Copy of earlier write-up

Specific serological substances in normal cattle serum.

Attempts to demonstrate the specific activity of cattle antisera against the cattle A blood factor upon WG15 O antigen were unsuccessful due to the presence in many, but not all, non-immune cattle sera of substances causing the agglutination of boiled WG15 cells to a high titer. The nature of these substances, their specificity, and mode of origin were considered sufficiently interesting to warrant a few exploratory experiments.

Cattle non-immune serum #1 (CNI-1) was titered on boiled preparations of Kauffmann O-types 1-25. At an effective dilution of 1/10, all 25 types were strongly agglutinated. At an effective dilution of 1/100, 14 were agglutinated to rx. grade #2, 10 were agglutinated to rx. grade #1, and one was barely agglutinated, if at all. At an effective dilution of 1/1000, one was agglutinated to rx. grade #1, while none of the others were agglutinated. Thus, little specificity of action was indicated, although the dilution steps were too large to detect anything but major differences.

Then, 15 different non-immune cattle sera (Blaine Farm) were tested at an effective dilution of 1/200 on boiled preparations of Kauffmann types. Tests were read at 20-24 hrs. at room temperature and are summarized below.

	CNI serum #														
Antigen	134	143	155	158	165	178	179	185	186	189	190	232	192	195	196
01	1	2	1	2	2	2	3	2	1	3	3	3	2	0	3
2	1	2	0	2	2	3	3	3							
3	0	0	0	2	0	0	3	2	0	2	1	0	2	0	3
4	0	2	0	3	1	1	3	3							
5	1	2	0	2	1	1	3	2	0	2	3				
6	0	0	0	1	0	1	3								
7	2	2	0	3	2										
8	0	0	0	2	1	0	1	1	0	1	0	1	0		
9	0	0	0	0	0	0	0	0	0	0					
10	2	2	1	1	2	2	3	3	1	3	3	1			
11	0	0	0	2	2	1	2	2	1	2	3	1	1	0	3
12	1	2	2	2	2	3	3	3	2	3	2	2			
13	0	0	1	2	1	2	3	2	1	3	2	0	2	1	3
14	0	0	2	0	1	0	1	1	1	1	1	1	0	0	1
15	0	0	3	2	1	0	3	1	3	3	3	2	1	1	3
16															
17	0	0	0	3	0	0	1	2							
18	0	1	0	2	2	2	3	2	1	3	3	3	3	0	2
19	0	2	3	2	2	3	3	2	3	3	2	2	2	1	2
21	0	0	3	0	1										
22	0	1	0	2	2										
23	0	1	0	2	2	1	3	2	0	3	2				
24	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
25	1	2	2	2	3	3	3	2							

Table 1. Interactions of CNI sera and Kauffmann O types. Mixtures of 0.1 ml of each of the boiled bacteria and effective 1/200 serum read after 20-24 hrs incubation at room temp. Grade 3 is strong agglut., grade 0 is no agg. Where no entry is made no test was made. Bacterial and serum controls showed no aggl.

These data indicate that, at least in this range of serum diltuion, there is a fair degree of specificity to the action of these substances in normal cattle serum. Readings made on these same test tubes after 48 hr. accentuated the differences alreasy apparent at 20 hr.

There is certainly a tendency of certain sera (e.g. # 158, 179) to agglutinate many O types, while others (e.g. #134) agglutinate few. Similarly, certain O types (e.g. 019) are agglutinated by many sera, while others (e.g. 09) are agglutinated by few. But the existence of reciprocally different interactions, such as are blocked off in the table, suggests some kind of specificity.

No adequate adsorptions upon these non-immune sera were performed which might have answered the question as to whether different and separable reactive substances were present.

Similar situations have been reported before, wherein mammalian sera have been shown to contain specific bacterial agglutinins, where there is no apparent reason for their presence in such diversity. It has been suggested that they are not immune bodies formed in response to an antigen from the particular bacterial source, but no very satisfacory altefnative explanation has been offered. Spicer (pers. comm.) reports that most normal rabbit sera which he has examined contain E. coli agglutinins, but this hashot been our experience.

