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ALTERATION OF CATTLE AND GOAT BLOOD TYPE ANTIGENS BY CARBODIIMIDE

by

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ABSTRACT

Carbodiimide (CDD) treatment of cattle and goat cells produced results similar to those of treated human cells, but with more variety of blocking (suppression), and significant reduction, or absence of hemolytic test reactions. There were 129 reagents used for cattle, including several replicates, and 48 for goats, including some cattle reagents crossreactive with goat red cells.

Reactions with B and Z system reagents of cattle were almost entirely blocked indicating that a carboxyl group is universally present in those systems and serologically of prime importance. The FV and J systems were unaffected and a COOH group is presumed absent or not significant in those specificities.

Drastic differential suppression of factors within the same system and of replicate reagents for the same factor in some systems, especially the A and C systems, points to phenogroup effects and to Landsteiner's old dictum about a variety of antibodies being elicited against one antigenic structure.

Keywords: blood types, cattle, goats, carbodiimide

Chemical analysis of blood groups has been slow and incomplete in humans (Kabat, 1968; Sharon, 1974; Watkins, 1974). Deliberate treatment of the red cells by chemicals or extracts to alter their reactivity and infer something about their structure may have started with Hubner (1925) using bacterial filtrates and continued (e.g., Kellner et al., 1950) with proteolytic enzymes such as trypsin and papain (Stone and Miller, 1955; Auditore, et al., 1979).

McNeil et al. (1972) advanced these efforts with a new chemical treatment using ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (CDD). This chemical is sufficiently toxic to result in caustic and very irritating severe dermatitis on moist skin and is dangerous inhaled or ingested, so caution is needed in its handling. CDD has unstable imino bonds, which will readily combine with carboxyl groups. This attachment then blocks or sterically hinders the specific antibody from reacting with its antigen, if that antigen had an available carboxyl group.

McNeil et al. (Ibid) tested for 20 factors in 8 systems of human blood groups. They found 11 factors suppressed and 9 not suppressed. The ABH system was not suppressed, and its structure lacks a known COOH group according to Sharon and Watkins (Ibid). The Rh, MN, and Jk systems did have a division of factors suppressed and not suppressed.

This report centers on McNeil's technic applied to blood types in cattle and goats.

Materials and Methods

The carbodiimide was obtained from Sigma Chemical Co. McNeil et al. (1972) recommended 12 mg of CDD dissolved in 1.6 ml of saline to treat 1 ml of packed, washed red cells for 10 min at 37°C. This ratio worked well on erythrocytes of cattle and goats. Treated cells were washed in saline twice and diluted to 3% for the bloodtyping tests. The typing tests are hemolytic for these two species and require the use of (rabbit) complement absorbed at 0°C by goat red cells to remove naturally occurring antibodies for that species and, sometimes, by cattle cells for their hemolytic tests.

There were 129 cattle bloodtyping reagents and 18 goat bloodtyping reagents prepared by the senior author over several years, including, in cattle, many replicates from different sources. Also 30 of the cattle reagents that crossreacted well with goats were also included in that combination test. More details of such tests may be found in previous publications such as by Miller, (1966).

Blood samples from 15 goats representing Saanen, Alpine, Nubian, and La Mancha breeds were tested. The cattle represented 4 Holstein Friesian, 26 Cebullaise, 2 Herefords, and 7 Jersey. The Cebullaise is a synthetic breed in preparation by the Nicholas family, Sacramento, California. It has genetic contributions from eight breeds, mostly Nelore, Brahman, and Charolais but also including Gelbvieh, Brown Swiss, Hereford, Angus, and Shorthorn.

The cattle samples included 51 bloodtyping factors, including several subtypes and "-" dash, the absence of a detected factor in 5 of the 10 genetic systems covered. This listing may be noted in the results section. The B system (Stormont et al., 1951) of cattle was represented by 24 phenogroups. The isoimmune goat factors have not yet been appropriately phenogrouped. Production of these goat reagents has

not yet been reported, but differs very little from that in cattle.

Results

CATTLE

Generally, the least effect of CDD treatment of the red cells was noted for the F-V and J systems. The most affected (blocked) systems were the B and Z systems (See Table 1).

