting substances failed to restore the antigen-binding capacity to the cells first exposed to the enzyme. Evidently the temporary disappearance from amniotic cells of the capacity to bind heterologous antigens following trypsin treatment was not caused by adherence of trypsin to the surface of the cells. The antigen-binding capacity was actually removed from the cells by the trypsin treatment. It would seem reasonable to assume that the capacity is a property of certain cellular components, presumably a protein, which can be resynthesized by the cells following destruction by trypsin.

pH effects. Once bound to amniotic cells the blood group antigens are firmly held by the cells; continuous agitation in PNS for hours failed to separate the antigens from the cells. However, upon exposure to an acetic acid solution of pH 3, the antigens were dissociated from the cells in a few minutes. Higher pH's, such as 5 and 6, were found to be ineffective.

Antigen-binding properties in other human cells. While the study reported here was in progress, we obtained 2 pieces of human kidneys, one from a patient of O blood group and the other from one of type A, and established cultures from both. The pieces were apparently normal and gave rise to cultures largely of epithelial type. Investigations with these cultures showed that kidney cells are also capable of binding heterologous blood group antigens present in human sera. Human erythrocytes were also tested for the same property. Group O red cells treated with a B serum or with a solution of B substance from animal tissues failed to agglutinate in the presence of anti-B typing sera. Furthermore, a B serum which was repeatedly absorbed with a large quantity of Group A or O erythrocytes did not lose B substance, since Group O cells exposed to this serum took up the B substance. Evidently human red cells are not capable of binding blood group antigens. Similarly, HeLa cells which originated from a cervical carcinoma of an O type patient also appear incapable of binding either A or B substance. O(H) antigen seems to be still present in these cells(11). Fibroblasts were found also to be unable to bind blood group antigens. A number of pure fibroblast cultures were established from human amnions and investigated for this property. It was found that regardless of the blood groups of the tissue donors, these cultures were always negative to mixed agglutination tests for A and B antigens as previously observed(6), and also they were not capable of binding heterologous blood group antigens in human sera.

Discussion. Holtman(12) has observed that certain bacteria are capable of taking up antigenic substance from horse serum when the latter is mixed with culture media. Stormont(13) demonstrated that J-negative erythrocytes of cattle cultured in the plasma of J-positive cows become J-positive, presumably owing to binding of J-substance present in the plasma. As far as we are aware it is not known whether the same property is present in other bovine tissues. As the tests here described demonstrate, human erythrocytes and fibroblasts lack the capacity to bind A or B substance, whereas epithelial cells of at least amnions and kidneys are endowed with this capacity. The property appears to be associated with a certain cellular component which disappears following exposure to trypsin, but reappears upon re-

cultivation of the cells. The cell component responsible for the binding of the blood group substances persists in the amniotic cells for a long period of time; in one culture of Group A cells the epithelial cells were still able to bind B antigen after 8 months of cultivation, although they were no longer able to grow and divide. A-substance was also present in these cells. One question that remains to be resolved concerns the specificity of the attraction of the blood group substances to heterologous cells. In other words, are there specific sites responsible for attracting A antigen and other sites that tend to react with B antigen, or can the same sites attract both antigens? An answer to this question might be obtained by exposing A and B cells to labelled A and B antigens, determining whether individual cells will bind only the heterologous antigen or both antigens. Such an experiment is in progress.

In various experiments dealing with amniotic cells of A blood group, cells of subgroups A_1 and A_2 were used without preference, because it was found early in this study that under comparable conditions A_1 and A_2 cells were indistinguishable with respect to the ability to bind B antigen in the serum. The percentages of cells capable of picking up B antigen were found to be not significantly different between cultures of cells of the 2 subgroups. With regard to their native blood group antigens, A_1 and A_2, the 2 subgroups were found to differ considerably. In 10 different primary cultures of A_1 cells tested by the mixed agglutination technic for A antigen, more than 50% of cells agglutinated in every culture, whereas in 4 different primary cultures of A_2 cells, agglutinable cells did not exceed 4%.

