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ON THE OCCURRENCE OF HUMAN-LIKE A-ANTIGENS IN CATTLE

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1. Introduction.

It is a well-known fact that antigens closely related to the A-antigen of the human organism are found in the organs and secretions of several of our domestic animals.

Thus A-antigen occurs in the saliva of horses (BRAHN and SCHIFF, LANDSTEINER, FRIEDENREICH and THYSSEN), in commercial pepsine, i. e. in the stomach wall of pigs, (SCHIFF and WEILER, BRAHN, SCHIFF and WEINMANN) and in peptone prepared by maceration of the stomachs of pigs (OTTENSOOSER). Further WITEBSKY and ZEIZIG have shown that alcoholic extracts of the duodenum and the abomasus from cattle exhibit large amounts of antigens reacting with immune anti-A. The secretions from cattle also, however, contain A-antigen, as established by FRIEDENREICH. The latter author examined saliva from 85 cows 49 % of which were found to react with human iso-serum anti-A. It should further be noted that cattle, although not belonging to the group of animals possessing the Forssman heterogenetic antigen, would seem to contain A-antigen in their blood corpuscles (WITEBSKY). This author found that blood corpuscles from about 25 % of the cows examined reacted with human immune anti-A sera while the remaining cows exhibited, in their sera, an antibody capable of agglutinating human A blood corpuscles with an intensity far greater than blood corpuscles of other groups.

The aim of the present investigation has been to examine, in some details, the content of A-antigen in the saliva and

organs of cattle in order to form an idea of the quantitative distribution and genetics of this antigen.

2. The Methods used for the Determination of the Antigen Concentrations.

For the examination of secretions (saliva) and aqueous extracts the agglutination-inhibition method was employed in the following form: Two series of small test tubes were used in the determination of the antigen content of each sample. In the first tube of each series 0.1 cc of the original antigen solution was introduced, (concentration 1). In the second tube of each series 0.1 cc of a concentration $^1/_2$, in the third 0.1 cc of a concentration $^1/_4 = \frac{1}{2^2}$, in the fourth 0.1 cc of a concentration $^1/_8 = \frac{1}{2^3}$, etc. Thus in tube number n 0.1 cc of an antigen solution of concentration $\frac{1}{2^{n-1}}$ was introduced. Hereafter 0.1 cc of a serum anti-A was introduced into all the tubes of one of the two series while 0.1 cc of a serum anti-B was added to the contents of all the tubes of the other series. Thus after this operation the antigen concentrations in the tubes of each of the two series were $\frac{1}{2^1}, \frac{1}{2^2}, \frac{1}{2^3}, \ldots\ldots \frac{1}{2^n}$. In the following experiments the same iso-anti-A (Ulla $^1/_{16}$) was employed throughout all the investigations. This serum proved fairly constant in strength. Different iso-anti-B sera were employed, but no reaction with anti-B was ever found. After the introduction of serum the test tubes were kept at about 20° C. for an hour when washed blood corpuscles of group A_1 and B were added, the A_1 blood corpuscles to the test tubes with serum iso-anti-A, the B blood corpuscles to the test tubes

with serum iso-anti-B. After two hours the tubes were shaken. In one of the two series agglutination is seen to take place in all the tubes (generally the series with anti-B), while in the other, agglutination occurs only in the tubes following a certain number n (the series with anti-A). The number n is taken as a measure of the antigen concentration in the original antigen solution and is termed the titer reading for this solution. It may be noted that n is the power of 2 in the expression for the antigen concentration in tube number n after the addition of serum.

For the determination of the antigen content in alcoholic extracts the complement fixation method (with sheeps' blood as test blood corpuscles) was employed. The technique adopted for this method is chiefly the same as that of the agglutination-inhibition method. The main difference is that with the former method the serum, instead of the antigen, is titrated in a series of tubes according to the scheme indicated above. The antigen concentration or the "titer reading" is the number of the last tube in which complete inhibition of the hemolysis of the sheeps' blood corpuscles still takes place. As antibody was employed an immune serum anti-A produced by immunisation of rabbits with human blood corpuscles of group A_1. The serum was used in a dilution $^1/_{10}$ and absorbed with blood corpuscles of groups B- and O-MN.

