of ether *vs.* dilute hydrochloric acid and ethyl acetate *vs.* dilute hydrochloric acid, for example, yielded partition ratios of 0.3 and 2, respectively, for hippuric acid, but less than 2 x 10^{-7} and 4 x 10^{-4}, respectively, for free glycine. These data suggest that quantitative separation of hippuric acid from glycine could be effected by either carefully controlled continuous liquid-liquid extraction with either of these solvent systems¶ or appropriate stage by stage extraction from a single cell with ethyl acetate versus dilute hydrochloric acid system.

Summary. Apparent free glycine and apparent hippuric acid in urine have been determined by microbiological analysis after fractionating it by a 4-cell, 7-transfer countercurrent distribution between *n*-butanol and 5% hydrochloric acid. The effectiveness of this procedure was demonstrated by separating hippuric acid from free glycine in known mixtures and by recovering known amounts of these metabolites added to urine before subjecting it to the fractionation procedure. The apparent concentrations of free glycine and hippuric acid in normal pooled urine were 3.1 and 4.6 μM/ml, respectively.

¶ Earlier investigators employed continuous liquid-liquid extraction with ethyl acetate(14) and ether (15,16) in connection with chemical determinations of urinary hippuric acid.

1. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., Baumann, C. A., *J. Nutrition,* 1947, v33, 209.
2. Woodson, H. W., Hier, S. W., Solomon, J. D., Bergeim, O., *J. Biol. Chem.,* 1948, v172, 613.
3. Thompson, R. C., Kirby, H. M., *Arch. Biochem.,* 1949, v21, 210.
4. Eckhardt, R. D., Davidson, C. S., *J. Biol. Chem.,* 1949, v177, 687.
5. Dunn, M. S., Camien, M. N., Shankman, S., Block, H., *Arch. Biochem.,* 1947, v13, 207.
6. Dunn, M. S., Camien, M. N., Akawie, S., Malin, R. B., Eiduson, S., Getz, H. R., Dunn, K. R., *Am. Rev. Tuberc.,* 1949, v60, 439.
7. Dunn, K. R., Getz, H. R., Dunn, M. S., Camien, M. N., Akawie, S., Malin, R. B., Eiduson, S., *ibid.,* 1949, v60, 448.
8. Malin, R. B., Camien, M. N., Dunn, M. S., *Arch. Biochem. Biophys.,* 1951, v32, 106.
9. Craig, L. C., Hausmann, W., Ahrens, E. H., Harfenist, E. J., *Anal. Chem.,* 1951, v23, 1236.
10. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry,* 1954, McGraw-Hill, N. Y.
11. Carsten, M. E., *J. Am. Chem. Soc.,* 1952, v74, 5954.
12. Evered, D. F., *Biochem. J.,* 1956, v62, 416.
13. Stein, W., Carey, G. C., *J. Biol. Chem.,* 1953, v201, 45.
14. Henriques, V., Sörenson, S. P. L., *Z. physiol. Chem.,* 1909, v63, 27.
15. Quick, A. J., *J. Biol. Chem.,* 1926, v67, 477.
16. Griffith, W. H., *ibid.,* 1926, v69, 197.

Received June 29, 1959. P.S.E.B.M., 1959, v102

A Spectrophotometric Technic for Measuring Erythrocyte Chimerism in Cattle.* (25159)

ARTHUR P. MANGE AND W. H. STONE (Introduced by M. R. Irwin)
Dept. of Genetics, University of Wisconsin, Madison

The vascular anastomosis known to occur frequently between pairs of cattle dizygotic twins(1) permits an interchange of blood-forming tissues resulting in chimerism of the erythrocytes(2). The 2 kinds of erythrocytes within each member of such twin pairs can be demonstrated by a technic known as differential hemolysis(3,4). Generally, cells of an individual sensitized by an appropriate antibody (reagent) are completely hemolyzed by addition of complement (normal rabbit serum) (5). However, cells of chimeras often are only partially hemolyzed by this treatment. Such partial reactions indicate presence of 2 distinct cell populations, those with and without the antigenic specificity against which the reagent reacts. Complete antigenic constitutions of the 2 cell types can usually be determined by testing residual cells (unhemolysed)