Two extreme interpretations of these substances are: 1) They are not true immune bodies, and the difference between different cattle sera are due to genetic differences in the cattle. 2. They are true immune bodies and differences between diff. sera are due to chance immunization with diverse antigens, probably of bacterial origin. These extreme views should be separable by a study of the normal sera of cattle twins.

Therefore, six sets of identical ~~twin~~ cattle twin sera and one set of non-identical twin sera we e tested as before for their ability to agglutinate Kauffmann types 1-25. Twenty-~~four~~ hour readings are summarized.

Twin pair:	I		II		III		IV		V		VI		Mix	VII	
Twin number:	1	2	10	11	18	19	20	21	22	23	24	25		N11	N12
Antigen															
01	3	3		0	3	3	2	3		3	3	3	3	1	1
2	2	3		3	3	3	2	3		3	3	3	3	2	3
3	0	(3)		0	0	0	0	0		0	(3)	(3)	0	3	0
4	(0)	3		2	2	(1)	(0)	(0)		3	3	3	3	3	0
5	3	3		3	2	2	(1)	2		3	3	3	3	2	2
6	0	(3)		0	1	(3)	0	0		0	0	(2)	0	0	
7	3	3	3	3	3	2	(1)	2	2	3	3	3	3	2	2
8	0	0	(3)	0	0	(3)	0	0	(3)	0	(2)	(3)	0	0	0
9	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0
10	2	3	3	3	3	3	3	3	3	3	2	3	3	1	2
11	1	0	0	1	0	0	0	0	(2)	0	(2)	0	0	0	0
12	(1)	2	3	2	3	(0)	2	2	3	3	3	3	3	2	3
13	3	3	3	3	2	(1)	3	3	3	(1)	3	3	3	3	2
14	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
15	3	(0)	(0)	(0)	(1)	(0)	3	3	3	3	3	3	3	0	0
16	3	3	2	(1)	3	3	2	2	3	3	3	3	3	1	3
17	0	1	0	0	1	0	0	0	0	0	0	(2)	0	0	0
18	2	3	3	3	2	(0)	(0)	(0)	3	3	3	3	3	2	2
19	2	(1)	3	3	2	(1)	(0)	3	3	2	3	3	3	0	2
20	0	0	0	0	1	0	0	0	(3)	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	1	(2)	(3)	(2)	0	0	0
22	0	(2)	1	0	(2)	0	0	0	1	0	(2)	(2)	0	0	1
23	1	(3)	1	0	1	0	0	0	1	0	(3)	(3)	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	3	3	(1)	(0)	3	3	(0)	2	3	2	3	3	3	0	3

Table 2. Tests of sera from sets of identical cattle twins (I - VI) and non-identical twins (VII) on bovine bacteria of Kauffmann types 01-25.

No clear tendency of twins to be alike in the agglutinating capacities of their sera is suggested by these data. No statsitical analysis has been attempted other than the following. Consider a difference in reaction grade of two units as significant. Then find the modal agglutination grade of each O type in the sera concerned (e.g. 019 is modally agglutinated to rx. grade #3). Then, in the array of identical twins, there are 50 instance of significant deviation from modal rx. grade (circled). Of these, 21 are in turn significantly ~~xxx~~ different from the twin reaction, 29 are not. More imporatntly, perhaps: While the frequency of overall deviation from mode is only 17% (50/288), the frequency of deviation from mode among twins of the 50 deviates is 40% (20/50). Among the non-identical twins, the comparable frequencies are 20% (10/49) and 40% (4/10), but these do not constitute fair controls in view of what is known of fetal circulation in cattle twins.) Thus, although no clear correlation between twins is demonstrated, the evidence is very suggestive of a tendency to similarity and may justify further investigation. Certainly the conclusion is not at present justified that differences between cattle ~~xxxx~~ sera in bacterial agglutinating capacity is divorced from cattle genotype.

not appropriate See calculation on next sheet

An attempt to correlate overall agglutinating capacity of cattle sera with presence of anti-cattle-J was unsuccessful, cf. Table 3.