If we consider a large number of samples from different subjects and want to know the range within which a certain quality, characteristic of these samples, varies, i. e. what is here termed the indefiniteness of this quality, it is first of all necessary to obtain an idea of the uncertainty of the method used for the measurement of the quality in question. In order to achieve this knowledge of the un-

certainty with the two methods indicated above, 10 titer readings with the same antigen, the same serum, the same test blood corpuscles and, in the case of the complement fixation method, the same complement and the same amboceptor were taken. The observations were further taken on the same day.

So the fluctuations in the results must be solely due to the uncertainty of the method. As a measure of this uncertainty the standard deviation μ for the ten observations was chosen. Two instances of these tests are given below, the one corresponding to the agglutination-inhibition method and the other to the complement fixation method. In both cases the standard deviation amounts to about half a titer.

Table I.

Repeated Observations on the Inhibition of the Isoagglutination by Saliva from Cow D. 11.

Exp. 22-7-39	⟨ Titers *n* ⟩									
Saliva from ...	2	3	4	5	6	7	8	9	10	11
Cow D. 11 ...	0	0	0	0	0	(+)	+	+++	+++	+++
— ...	0	0	0	0	0	0	+	+(+)	++	+++
— ...	0	0	0	0	0	0	+	++	+++	+++
— ...	0	0	0	0	0	+	+(+)	++	++	+++
— ...	0	0	0	0	0	0	+	+++	+++	+++
— ...	0	0	0	0	0	(+)	+	++	++	+++
— ...	0	0	0	0	0	0	+	++	+++	+++
— ...	0	0	0	0	0	(+)	+	++	+++	+++
— ...	0	0	0	0	0	0	(+)	+	++	+++
— ...	0	0	0	0	0	0	+	+(+)	++	+++

Average Titer: 6.6
Standard Deviation μ: 0.52

Table II.

Repeated Observations on the Inhibition of the Hemolysis of Sheeps' Bloods by Complement Fixation with an Alcoholic Extract of Duodenum from Cow C. 3.

Exp. 7-11-39	Titers *n*						
Alcoholic Extract of	1	2	3	4	5	6	7
Duodenum from Cow C. 3 ..	0	0	0	10	100	100	100
— ..	0	0	0	10	30	100	100
— ..	0	0	0	0	20	100	100
— ..	0	0	0	0	80	100	100
— ..	0	0	0	100	100	100	100
— ..	0	0	0	0	60	100	100
— ..	0	0	0	10	80	100	100
— ..	0	0	0	10	80	100	100
— ..	0	0	0	20	80	100	100
— ..	0	0	0	0	10	80	100
	Average Titer: 3.4 Standard Deviation μ: 0,52						

So the two methods are judged to be fairly satisfactory and suited for the quantitative investigations here dealt with.

It may be noted that two similar tests on the agglutination inhibition method were performed and gave $\mu = 0.53$ and 0.88 respectively. Quite obviously the close agreement between the values for μ derived from Table I and Table II is accidental.

3. The Concentration of A-Antigen in Saliva from Cattle.

A considerable number (562) of saliva samples from cows was examined in order to elucidate the fluctuations

in the concentration of the A-antigen within a random group of cows. Before the individual fluctuations or "the sample to sample indefiniteness" could be derived, it was, however, necessary to find out how large might be the normal fluctuations in the course of time for the saliva from a single cow. The latter fluctuations were determined from observations on four cows, the observations extending over one week and the samples being collected at different times of the day. The samples were boiled immediately after collection and then kept in a cool place to avoid destruction of the antigen. All the samples thus collected were tested at the same time by the agglutination-inhibition test, identically the same serum being employed in all the tests.

Table III shows the titer readings for all the samples in question. From the observations on "cow 23" and "cow 1" were derived the standard deviations μ_R; they were found to be 0.88 and 0.70 respectively. The observations on "cow 6" and "cow 18" were less suited for the determination of the indefiniteness, seeing that they include zero readings, i. e. readings covering all values below titer 1. Now, what has here been determined is not solely the fluctuations in the course of time or the pure "time to time indefiniteness". The fluctuations also reflect the uncertainty μ_M of the method. The latter was found to be $\mu_M = 0.53$ titers expressed in terms of the standard deviation. The actual time to time indefiniteness μ_T is determined by:

$$\mu_R^2 = \mu_T^2 + \mu_M^2 .$$

Introducing $\mu_M = 0.53$ and $\mu_R = \frac{0.88 + 0.70}{2} = 0.79$ we find $\mu_T = 0.59$ titers. This figure will be used below in

Table III.

