obtained only with bloods known to possess the Mi^a factor.

Titrations of anti-Mi^a with the indirect anti-globulin technic on the four positive bloods of the Miltenberger family showed uniform values of 1:16-1:32. Thus, the double-dose effect indicating homozygous individuals could not be demonstrated, but this was not surprising since all four individuals are obviously heterozygous. As is to be expected on the basis of gene frequencies of any factor with such low incidence in the general population, bloods homozygous for the factor must be exceedingly rare, and by the same token the allelic factor Mi^b should be present in almost all bloods tested. Whether or not Mi^a is genetically related to either of the two factors characterized by unusually high incidences, Tj^a (Jay)(3) or k (Cellano)(4) is still to be determined.

Three blood factors of low incidence (Levay)(5), Gr(o), and Jobbins(7) occurring mainly in members of particular families have been reported recently, but their possible relationship to each other and Mi^a cannot be determined because of lack of antibodies for simultaneous studies.*

In contrast to the antibodies for the 3 factors mentioned above, anti-Mi^a is the only one held to be responsible for hemolytic disease and hence is clinically important. Both anti-Mi^a and anti-Gr are the only atypical antibodies present in their respective sera, but they differ from each other since anti-Gr is probably of the naturally occurring variety, its origin by antigenic stimulus of pregnancy or transfusion having been excluded.

The occurrence of antibodies for factors such as Mi^a makes it necessary to exclude their presence in the serum of all instances of hemolytic disease presumed to be due to ABO incompatibility. In this latter group of cases, antibodies reacting only on occasional bloods can be excluded if absorption of the mother's serum with a number of bloods of group A (or B) removes all activity for the father's blood of group A (or B). Only then can one consider the possibility that isoimmunization with resulting hemolytic disease was brought about by the action of anti-A or anti-B.

Summary. A new blood factor, Mi^a, demonstrable with the aid of an immune isoantibody responsible for hemolytic disease of the newborn is briefly described. Its hereditary nature is indicated by its presence in four out of 10 individuals in three generations of a particular family, in contrast to its absence in 425 random bloods.

3. Levine, P., Bobbitt, O. B., Waller, R. K., and Kuhmichel, A. B., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 403.

4. Levine, P., Wigod, M., Backer, A. B., and Ponder, R., *J. Hemat.*, 1949, v4, 869.

5. Callendar, S. T. and Race, R. R., *Ann. Eugenics*, 1946, v13, 102.

6. Graydon, J. J., *Med. J. Australia*, 1946, v2, 9.

7. Gilbey, B. E., *Nature*, 1947, v160, 362.

* According to Race, anti-Levay cannot be identical with anti-Mi^a since the latter antibody failed to react with blood known to contain the Levay factor. Tests of anti-Mi^a with bloods positive for the Jobbins and Gr factors are still to be made.

Received May 22, 1951. P.S.E.B.M., 1951, v77.

Isoimmunization by a New Blood Factor in Tumor Cells. (18794)

PHILIP LEVINE, O. B. BOBBITT, R. K. WALLER, AND A. KUHMICHEL.
From the Ortho Research Foundation, Raritan, N. J., and Department of Clinical Pathology, University of Virginia Medical School, Charlottesville, Va.

In the course of preparing a 66-year-old female patient, Mrs. D. S. J., for gastric resection of a tumor later diagnosed as adenocarcinoma, difficulties were encountered in selection of compatible donors.* The patient was in group O, Type MNS, presumably of

* The initial observation on an atypical antibody in the patient's serum was made by Dr. O. B. Bobbitt.

the Rh genotype *DCe/DCe* (R_1R_1), Duffy and Cellano positive. The factors P, Lewis, and Lutheran could not be demonstrated in her blood. Among 3000 random group O individuals, all bloods were hemolyzed by the unheated serum or agglutinated by the inactivated serum. The hemolysin could also be demonstrated in the patient's inactivated serum by adding sensitive red cells suspended in pooled fresh group compatible unheated serum from normal individuals. The unusually high incidence of positive reactions is apparently attributable to a single antibody since all activity is removed after treatment of the serum with each of 10 different bloods.

These observations suggested the presence of a new hereditary factor which is present in the red blood cells of almost all individuals tested. For identification purposes, the blood factor and its antibody may be designated as "Jay" and "anti-Jay" respectively. Because of the relationship to the tumor described below, the abbreviated symbols for the gene, blood factor, and antibody are *Tj*^{a}, Tj^a, and anti-Tj^a, the letter "T" referring to the tumor. The corresponding symbols for the theoretical allelic gene, factor, and antibody can be designated as *Tj*^{b}, Tj^b, and anti-Tj^b, respectively.

Tests of anti-Tj^a with the blood of her 3 siblings, all in group O, showed that the blood of one of her sisters did not contain the factor, but it was present in the red cells of the sister's 4 children.† The factor could be demonstrated in the blood of the patient's husband and their 4 children, all in group A, since their red cells absorbed anti-Jay.

The titer of the antibody, 1:8, when first studied increased to a titer of 1:512 as result of a so-called biologic test, *i.e.* an intravenous injection of 25 cc of a random sensitive group O blood. This was followed by an immediate severe reaction and her grossly pink serum, obtained 15 minutes later, contained 180 mg % free hemoglobin.