In the A system Z' was not blocked by the CDD treatment, but H was significantly reduced. Replicate reagents sometimes produced different treatment results. Three A reagents separated into three classes: blocked, significantly reduced, and not affected for the hemolytic reactions. Three D reagents separated into those with essentially no effect versus significantly reduced classes. A fourth D reagent divided cell types into those with no effect and those completely blocked. Other replicates to behave this way were those for C_1, R, and S factors. Four S reagents had no effect noted, but two showed significantly reduced reactions. Reactions of one C_1 reagent was not affected, and another was significantly reduced by CDD treatment of the red cells.

Three of the subtyping reagents showed a block for the more "narrow" specificity (antibody portion designated by a lower number), but allowed the broader specificity to react. C_1 cells still reacted with C_2 reagent, but the C_1 reagent reaction was blocked. Similarily, R_1 and R_2 specific combinations were completely blocked, but R_1 cells still reacted strongly with R_2 reagents. Also, after CDD treatment, Y_1 cells were still reactive with Y_2 reagents. To emphasize by repetition of these interesting observations, the reactions of the C_2 reagents are blocked for C_2 cells but not for C_1 cells, R_2 reagents are blocked for R_2 cells but not for R_1 cells, and Y_2 reagents are blocked for Y_2 cells but not for Y_1 cells!

CDD-treated Z cells were significantly reduced in reactivity with Z reagents. But, after being washed in saline, if they were allowed to stand at refrigerator temperatures for 2 days, were now completely blocked!

The B system reagents were almost completely blocked in reacting with CDD treated red cells. Only a B', some I', and Y_1 cells with Y_2 reagents escaped reasonably complete blockage.

The C system divided rather clearly into C_1, C_2, and W being unaffected, but R_1, R_2, X_1, X_2, and L' were blocked by the treatment.

Different samples reacted differently with L (and the already mentioned D) reagents in treatment effects. Reactivity was reduced for M' (formerly M_2) cells but not for M_1 cells.

Four S reagents, had no change in reaction after treatment, but two other S reagents were strongly reduced in reaction. New factors temporarily designated 33, C15, C110, and C113 were blocked by the CDD.

Replicate reagents behaving essentially alike were 3 B, 4 G, 2 T, 5 Y_2, 2 D', 2 I', 4 R_1, 2 F, 4 V, 2 J, and 2 Z reagents.

GOATS

Treated red cells of the 15 goats were tested with 30 crossreactive cattle reagents, including some replicates (see table 2). The 18 isoimmune goat reagents and some of their original unabsorbed antisera (numbers) also had divided reaction effects as did the cattle cells, table 2. But only one of the goat isoimmune antisera was crossreactive with cattle cells and that antiserum (#1) was not crossreactive with these treated cattle cells.

The reagents C and H were both derived from the same antisera, yet they gave quite different CDD treatment results. Similarily, I was derived from the unabsorbed antiserum 29, the I component reagents being unaffected by the CDD treatment, but the extra rections of 29 were blocked. Others were in line with expectations, G derived from 15 and F from 22 being basically equal in supression.

Discussion

Different serological specificities in the same genetic system of red cell factors could behave the same after CDD treatment or they could differ drastically. The former situation is nicely represented by the B system of cattle, with practically all reagents, even replicates, being blocked, and the FV and J systems, wherein reactions of all reagents were unaffected by the treatment. The latter category of differences with the greatest contrast can be noted in the A and C system results in cattle. For example, contrast the unaffected W with blocked R, X, and L' specificities of the C system.

Even more significantly, the differential results could occur with the same "factor specificity" among replicated reagents from different sources; e.g., A and D and C_1. The observation that the named factor may be detected by different antibody specificities (here with some evidently including COOH and others not) has been noted previously (Landsteiner, 1945). Miller (1959) also noted phenogroup effects by the same "factor" in different groups.

The observation that some L factors are suppressed and others not by a single reagent is the first evidence for the existence of two kinds of L. Heretofore, L has been the simplest cattle blood group system with only L and its absence demonstrated, although L_2 subtype or L_x was suspected by some laboratories.