Fluctuations of the Antigen Content in the Course of Time for Salivas from 4 Cows.

Cow 23 Nøruplund		Cow 6 Nøruplund		Cow 18 Nøruplund		Cow 1 Nøruplund	
Sample taken:	Titer	Sample taken:	Titer	Sample taken:	Titer	Sample taken:	Titer
2—8. 11^{30} a.m.	4	2—8. 11^{30} a.m.	0	2—8. 11^{30} a.m.	0	2—8. 11^{30} a.m.	1
— 5 p.m.	2	3—8. 5 a.m.	1	3—8. 5 a.m.	0	3—8. 5 a.m.	1
3—8. 5 a.m.	5	4—8. 5 p.m.	1	4—8. 5 p.m.	1	4—8. 5 p.m.	3
— 5^{30} p.m.	4	5—8. 5 a.m.	1	5—8. 5 a.m.	0	5—8. 5 a.m.	1
4—8. 5 a.m.	5	— 5 p.m.	1	— 5 p.m.	2	— 5 p.m.	2
— 4 p.m.	4	6—8. 5^{30} a.m.	2	6—8. 5^{30} a.m.	1	6—8. 5^{30} a.m.	2
5—8. 5 a.m.	4	7—8. 5 a.m.	1	7—8. 5 a.m.	1	7—8. 5 a.m.	2
— 5 p.m.	5	— 5^{30} p.m.	1	— 5^{30} a.m.	2	— 5^{30} p.m.	1
6—8. 5^{30} a.m.	4	8—8. 6 a.m.	2	8—8 6 a.m.	1	8—8 6 a.m.	2
7—8. 5 a.m.	3	4^{30} p.m.	3	— 4^{30} p.m.	2	— 4^{30} p.m.	1
— 5^{30} p.m.	5						
8—8 6 a.m.	5						
— 4^{30} p.m.	4						
Average Titer: 4.2		Average Titer: 1.3		Average Titer: 1.0		Average Titer: 1.6	
Standard Deviation:		Maximum Deviation:		Maximum Deviation:		Standard Deviation:	
μ_R = 0.88 Titers		1.7 Titers		1.0 Titers		0.70 Titers	

the determination of the actual sample to sample indefiniteness.

We now proceed to consider the main results comprising the 562 observations on the random group of cows referred to above. The material was obtained from cattle in "Kødbyen" in Copenhagen. (The Municipal Slaughter Houses, Copenhagen). The saliva was collected with a spoon immediately after the animal was killed. The cows were of different breeds, most of them adult animals, but the material also included some not quite young bull calves. The salivas were boiled, centrifuged and then titrated for

their inhibition of the isoagglutination. The results are represented in Table IV and in the distribution diagram fig. 1 where the percentages are plotted against the corresponding titer readings.

Judging from the diagram it would seem that A-antigen is a constituent in all cattle, in contradistinction to the

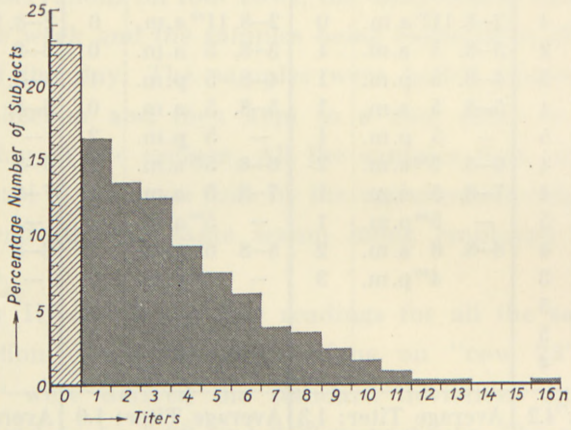

Fig. 1. Distribution Diagram for the Concentration of A-Antigen in Saliva from Cattle. (562 Samples from different Subjects).

opinion held by former authors. It should here be noted that the titer reading 0 does not indicate an absolute lack of A-antigen but only a concentration lower than the concentration corresponding to the titer reading 1. It appears further from fig. 1, that in relatively few cows only there is a higher amount of A-antigen. The shape of the block diagram suggests that the actual distribution curve for the antigen concentration may have the form shown in fig. 2.

