and guinea pigs, blood levels of cholesterol and per cent of cholesterol esters are similar in all 4 species(9). This suggests that significant cholesterol-esterifying activity is needed to maintain high levels of cholesterol esters in the adrenal gland and that the blood level *per se* is not the determining factor.

These findings correlate well with those of Lossow, Brot and Chaikoff(10). These workers injected 24-C^{14}-cholesterol intravenously into rats and noted that initially the adrenal contained large amounts of labeled free cholesterol, but subsequently the label was found largely in the ester fraction. They suggested that cholesterol esters of the blood were hydrolyzed prior to entry into the adrenal cells and were subsequently esterified *in situ*. Our finding cholesterol-esterifying activity in adrenals that normally contain large amounts of cholesterol in the ester form, and our failure to demonstrate this activity in adrenals that do not contain significant amounts of cholesterol ester, would seem to support this thesis.

Summary. Cholesterol-esterifying activity was demonstrated in homogenates of rat, rabbit and guinea pig adrenals. Cholesterol-esterifying activity was found to be deficient in homogenates of bovine adrenal tissue. This suggests that the amount of cholesterol esters in the adrenal gland is dependent on the level of cholesterol-esterifying activity in the gland, rather than on blood levels of cholesterol, free or esterified.

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Transferrins of Cattle Twins.* (28482)

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About 90% of dizygotic (DZ) cattle twins are chimeras and have a mixture (mosaicism) of 2 antigenically distinct kinds of erythrocytes(1). Erythrocyte mosaicism or chimerism is believed to arise from *in utero* vascular anastomosis between dizygotic twins, permitting an interchange of primordial erythropoietic cells and the persistence of mixed blood types in each twin(2), consequently members of a twin pair show identical blood types. Anastomosis of the vessels of unlike-sex twins presumably allows for the exchange of hormones resulting in the freemartin condition(3). Tissues that give rise to histocompatibility antigens are likewise exchanged, since dizygotic twins with erythrocyte mosaicism (DZM) usually accept each other's skin grafts(4,5). In contrast, the tissues that produce the J factor of cattle blood are presumably not exchanged, since DZM twins may be phenotypically different in J even if they had communal circulation as

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fetuses(6). Further, there is evidence that neuron precursors are not exchanged, since males which are twinned to freemartins do not show an increase in the number of cells with sex chromatin(7).

The observation that DZM twins may differ in their transferrin (iron-binding β-globulins) types suggested that the transferrins may not be influenced by fetal vascular anastomosis(8). This report presents some data which raise the possibility that chimerism for transferrins may occur in a small proportion of DZM twins.

Materials and methods. Blood and serum samples were obtained from 91 like-sex and 5 unlike-sex pairs of cattle twins, varying in age from 3 months to 3 years; the majority were Holstein-Friesians.

Transferrin types were determined by vertical starch gel electrophoresis(9) using borate buffer. Electrophoresis was carried out at 6 v/cm for 16 hours, after which the gel was sliced and stained with amido black. For autoradiographs, 0.8 μc of radioactive iron as Fe^{59} citrate was added to 0.1 ml of the sample, before electrophoresis. The sera from co-twins were run on the same gel, either side by side or interspaced with sera from single-born individuals of known types.

Cattle transferrins migrate in starch gel as a series of 3 or 4 closely spaced protein bands whose position is characteristic for each allele. The 5 alleles known at the transferrin locus are Tf^A, Tf^B, Tf^D, Tf^F and Tf^E in order of decreasing mobility of corresponding transferrin(10). In each homozygous type (3-4 bands) the fastest moving band stains faintly, whereas the other bands are characteristically heavily stained. In heterozygotes (4-5 bands), the slowest moving band is faintly stained also.