* Paper No. 741 Dept. of Genetics. Published with approval of Director of Agri. Exp. Station, Univ. of Wisconsin. This project supported in part by Research Committee of Graduate School from funds supplied by Wisconsin Alumni Research Fn.

with a battery of blood typing reagents. The proportion of the 2 cell types can be determined by measuring the fraction of cells not lysed: Owen(3) obtained reasonably precise results using a microscopic cell counting technic, but this method was lengthy and tedious, and required as much as 4-6 ml of reagent. More recently Rendel(4) obtained comparable results using hematocrit tubes. This method was much less time-consuming, but did not conserve on reagent. Precision was limited, especially when the fraction of residual cells was small. This report describes a new technic for determining the fraction of residual cells by measuring transmittance of hemoglobin solutions derived from cell samples before and after treatment with those reagents yielding partial hemolysis. This method gives reproducible results, conserves on reagent, and requires little more time than the hematocrit technic.

Materials and methods. Blood was drawn from the animals into tubes containing 2% sodium citrate. Erythrocytes were washed 4 times in saline (0.9%) and final suspension adjusted so that ½ ml of cell suspensions lysed by diluting to about 4 ml with distilled water gave about 25% transmittance in 13 x 100 mm tubes. From knowledge of blood types, the antigenic factors for which the animals were probably mixed were determined and appropriate reagents selected for use. The reagents were used at a concentration that would give complete hemolysis with known positive cells within 4 hours' incubation at room temperature (24 ± 4°C) in a standard hemolytic test(5). Ingredients for differential hemolysis tests were added to 13 x 100 mm tubes (matched for the spectrophotometer and marked at 3 ml) according to the following Table:

	Exp. tubes	Complement control tubes	Saline control tubes
No. of tubes	1/reagent	3 (total)	3 (total)
Erythrocyte suspension, ml*	.50	.50	.50
Saline, ml		1.0	1.5
Reagent, ml	1.0		
Complement (undiluted), ml	.5	.5	

* Volumetric pipette.

The tubes were twirled at frequent intervals during incubation. Cells remaining after treatment with reagent and those in control tubes were washed 4 times in saline. The pellet of cells obtained was then lysed by diluting to the 3 ml mark with distilled water. Tubes were capped to prevent evaporation and centrifuged 20 minutes at 3000 rpm to sediment the stroma. Tubes were wiped clean and transmittance of each read twice with a Bausch and Lomb *Spectronic 20* set at 550 mμ. Percentage cells remaining was calculated from the formula $P = 100\frac{\log T_e}{\log T_c}$ where T_c is average transmittance (expressed as decimal fraction) of those tubes yielding approximately the same result, and T_c is average transmittance of complement control tubes. Since complement is not entirely non-lytic, complement control tubes represent more accurately than saline control tubes the base for calculating percent cells remaining. If complement were non-lytic the formula $P = 100\frac{\log T_e}{\log T_s}$ would be correct. T_s is average transmittance of saline control tubes. Under the assumption that complement acts uniformly upon only the cells remaining after lysis by reagent, it can be shown that this formula transforms into the formula stated before. If the 2 cell types do not occur in approximately equal proportions, T_e values of the experimental tubes fall into 2 distinct groups defined by antigens unique to each of the cell types in the chimera. However, when the 2 cell types occur in approximately equal proportions, it is not possible to determine immediately which T_e values should be grouped for averaging. The residual cells must then be blood typed to delineate the 2 groups. Two additional groups which yield no information (but are useful indicators of correct procedure) are defined by antigens common to both types ($P = O$), and lacking on both types ($P = 100$). The computed value of P is subject to error due to errors in measurements of transmittance. An estimate of this error (E) was obtained by differentiating the equation for P, and substituting for the differential terms the standard deviations of $\log T_e$ and

TABLE I. Percentage Admixture in One "Artificial Twin" and 4 Naturally Occurring Twin Sets Showing Erythrocyte Chimerism.