The curve below titer 1 has been drawn in such a way that the area between this curve and the axis of abscissa is about 22.6 % of the whole area under the curve, corresponding to the relative number of the titer readings 0,

and otherwise drawn as simply as possible. The fictive titers 0, —1, and —2 would correspond to antigen solutions produced from the original solution in tube 1 by evaporising the latter (without serum) to $^1/_2$, $^1/_4$ and $^1/_8$ of its original volume. The curve thus obtained greatly resembles other distribution curves for biological qualities when a logarithmic scale is used for the quality. Such curves are very often characterised by a steep rise to a maximum followed by a slower fall in the direction of higher values for the quality in question. This is true for instance for the distribution of antigen concentrations in saliva from man (the secreter type). From fig. 2 the standard deviation μ_R' was found to be 2.6 titers. This, however, is not, as will now be understood, a true measure of the individual fluctuations, or the "sample to sample indefiniteness" μ_S of the antigen concentration within a random group of cows, seeing that μ_R' also includes the "time to time inde-

Table IV.
Titer Distribution for 562 Saliva Samples from Cattle.

Titer n	Saliva Samples	
	Absolute Number	Percentage Number
0	127	22.6
1	92	16.4
2	75	13.3
3	69	12.3
4	52	9.2
5	41	7.3
6	34	6.0
7	22	3.9
8	20	3.6
9	13	2.3
10	9	1.6
11	5	0.9
12	1	0.2
13	1	0.2
14	0	..
15	0	..
16	1	0.2

finiteness" μ_T for the single subject and further the indefiniteness μ_M due to the uncertainty of the method. The resultant μ_R of the latter two indefinitenesses was found

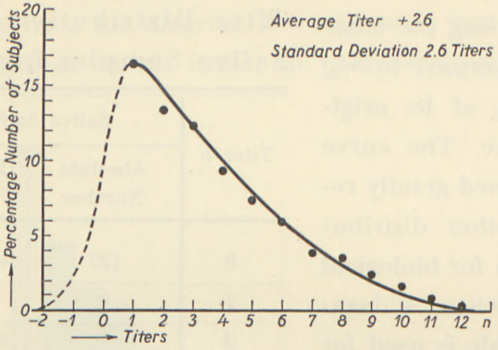

Fig. 2. Presumed Distribution of the Concentration of A-Antigen in Salivas from Cattle.

to be 0.79 titers. Thus the actual "sample to sample indefiniteness" μ_S is to be determined from:

$$\mu_R'^2 = \mu_S^2 + \mu_R^2 \qquad \text{or}$$

$$\mu_S = \sqrt{2.6^2 - 0.79^2} = 2.48 \quad \text{or 2.5 titers,}$$

which means that μ_R' may practically be taken as a measure of the "sample to sample indefiniteness" for the group in question.

4. The Heredity of the A-Antigen in Cattle.

The simple shape of the curve in fig. 2 suggests several possibilities with regard to the genetics of the A-antigen in cattle. In the first instance the possibility obtains that the faculty of developing A-antigen depends on genes present in all subjects. The whole interval of fluctuations in fig. 2

would in that case simply represent the width of the interval of indefiniteness for these genes, and family investigations would not then probably disclose any relation between the antigen concentration in parents and offspring.

Because of the great variation of the antigen concentration in the different subjects it would perhaps seem more likely that the development of antigen depends on multiple factors just as many other qualities which, within a group of subjects, exhibit a continuous quantitative variation. In that case family investigations should as a rule disclose an offspring with an antigen concentration between those of the parents.

Finally the possibility obtains that the heredity of the A-antigen in cattle is the same as that of blood groups in man, i. e. a heredity depending on multiple allelomorphs similar to the genes of the A_1, A_2, A_3, A_4 system of man. (THOMSEN, FRIEDENREICH and WORSAAE, FRIEDENREICH, GAMMELGAARD and MARCUSSEN). The objection might be raised that in the latter case a distribution curve with several maxima might be expected. In this connection it must, however, be noted that distribution curves may overlap to such a degree as to render impossible or at least difficult the distinction between them. This is for instance true in the case of curves representing the distribution of the antigen concentration in saliva from A_1 and A_2 persons (secreters). Here the joint distribution curve for the two groups has only one maximum, although each group may be shown to possess a separate curve, the two curves, however, lying very close to each other.