For at least several passages, neither amnion nor kidney cells with heterologous blood group antigens attached to their surface appear to exhibit any marked retardation of growth or abnormal appearance as compared to sister cells cultured in homologous sera. Nevertheless, it might be desirable to avoid heterologous sera for long term cultivation of epithelial cells, so that cell surface will remain free of exogenous antigens and antibodies that might affect cell metabolism if bound to the cells for prolonged periods of time. For cultivation of fibroblasts in which blood group antigens as well as the antigen-binding property appear to be lacking, such precautions seem unnecessary.

The inability of HeLa cells to bind blood group antigens poses an interesting question. Is the loss of the antigen-binding property from the epithelial cells related in any way to the neoplasia of the cells? HeLa cells cannot provide an answer to this question, since it is not possible to find whether the property was present in the carcinoma tissue from which HeLa cell cultures were originally derived. It is also not known whether the normal epithelial cells of the cervix possess the property. In the kidney the epithelial cells are endowed with the ability to bind blood group antigens. Therefore, tests for this property applied on primary cultures initiated from carcinomas of the kidney without subjecting the cells to conditions favoring loss of antigen-binding capacity should indicate whether the capacity is already lost in the tissues *in situ*. A method has been developed in this laboratory for establishing cell cultures from neoplastic tissues of the kidney and other organs without the use of trypsin, and it is being used for initiating cultures from hypernephromas for the purpose of resolving the above question.

The finding of the antigen-binding property in human cells suggests certain precautions that must be taken if the ABO blood group locus is used as marker for genetic recombination tests. DNA extracts to be used for this purpose should be as free as possible of blood group substances, and human sera for supplementing media should be of types homologous to the cells, so that alteration of blood group phenotypes that might occur after cultivation of the cells in the presence of DNA extracts in media could not be caused by cellular binding of exogenous blood group substances. Alternatively, it should be possible to circumvent possible misinterpretation arising from such phenotypic alterations by treating cells exposed to DNA extracts with trypsin before they are tested for alteration of the blood group phenotypes. This would avoid a requirement for repeated puri-

fication of DNA which might cause loss of desirable qualities of the extracts. With the aid of such procedures attempts are now being made to study genetic recombination at ABO locus in human amniotic cells cultured *in vitro*.

Summary. Evidence is presented that epithelial cells of human amnion and kidney are capable of binding heterologous blood group substances in human sera and in animal tissue extracts, thereby displaying altered blood group phenotypes following short exposures to such sera and extracts. The antigen-binding property of such cells disappears following treatment with trypsin, but reappears gradually upon appropriate recultivation of the cells. The property is apparently lacking in fibroblasts, erythrocytes and HeLa cells.

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Glutamic Pyruvic Transaminase in Rat Liver after Single Injection of Carbon Tetrachloride. (27171)

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It is known that carbon tetrachloride intoxication decreases the following enzymes of the liver: pyridine nucleotides(1), adenylnucleotides(2), cytochrome C(3), cytochrome oxidase(4), succinic oxidase(4,5), acid and alkaline phosphomonoesterase and liver esterase(4). Koch-Weser *et al.*(6) have found an increase of alkaline phosphomonoesterase. According to Rahman *et al.*(5) xanthine, choline and d-amino acid oxidases are unaffected.

The transaminases in the liver are also known to decrease in various conditions of liver damage. The release of these enzymes from the hepatic tissue is easily determined by the increase in their serum levels. It is common practice to estimate the serum level of glutamic pyruvic transaminase (GPT) in liver diseases in man, as GPT activity in hepatic tissue is much greater than in any other tissue of the body, whereas glutamic oxaloacetic transaminase (GOT) activity is high in many other organs. The enzyme in the hepatic tissue, however, has not been studied as intensively as have the serum levels of the enzyme. Sommerville *et al.*(7) and Zelman *et al.*(8) have found a significant decrease of hepatic GPT in various liver diseases in man, using different methods. Molander and Friedman(9), studying the transaminase tissue level in experimental carbon tetrachloride injury in rats, could find no decrease in activity. There is, however, no information as to which of the transaminases was determined. In a dog which died 4 days after gastric instillation of 2 ml of carbon tetra-