Results. Table I shows the results of intra-pair comparisons of blood and transferrin types of 96 pairs of twins. There were 48 pairs of twins in which the co-twins had the same blood types but no mosaicism. On the basis of the blood types alone, these twins could be monozygotic (MZ). However, in 4 of these 48 pairs, the co-twins had distinctly different transferrin types and therefore were dizygotic. Thus the transferrin typing revealed dizygosity where the blood typing had failed to do so. There were 44 pairs of twins in which the co-twins had the same blood types but mosaicism was detectable; consequently these twins were clearly DZM. Eighteen of the 44 pairs showed intra-pair differences in transferrin types. Among these 18 pairs, differences in the transferrins of the co-twins of 10 pairs were clearly discernible in stained gels or by autoradiographs. In the other 8 pairs the differences were not so clear in stained gels but were clear by autoradiograph. Fig. 1A illustrates a typical example of the β-globulin region of the latter group of 8 pairs. Considering the faintly staining bands, represented diagrammatically by dotted lines (Fig. 1B), the co-twins appear to have similar but not identical types. However, in autoradiographs the faint bands did not appear and thus it was concluded that the co-twins had different transferrin types. Also among these 44 pairs were 26 in which the co-twins had the same transferrin types. However, a close examination of the transferrin patterns of these 26 pairs revealed that they fell into 2 distinct groups: 22 pairs whose co-twins had identical patterns and 4 pairs whose co-twins had very similar, but not identical patterns. In this group of 4 pairs, the electrophoretic patterns of the co-twins showed differences in the intensity of one or more iron-binding bands (Fig. 1C, 1D). Slow α-proteins (not shown in the Figures) were the only other serum proteins that showed intensity differences. These differences were repeatable using different amounts of the same serum sample as well as sera from 3 additional bleedings of these twins obtained at intervals of 3-6 months. All 8 individuals in this group were phenotypically AD type. Sera of 58 single-born cattle of the Tf^A/Tf^D genotype did not show

TABLE I. Intra-Pair Comparison of Blood and Transferrin Types of 96 Pairs of Cattle Twins.

Blood types co-twins	Erythrocyte mosaicism	No. of pairs	Transferrin types* Same	Different
Same	No	48	44	4
"	Yes	44	26	18
Different	No	4	2	2

* See text.

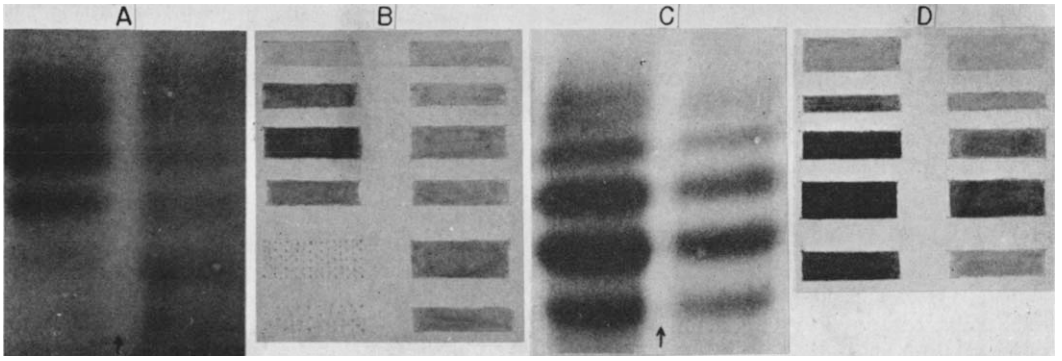

FIG. 1. Photographs and diagrams of β-globulin region of electrophoretic patterns of sera from co-twins. (A) and (B) show a DZM pair with different transferrin types, and with non-iron binding proteins in the transferrin region; (C) and (D) show a DZM pair with differences in intensity of iron-binding bands.

these variations in band intensity.

To determine the efficiency of our technic in detecting mixtures (mosaicism), sera from single-born individuals of Tf^A/Tf^A, Tf^D/Tf^D, Tf^A/Tf^D genotypes were mixed in varying proportions and run in parallel with sera of known types. Three different experienced workers independently classified them as to their phenotypes without knowing whether the sera were from twins, single-born or were artificially mixed. These mixtures could be classified into 3 groups: (a) those indistinguishable from AD type in stained starch gels and in autoradiographs, (b) those distinguishable from AD type because of differences in the intensity of one or more protein bands in the starch gels, and (c) those that were indistinguishable from either A or D types in stained gels, but were similar (not identical) to AD type by autoradiographs. The classification of an artificial mixture into one of these 3 groups depended upon the proportions of the components. For example, in various mixtures of Tf^A/Tf^A and Tf^D/Tf^D types, those containing 45-65% of D type serum were in group (a), those with 25-45% and 65-85% of D type were in group (b), and those with more than 85% or less than 25% of D type were in group (c). These results indicate that chimerism of transferrins may not be detected if certain proportions of different types are involved in a mixture. Consequently, any pair of heterozygous twins among the 22 pairs whose members showed identical transferrin types may have had a mixture as described in (a) above, but such a mixture would not be detectable. In contrast, the 4 pairs which showed intensity differences might represent mixtures as described in (b) above.