In order to find out which of the three genetical theories is the most likely a number of family investigations was carried out. Three of the most common races in Denmark

were examined. None of these three races differed from the others with regard to the antigen content, as will be seen from Table V.

Table V.

Family Investigation on the Concentration of A-Antigen in Salivas from Cattle.

Farm	Parents				Offspring				Race
	Bull	Titer	Cow	Titer	Cow	Titer	Calf	Titer	
Langø	Terp	8	149	8	..	..	T 149	10	
(Lundstein)	Jan	0	—	—	..	..	J 149	4	
	Sten	..	116	0	143	1	..	..	
	Terp	8	143	1	..	..	T 143	9	Guernsey
	—	—	91	0	20	3	T 91	6	
	—	—	20	3	..	..	T 20	3	
	—	—	1	7	..	..	T 1 a	11	
							T 1 b	16	
	Jan	0	117	11	..	..	J 117	1	
	—	0	96	1	22	9x	..	..	
	Terp	8	9	0	21	0	T 9	5	
	Jan	0	—	—	23	1	J 9	3	
	Sten	..	—	—	89	0	..	..	
	Terp	8	21	0	..	..	T 21	3	
	Jan	0	38	4	..	..	J 38	3	
	Terp	8	87	2	137	3	T 87	4	
	—	—	137	3	164	3	T 137	5	
	Sten	..	—	—	170	1	..	..	
	Terp	8	164	3	..	..	T 164	8	
	—	—	97	9	..	..	T 97	10	
	Sten	..	—	—	168	10	..	..	
	Jan	0	168	10	..	..	J 168	7	
Gydegaard	Bonde	1	36	6	..	..	B 36	4	Guernsey
(Bonde)	—	—	37	5	..	..	B 37	15	(Breed:
Drammelstrup	—	—	77	5	..	..	B 77	4	Højager)
Skovly	Poul	7	1	1	..	..	P 1	9	
(Hytting	—	—	2	0	..	..	P 2	6	Guernsey
Petersen)	—	—	3	6	..	..	P 3	6	(Breed:
Tirstrup	—	—	4	0	..	..	P 4	5	Højager)
	—	—	5	4	..	..	P 6	6	

Table V (continued).

Farm	Parents				Offspring				Race
	Bull	Titer	Cow	Titer	Cow	Titer	Calf	Titer	
Bertel Lassen, Tversted	Bertel	9	64	5	..	..	B 64	3	Cross between: Guernsey and Jerseys
	—	—	54	1	..	..	B 54	1	
	—	—	66	4	..	..	B 66	9	
Kallehave	Tyr	8	17	1	..	..	T 17	2	Jersey
Herman Andersen, Raabjerg	Peter	2	12	1	..	..	P 12	10x	Jersey
	—	—	27	1	..	..	P 27	1	
	—	—	26	2	..	..	P 26	2	
Mandrup Andersen, Raabjerg	Mandrup	2	14	1	..	..	M 14	1	Jersey
	—	—	16	8	..	..	M 16	13	
Christen Winther, Tversted	Kræn	0	1	7	..	..	K 1	10	Holstein
	—	—	7	2	..	..	K 2	2	
	—	—	8	2	..	..	K 8	2	
	—	—	5	3	..	..	K 5	3	
	—	—	21	2	..	..	K 21	2	
	—	—	66	4	..	..	K 66	8	
Mrs. Graff, Raabjerg	Graff	1	7	2	..	..	G 7	3	Jersey
	—	—	9	4	..	..	G 9	5	
	—	—	75	8	..	..	G 75	2	

In this table 49 families are considered, each comprising the parents and at least one of the offspring. The latter was in most cases a very young calf sometimes not more than a few days old. Within the Langø-stock only, adult subjects were found among the offspring. These subjects are indicated in the table. The denomination "calf" in the table also covers some cow-calves. For instance T 1 a was a cow-calf, while T 1 b was only five days old. Summing up the results of the investigations it was found that out of 49 of the offspring 14 had the same antigen concentration

as one of the parents; in seven of the cases the same as the strongest of the parents, in the other seven cases the same as the weakest of the parents. Again, 19 had a concentration lying between the parents and 16, i. e. about 33 %, had a concentration higher than the strongest of the parents. None of the calves exhibited a lower antigen concentration than the weakest of the parents. This fact would seem to indicate that the antigen concentration of the young subject lies on a higher level than that of the adults. This difference in concentration is most clearly illustrated in the block diagram fig. 3 a—b representing separately the distribution curves for the calves and their parents. The average titer for the calves would seem to be displaced by about two titers upwards relatively to that for the parents.