Serum	Frequency of grades among 20 O-types 0	1	2	3	Anti-J rx.
134	14	5	1	0	weak
143	10	2	8	0	strong
155	12	3	3	2	very weak
158	3	2	13	2	very strong
165	4	7	8	1	none
178	7	5	4	4	"
179	2	3	1	14	"

Table 3. Relative freq. of rx-grades 0-3 in tests of 7 cattle sera against 20 O types, as compared to strength of anti-J component (data of W.Stone).

Similarly, no apparent relation could be seen between anti-J strength and capacity for agglutinating any particular O type, 1-7 serious exceptions could be found in every case.

A limited test was made of the aggl. capacity of these cattle sera upon unboiled Kauffmann strains. No clear flagellar-type aggl. was observed, but the bacteria were not intentionally motilized prior to test. In the table below, are given deviations in rx-grade from those observed for boiled bacteria tested similarly (in Table 1).

Antigen	Serum # 179	185	186	189	190	232	192	195	196
~~012~~ 04	-1	-2							
08	*1	0	*1	*1	*1	0	*1		
012	0	-1	*1	0	0	0			
013	-1	-2	*1	-1	-1	0	*1	-1	-2
018	-3	-2	-1	-2	-3	-3	-3	0	-2
019	0	*1	0	0	*1	*1	*1	0	0
025	-1	-1							

Table 4. Reaction grade differences between boiled and unboiled O-types when tested with selected cattle sera (* equals plus).

It had been hoped that these expts. would indicate that the action of the cattle sera was against the specific O types, and that this action would be blocked by the presence of K antigens in the unboiled preparations. The significance of the one apparently blocked aggl. (of 018) is obscure, however, since this one of the few Kauffmann strains that does not possess a K antigen.

For any given antigen:

a = frequency of rx. grade 0 in array of cattle sera
b = " " " " 1 " " "
c = " " " " 2 " " "
d = " " " " 3 " " "

Exp. Freq. 3-0 pairing by chance = 2ad
3-1 " " " = 2bd
2-0 " " " = 2ac

} these arbitrarily selected as different pairings. Like pairings are 33, 32, 22, 21, 11, 10, 00.

Exp. freq. of pairing differing by 2 rx. grade = 2ad + 2bd + 2ac = 2(ad + bd + ac)

Antigen	(from Table 2 mg) Exp. freq. diff. pairs 2(ad+bd+ac)	Living pairs examined	Exp. # different	Obs. # different
01	0.35	4	1.40	0
02	0	4	—	0
03	0.44	4	1.76	1
04	0.46	4	1.84	1
05	0.08	4	0.32	0
06	0.38	4	1.52	3
07	0.08	6	0.48	0
08	0.46	6	2.76	3
09	0	6	—	0
010	0.10	6	0.60	0
011	0.20	6	1.20	2
012	0.19	6	1.14	1
013	0.20	6	1.20	1
014	0	6	—	0
015	0.50	6	3.00	1
016	0.18	6	1.08	0
017	0.11	6	0.66	1
018	0.34	6	2.04	1
019	0.33	6	1.98	1
020	0.13	6	0.78	1
021	0.31	6	1.86	0
022	0.29	6	1.74	2
023	0.34	6	2.04	1
024	0	6	—	0
025	0.39	6	2.34	1
			31.74	21

	like	diff.
Exp	18.3	31.7
obs.	29	21

$$\chi^2 = \frac{(10.7)^2}{18.3} + \frac{(10.7)^2}{31.7} = \frac{114.5}{18.3} + \frac{114.5}{31.7}$$

$$= 6.3 + 3.6 = 9.9$$