Because goat blood typing is not as far developed as that for cattle, one can best conclude that the results agree in general with that for cattle. The C, H, and G goat specificities do seem to represent subtyping relationships (Miller, 1958) and, therefore, may well differ in CDD treatment results. The phenogroup effect and Landsteiner's observations about a variety of antibodies resulting from immunization with one simple antigenic structure also could explain such results.

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Literature Cited

Auditore, K. J., B. G. Morris, Y. Suzuki and C. Stormont. 1979. Subtypic linearity in the bovine B blood group system. Vox Sang, 36:236-239.

Hubner, G. 1925. Umtersuchungen uber isoagglutination, mit besonderer Berwcksichtigungscheinbarer Abweichungin vom Gruppenscheance. Ztochi f. Immune tats v. exper therop. 45:223-248.

Kabat, E. A. 1968. Structural concepts in immunology and immunochemistry. Hole, Rinehart and Winston, N.Y., pp. 310.

Kellner, A., E. S. Hedal, and J. M. Haber. 1950. Effect of proteolytic enzymes on the agglutinating properties of eythrocytes. Proc. Am. Assoc. Blood Banks 3rd Annu. Meet. 102-120.

Landsteiner, K. 1945. The specificity of serological reactions. Harvard University Press, Cambridge, MA 310 pp. (Rev. ed.)

McNeil, C., E. Sax, Kay J. Nielsen, and J. D. Wahlen. 1972. The alteration of blood group antigens by carbodimide. Proc. Soc. Exp. Biol. Med. 139:1144-1147.

Miller, W. J. 1958. Subtypes in cattle blood-typing. Abstr. X Int. Congr. Genet. Vol. II:190.

Miller, W. J. 1959. The Immunogenetics Letter. Vol. 1 No. 2, p. 10., Univ. of California at Davis. 1972. .

Miller, W. J. 1966. Evidence for two new systems of blood groups in cattle. Genetics 54:151-158.

Sharon, N. 1974. Sci. Am. 230:78-86.

Stone, W. H. and W. J. Miller. 1955. Alterations in the reactivity of cattle erythrocytes following treatment with enzymes. Genetics 40:599. Abs.

Stormont, C., R. D. Owen, and M. R. Irwin. 1951. The B and C systems of bovine blood groups. Genetics 36:134-161.

Watkins, W. M. 1974. The red blood cell. Vol. I. 293-360.

Table 1. Effects of CDD Treatment on cattle red cell typing factors.

	Hemolytic Typing Reaction			
Genetic System	Blocked	some not blocked; differential suppression	significantly reduced	Not Affected
A	A, D	D	H,(A),(D)	Z',(A),(D)
B	B, G, I, I2, O1 O3, P, Q, T, Y2 Y', A', D', E'2 E'3, G', I', K', O'		B', I' Q*	
C	C1, R1 R2 X1, X2 L'			(C1), C2, W
FV				F, V
J				J
L		L		
M			M'	M1
S	H'	S	U1, U'	(S)
Z	Z	[Z]		
R',S'			R', S'	
New Factors	33, C15, C110, C113			

[ ] Complete block delayed

( ) replicate reagents different in results

* for one phenogroup $BI_1O_1QD'I'$

Table 2. Effects of CDD treatment on goat red cell typing factors.

		Hemolytic typing reaction			
		Blocked			
		Some not blocked; differential suppression	significantly reduced	slightly reduced	Not Affected
cattle reagents	NF 34	G, 03, Y2,	B, (G), (03)	E'3	P,(A')
		A', E'2	(E'2)	(C1)	(J)
		C1, W R1	(C1)	J	V
		A		NF C43	U
		L		(A)	M
					6 N.S.
		F	B		
Goat reagents	C	14	D	H	E
	G	19	186		I
	9	20			1
	10	21			
	13	22			
	15	23			
	16	24			
	25	26			
		29			
	27	31			

( ) =~ replicate reagent different in results

6 N.S.= six different normal sera from cattle reactive with goat cells.

* 9 goat reagents were not reactive with this sample of 15.