Now, however, no definite answer as to the question of the heredity of the A-antigen can be derived from the investigations considered above, simply because of the difference in the levels of antigen concentrations of "infants" and adults. Unfortunately it proves practically impossible to obtain a material of parents and adult offspring. It would seem, however, that a certain quantitative relationship obtains between the antigen concentrations of parents and offspring. Two exceptions only, indicated by a cross in the table, were found in which two parents with practically no antigen content had offspring with a high antigen content. In one of the cases, that of the Langø stock, there was, however, some doubt with regard to the paternity in so far as the period of gestation was rather long. The stock was bred on a little isolated Danish island and at the time of conception two bulls were found on the island, "Jan", the assumed father, and "Sten" a bull killed before the

investigation took place. Examination of "Sten's" descendants did not, however, suggest any greater antigen concentration for this bull, (compare Table V). In the second case, that of the Herman Andersen stock, there was no

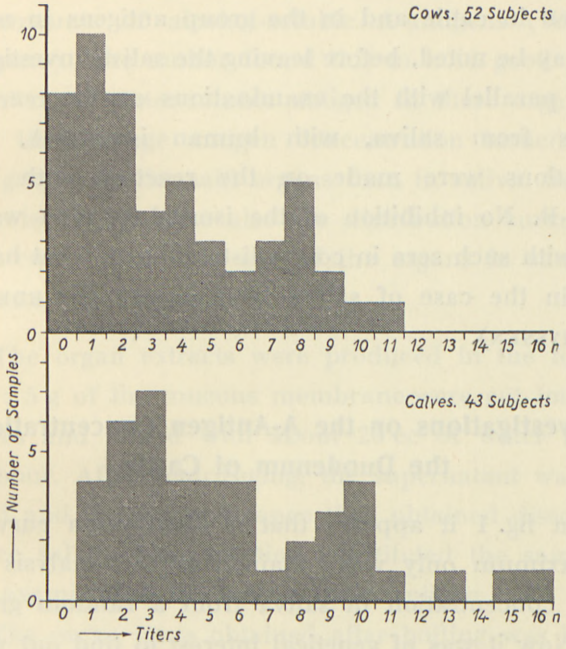

Fig. 3. Distribution Diagrams for the Concentration of A-Antigen in Salivas from Cattle.

other reason for suspicion as to the paternity than the fact that the neighbour's bull, also a Jersey bull, was considered by Mr. Andersen a better breeding animal than his own, and then perhaps that the pastures of the two stocks were adjacent to each other.

The experience that a certain quality, here the antigen concentration, was more pronounced at an early age than later on was found by the author in the group antigens

in saliva from man. Here the group antigen of the so-called non-secreters is as a rule distinctly developed at the time of birth, and disappears in the course of the first year[1]. Thus, on this point, the family investigations disclosed an interesting analogy between conditions as revealed in the A-antigen in cattle and in the group antigens in man.

It may be noted, before leaving the saliva investigations, that in parallel with the examinations on the reaction of antigens from saliva, with human iso-anti-A, similar observations were made on the reaction with human iso-anti-B. No inhibition of the isoagglutination was ever found with such sera in contradistinction to what has been found in the case of saliva from horses (Friedenreich and Thyssen).

5. Investigations on the A-Antigen Concentration in the Duodenum of Cattle.

From fig. 1 it appears that a distribution curve with one maximum only was obtained in the analysis of the antigen concentration in saliva from a random group of cattle. Now it was of genetical interest to find out whether this curve is made up of two or more distribution curves slightly displaced with regard to each other, or whether it constitutes one single distribution curve only. In this connection the following experience of the author should be recalled. Between the two human types "secreters" and "non-secreters", with regard to group antigens, there is only a quantitative difference in the antigen content. A particular organ thus exhibits two distribution curves, one

[1] The Paper on this subject has not yet been published.

for "non-secreters" and another for "secreters". Now, if the antigen content is comparatively high the two curves are distinctly separated, while in the case of a comparatively low concentration they will almost coincide. It is known that the duodenum and the abomasus in cattle contain great amounts of alcohol-soluble A-antigens (WITEBSKY and ZEIZIG). The author found this to hold good also for the content of water-soluble antigen in these organs, and further the average antigen concentration turned out to be far greater in the said organs than in saliva. So it was found wise to examine also the distribution curve of the antigen concentration in one of the organs in cattle. The duodenum was chosen.