Since a mixture of human haptoglobulins 1-1 and 2-2 is distinguishable from the heterozygote type 1-2(9), serum from a human twin exhibiting blood group chimerism(11) was studied for a possible mixture. The electrophoretic pattern of serum of Mrs. McK. (obtained through the courtesy of Dr. I. Dunsford, Nat. Blood Transfusion Service, Sheffield, England) showed that she had normal haptoglobulin 2-2. However, in the transferrin region, besides the band for transferrin C, there was another distinct slow-moving band, but this band did not bind iron and therefore was not transferrin. Dr. W. Nance (Dept. Medical Genetics, Univ. of Wisconsin) kindly consented to check our results on this serum and confirmed them.

Discussion. These results confirm those of Gahne(12) that the transferrins are useful for diagnosing the zygosity of cattle twins. Using both transferrin and blood types, we found that the diagnosis was about 4% more efficient than using the blood type alone.

Sera of some DZM cattle twins contained non-iron binding proteins that tended to increase the intra-pair similarity in the β-globulin region of the electrophoretic patterns (Fig. 1A, 1B). Serum from Mrs. McK. also contained a non-iron binding protein which migrated into the transferrin region. The na-

ture and source of these proteins are not known, but since they were observed *only* in DZM twins, they may be a consequence of fetal vascular anastomosis.

The intra-pair differences in the intensity of transferrin bands (Fig. 1C, 1D) in a small proportion (about 9%) of DZM twins may be due to differences in total transferrin concentration. Since certain artificial mixtures were detectable because of differences in intensity of transferrin bands, the intensity differences observed in the DZM twins may be suggestive of transferrin chimerism. The seemingly unique occurrence of these differences in DZM twins also raises the possibility of chimerism. A consequence of transferrin chimerism would be an excess of DZ pairs in which the co-twins have the same transferrin types. Using the frequencies of *Tf* alleles in American Holstein-Friesian cattle (unpublished: $Tf^A = 0.46$, $Tf^D = 0.53$, $Tf^B = 0.01$) it can be calculated, assuming random mating, that in 58% of DZ pairs, the co-twins would inherit identical transferrin types. The observed (28 pairs) and expected (30 pairs) values were in agreement. Gahne(12) also found a close agreement. Thus, if transferrin chimerism exists, the frequency must be very low.

If transferrin chimerism exists, its rarity in contrast to the high frequency of erythrocyte chimerism may be due to limitations in detection, or to differences between the primordial erythropoietic cells and the embryonic precursors of transferrin synthesizing cells to establish and maintain themselves in the host. Such a difference is suggested by Shreffler's(13) observation that in post-radiation homologous bone marrow or fetal liver chimeras, the transient transferrin mixture was lost even though the animals progressed to 100% donor type red cells. It is conceivable therefore that in most DZ cattle twins the fetal vascular anastomosis develops either at a time when the primordial erythropoietic cells, but not the precursors of transferrin-synthesizing cells, still retain the ability to migrate and take root in the alien milieu; or at a time when the hosts still accept the primordial erythropoietic cells but have become less tolerant to precursors of transferrin-synthesizing cells.

Summary. Blood and transferrin types of 96 pairs of cattle twins were investigated. For diagnosing zygosity, both transferrin and blood types were more efficient than either alone. In intrapair comparisons of 44 twin pairs with erythrocyte mosaicism, 18 pairs had different transferrin types and 22 pairs had identical types. Four pairs had similar but not identical types; they exhibited definite intensity differences in one or more iron-binding bands suggesting the possibility of transferrin chimerism.

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