The gastric tumor, a grade II adenocarcinoma invading the muscularis, was uneventfully removed by a gastric resection and transfusions were not required. It is significant that the mucosa over the tumor was not ulcerated. The tumor was finely divided and when lyophilized was found to be more or less suitable for absorption experiments. Remarkably enough, 20 mg of the dried tumor specifically absorbed 16-32 units of the antibody. These experiments were properly controlled since under the same conditions the dried tumor cells failed to diminish the activity of anti-D, although the D antigen is present in the patient's red cells. The tumor cells also failed to diminish the activity of anti-A, anti-B, or anti-E. No activity could be demonstrated in concentrated saline extracts, and the tumor cells after extraction retained their affinity for the antibody. Unfortunately normal gastric tissue was not available to test for the presence or absence of the factor.

As in the case of Levine and Stetson(1)—an intragroup hemolytic reaction in a pregnant woman at the very first transfusion—the main interest here lies in the source of the immunization. The last pregnancy had occurred 32 years previously, and there is no history of previous transfusions or hemolytic disease in any of her four children. Since the tumor cells contain the antigen, one may assume that they are also responsible for the isoimmunization. Apparently, the tumor cells contain the Jay antigen which is present in the form of a blood factor in the red blood cells of almost all individuals except the propositus and her sister.‡

If the Jay factor is inherited, its presence and absence are determined by two genes of equal dominance as in the case of the MN, Rh-Hr, and Kell-Cellano systems. Since the

† The antibody could not be demonstrated in the sister's serum. Since submitting the manuscript, an antibody of identical specificity *i.e.* anti-Jay was found in the serum of a patient, Mrs. Mc, living in South Africa. In this case, the antibody was of the saline variety (titer 1:128) and the antigenic stimulus was probably derived from four pregnancies, each of them ending in spontaneous abortions after 3 or 4 months. With both sera, there were only 3 bloods (Mrs. Jay, her sister and Mrs. Mc.) which were not agglutinated. These findings will be reported by Zoutendyk and Levine.

1. Levine, P. and Stetson, R. E., *J.A.M.A.*, 1939, v113, 126.

factor is not present in the patient's blood, her genotype may be represented as Tj^bTj^b and paradoxically she has been harboring, at least in her tumor cells, the Jay gene Tj^a not transmitted from either of her two parents. In the absence of external sources for the blood factor in the tumor cells, one is led to assume that its unexpected presence may be brought about by intrinsic changes in the body, *i.e.*, a genetic mutation.

If only one locus—the site of the gene for the blood factor—is affected, then the mutation may be held responsible for the presence of the blood factor in the tumor. Because mutations have been frequently discussed in the pathogenesis of malignancy, one is tempted to suggest that the mutation may have also induced the tumor. This question, however, must be left open, in view of the importance of the issue involved and the absence of any direct evidence.

In any event, the highly exceptional circumstances in the case here reported provided specific agglutination reactions, the interpretation of which has led to a rather unexpected conclusion that a blood factor in tumor cells may arise from a genetic mutation.

‡ For production of immune isoagglutinins resulting from transplantable tumors in mice and rats cf. Gorer(2) and Lumsden(3). Isoimmunization by tumor cells is also a possible explanation for the origin of a very weak anti-Duffy which caused a hemolytic transfusion reaction at the first transfusion of a 64-year-old patient suffering from benign prostatic tumor. This case was recently reported by Rosenfield, Vogel, and Race(4) who made no comments on the origin of a presumably immune antibody in the pre-transfusion specimen.

2. Gorer, A. P., *J. Path. and Bact.*, 1937, v44, 691.

3. Lumsden, T., *Am. J. Cancer*, 1938, v32, 395.

4. Rosenfield, R., Vogel, P., and Race, R. R., *Rev. D'Hem.*, 1950, v5, 315.

Received May 22, 1951. P.S.E.B.M., 1951, v77.

Excretion of Coxsackie Virus (Conn. No. 5 Strain) in the Urine of Infected Mice.* (18795)

ALWIN M. PAPPENHEIMER, LAWRENCE J. KUNZ, AND SHEILA RICHARDSON.
From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

In the already abundant literature on infection with viruses of the Coxsackie group, we have found but one casual reference to the recovery of virus from the urine of human cases(1). Howitt, in listing the sources from which Coxsackie virus had been recovered includes urine from one case. No details are given. There have been no reports concerning the elimination of virus in the urine of infected animals. Because of the possible epidemiologic importance, the following observations demonstrating viral excretion in the urine of infected mice seem worth placing on record.

Exp. I. Several 0-day-old mice were inoculated intraperitoneally with 10^{-3} dilution of Conn. No. 5 passage virus in buffered saline. After 48 hours, at which time the mice showed the usual signs of illness, urine was obtained from several animals by direct aspiration of the bladder with a tuberculin syringe. The urine was kept frozen in the syringe for 5 days, then thawed, and penicillin and streptomycin (500 units per cc) added. The final dilution was approximately 1 to 3. Ten mice, 1 to 2 days old, were inoculated intraperitoneally with 0.02 cc of this diluted urine. Three days after injection, 9 of the 10 mice were dead or missing; the moribund survivor was sacrificed, and a 10% suspension of carcass, to which 500 units of penicillin and streptomycin per cc had been added, was frozen for further passage. Liver and pancreas were examined histologically and showed the usual lesions found in suckling mice infected with the Conn. No. 5 strain

* Aided by a grant from the National Foundation for Infantile Paralysis.

1. Howitt, B. F., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 443.