The organ extracts were produced in the following way: 5 g of the mucous membrane were cut into small pieces and boiled with about 20 cc of water for half an hour. After centrifuging, the supernatant was vaporised and the dry substance thus obtained dissolved in 2.5 cc saline. This solution constituted the sample for the examination of water-soluble antigen.

The organ mass obtained after boiling was kept for ten days in 55 cc of 99 % alcohol when the organ mass was removed. The remainder formed the extract containing the alcohol-soluble antigens. Before the test the alcoholic extract was vaporised and the dry substance suspended in a volume of saline equal to that of the original alcoholic extract.

The investigation comprised 150 aqueous extracts and 105 alcoholic extracts from the duodenum. In Table VI the titers obtained with the different extracts are given.

Table VI.

Concentrations of A-Antigen in the Duodenum from a random Group of Cows. In the Titer Columns the left Hand Figures correspond to aqueous Extracts, the right Hand Figures to alcoholic Extracts.

Duodenum No.	Titer n		Duodenum No.	Titer n		Duodenum No.	Titer n		Duodenum No.	Titer n	
K. 3	13	4	C. 1	4	3	H. 10	1	3	J. 12	12	2
K. 6	12	3	C. 2	6	2	I. 1	16	3	J. 13	15	0
K. 71	17	4	C. 3	17	4	I. 2	13	3	J. 14	7	1
K. 83	13	3	F. 1	6	5	I. 3	17	4	J. 15	13	4
U. 1	15	3	F. 2	17	5	I. 4	19	5	J. 16	16	0
U. 2	15	5	F. 3	8	1	I. 5	9	2	J. 17	8	2
U. 3	15	4	F. 4	6	0	I. 6	14	5	J. 18	10	1
U. 4	6	4	F. 5	19	5	I. 7	7	4	J. 19	16	1
U. 12	15	3	F. 6	6	6	I. 8	10	1	J. 20	18	2
U. 13	1	3	F. 7	20	4	I. 9	16	3	L. 1	0	
U. 14	3	3	F. 8	5	6	I. 10	20	0	L. 2	7	
U. 15	3	0	F. 9	11	3	I. 11	9	4	L. 3	11	
U. 16	6	0	F. 10	5	3	I. 12	17	0	L. 4	10	
U. 17	14	4	G. 1	4	0	I. 13	5	5	L. 5	3	
U. 18	4	5	G. 2	10	3	I. 14	5	3	L. 6	4	
U. 19	16	4	G. 3	3	1	I. 15	14	5	L. 7	10	
U. 20	4	0	G. 4	5	1	I. 16	13	0	L. 8	3	
U. 21	5	5	G. 5	18	2	I. 17	9	0	L. 9	14	
U. 22	3	5	G. 6	5	1	I. 18	15	3	L. 10	5	
U. 23	14	5	G. 7	9	0	I. 19	13	2	L. 11	2	
B. 1	9	4	G. 8	4	0	I. 20	12	3	L. 12	3	
B. 2	6	4	G. 9	19	3	J. 1	15	5	L. 13	7	
B. 3	16	5	G. 10	1	0	J. 2	5	0	L. 14	2	
B. 4	7	4	H. 1	4	3	J. 3	16	0	L. 15	0	
B. 5	3	1	H. 2	13	3	J. 4	4	4	L. 16	12	
B. 6	14	4	H. 3	10	4	J. 5	11	2	L. 17	2	
B. 7	4	2	H. 4	5	0	J. 6	6	2	L. 18	12	
B. 8	4	1	H. 5	4	0	J. 7	14	3	L. 19	11	
B. 9	3	0	H. 6	7	4	J. 8	5	0	L. 20	8	
B. 10	2	1	H. 7	5	0	J. 9	9	0	L. 21	2	
B. 11	14	4	H. 8	2	2	J. 10	18	3	L. 22	11	
B. 12	10	2	H. 9	3	2	J. 11	8	1	L. 23	16	

Table VI (continued).