Twin set	Cell type	% of each cell type in twin a	b	Antigenic constitution by loci (abbreviated) AH	B	C	FV	L	SU	Z	Reagents used specific for cell type I	II
A1* (1st test)	I	28.0 ± 3.0†		—/—	$B_1O_1/—$	$C_1WX_2/X_2L'E$	V/V	—/—	—/—	Z/—		
	II	63.1 ± 3.8		—/—	$GY_2E_1'/O_3J'K'$	C_1X_3E	F/F	,,	,,	—/—	B_2 L' Z	Y_2 E_3'
A1* (2nd test)	I	30.8 ± 1.2				as above					,,	(J'K')
	II	66.9 ± 1.6										
N1	I	12.7 ± .6	20.3 ± .6	A /—	$B_1O_1'/Y_1D'E_3'$	C_2RX_1E/WX_2	F/V	,,	S /—	Z/Z	B I' V	E_1' E_2' E_2'
	II	84.8 ± 1.7	79.0 ± 2.2	,,	$QD'E_1'/Y_1D'E_3'$	,,	F/F	,,	,,	Z/—		
N2	I	5.0 ± .3	7.1 ± .3	,,	B_1O_1D'/GY_2E_1'	$C_1X_1/—$	F/V	,,	,,	,,	A B G Y_2	J'K'
	II	95.8 ± 1.8	92.9 ± 2.0	—/—	$D'E_3'/O_3J'K'$	,,	F/F	,,	—/—	,,	E_2' V S	(,,)
N3	I	40.2 ± 1.2	45.5 ± 1.0	A /—	$B_1GKO_3Y_2A'/O_1A'$	C_1W_2E	F/F	,,	SU_2	—/—	B G	D' I' Q
	II	58.6 ± 2.7	56.9 ± .5	,,	$Y_1D'I'/Q$	X_2E	F/V	L /—	,,	,,	C_1 W_2	X_2 V L
N4 (1st test)	I	28.8 ± 3.6	42.6 ± 1.5	,,	$I_2/—$	C_1E	F/F	,,	—/—	Z/—		
	II	77.2 ± 1.7	61.9 ± 1.2	,,	,,	C_1X_2E	F/V	—/—	,,	—/—	L Z	X_2 V
N4 (2nd test)	I	26.4 ± 1.2	39.1 ± 3.2			as above						
	II	75.5 ± 2.6	61.1 ± 2.9									

* Actually mixed 33% cell type I and 67% cell type II. † E value, see text.

$$\log T_c$$. Denoting these quantities by S_e and S_c respectively the value of this "propagated standard deviation in P" due to errors in measurements is given by:

$$E = 100\left[\frac{1}{\log T_c}S_e + \frac{\log T_e}{(\log T_c)^2}S_c\right]$$

Results. The results of differential hemolysis tests are reported here for 2 tests on an "artificial twin" (made by mixing cells from 2 different cows), and for 4 naturally admixed twin sets. The artificial twin was made by combining 1 part of cell type I with 2 parts of cell type II. Results of all tests are summarized in Table I and Fig. 1. Proportion of the 2 cell types determined by tests on the artificial twin (Twin A1) closely approximated the proportions in which the 2 cell types were originally mixed, suggesting that the technic gave reasonably accurate results. Evidence for reproducibility was provided by results of duplicate tests on both artificial twin (A1) and natural twin (N4). The results of first and second tests were similar for both sets of twins.

Among the majority of twin pairs, each member appears to have approximately the same proportion of the 2 cell types as its cotwin. Exceptions to this generality have been noted by Rendel(4), and further examples are afforded by twin sets N1 and N4.

FIG. 1. Percentage admixture of 2 cell types in cattle chimeras. A1 is an "artificial twin" mixed 33% cell type I, 67% cell type II. N1, N2, N3 and N4 are natural twin sets showing chimerism. Open bars represent computed percentages plus and minus E, "propagated stand. dev. in P."

Discussion. The method of measuring the percentage admixture in cattle chimeras presented here is less tedious than cell count methods, has greater precision and is no more tedious than hematocrit methods, and requires much less reagent than either of these methods. All 3 methods depend upon the measurement of the percent cells remaining after hemolysis of one or the other cell types by appropriately chosen reagents, and each is subject to certain errors in this measurement. The spectrophotometric method requires that bovine oxyhemoglobin follow the Beer-Lambert Law of absorption, and that solutions of oxyhemoglobin in distilled water be stable in time with respect to absorption. Both these conditions have been carefully checked and found satisfactory.

By extensions of this method it should be possible to perform the differential hemolytic tests with less than 1.0 ml reagent (thus conserving further), and to measure the percent cells hemolyzed, as well as percent cells remaining, thus gaining twice as much information per test with little more work. Owen(6) used a spectrophotometer to measure transmittance of hemoglobin solutions after differential hemolyses of the red blood cells of irradiated mice injected with homologous erythropoietic tissue. It should be possible to apply our technic to studies of human blood cell chimeras(7) using a hemolytic rather than a hemagglutination system.

Summary. An easily performed and precise method for measuring degree of erythrocyte chimerism in cattle is presented. A spectrophotometer is used to measure transmittance of solutions containing hemoglobin of those cells remaining after differential hemolysis by appropriately chosen reagents.

The authors wish to thank Janis Beckstrom for performing several of the differential hemolytic tests.

1. Lillie, F. R., *Science,* 1916, v43, 611.
2. Owen, R. D., *ibid.,* 1945, v102, 400.
3. Owen, R. D., Davis, H. P., Morgan, R. F., *J. Hered.,* 1946, v37, 291.
4. Rendel, J., *Acta Agric. Scand.,* 1958, v8, 162.
5. Stormont, C., Cumley, R. W., *J. Hered.,* 1943, v34, 34.
6. Owen, R. D., *Radiation Research,* 1958, v9, 164 (abstract).
7. Race, R. R., Sanger, R., *Blood Groups in Man,* Blackwell, Oxford, 1958, 3rd Edit., 303-309.

Received July 1, 1959. P.S.E.B.M., 1959, v102.

Effect of Secretin Given into Portal or Peripheral Vein.* (25160)

H. NECHELES, T. OGAWA, TH. CHILES AND M. LEVINSON
Dept. of Gastrointestinal Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.

Intravenously injected secretin is considered generally to equal the physiological effect of the hormone as liberated during digestion. However, there is reason to assume a difference in effect on the pancreas between these 2 ways of entrance of the hormone into the circulation, because a number of hormones seem to be inactivated in the liver. The naturally secreted secretin most probably enters the portal circulation and passes through the liver first, while in intravenous injection it passes the liver only after it has been diluted in the general circulation(1). This concept is supported by results obtained with vivdialysis by Necheles and Lim(2). Previous workers, using rather crude preparations of secretin, found a weaker response of pancreatic secretion following its injection into the portal vein than into a systemic vein(3). However, Mellanby(1) using a more purified secretin, found no such difference. Our attention was focused on this question when we were able to obtain a highly purified secretin labelled with I^{131}†; following its intravenous

* Supported by grant from Merton Davis Club.

† I^{131} kindly supplied by S. Bocchieri, Abbott Radio Pharmaceuticals, Oak Ridge, Tenn, and Dr. R. K. Richards, Abbott Labs., North Chicago, Ill. Secretin was kindly supplied by Dr. R. M. Rice, Lilly Lab. for Clin. Res., Product E. G.-110-33A.