Duodenum No.	Titer *n*	Duodenum No.	Titer *n*	Duodenum No.	Titer *n*	Duodenum No.	Titer *n*
L. 24	4	L. 33	15	L. 39	12	L. 42	2
L. 25	7	L. 34	17	L. 27	1	L. 43	0
L. 26	3	L. 35	6	L. 28	2	L. 44	12
L. 30	3	L. 36	4	L. 29	11	L. 45	7
L. 31	9	L. 37	14	L. 40	13	L. 46	3
L. 32	3	L. 38	5	L. 41	4		

Both the titers for the aqueous extracts and for the alcoholic extracts are stated, the first in the column to the left, the last in the column to the right. Considering the aqueous extracts, i. e. the titers measuring the content of water-soluble antigen in the duodenum, it should be noted that scarcely any samples, at least only 3 out of 150, show the titer 0. This is in good agreement with the conception stated above that all cows contain A-antigen. A block diagram and a smoothed-out distribution curve was drawn for the titers of the aqueous extracts. In contradistinction to the block diagram drawn for the saliva samples the duodenum diagram shows a rather irregular shape suggesting that it is, in fact, to be considered the resultant of two or perhaps three curves with maxima at about titer 4, (titer 9), and titer 14. This is indicated in the smoothed-out curve above the block diagram. Thus the suspicion that several quantitatively different A-types obtain in cattle would seem justified.

These investigations, however, were carried out with aqueous extracts, i. e. on antigens in the same, water-soluble form as the antigens found in saliva, while WITEBSKY'S observations on A-antigen in the duodenum were made on alcoholic extracts. Now, passing on to the alcoholic

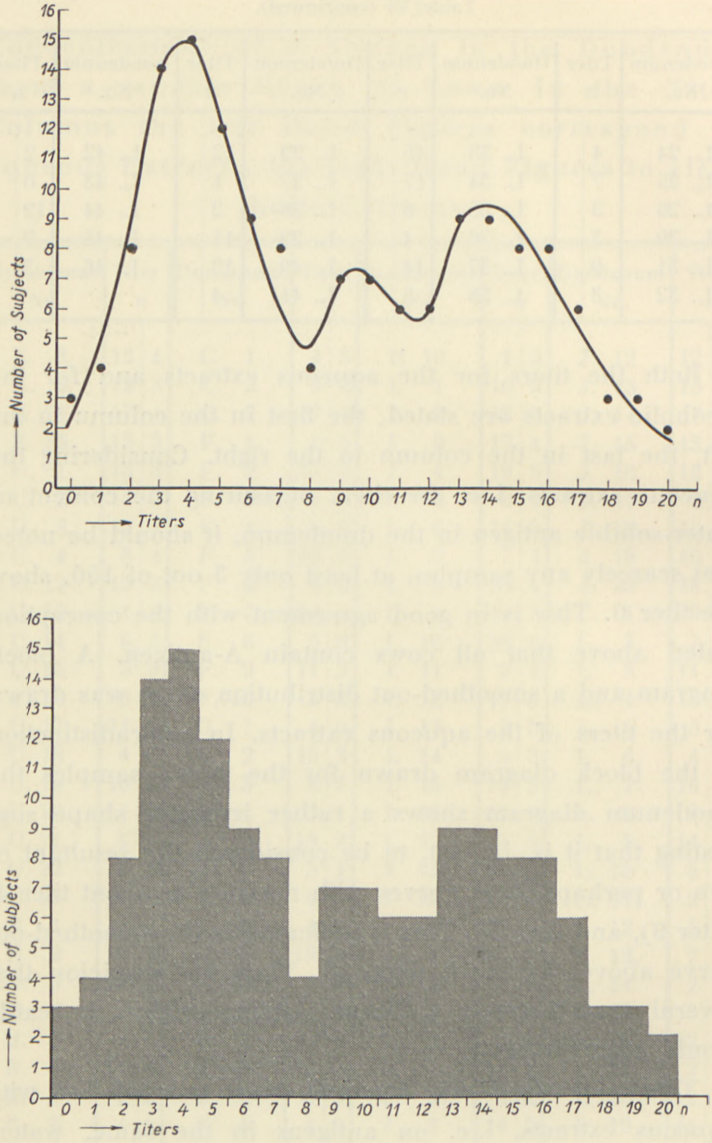

Fig. 4. Distribution Diagrams for the Concentrations of A-Antigen in aqueous Extracts of the Duodenum from Cattle. (Samples from 150 Subjects).

extracts of the present investigation, it appears from Table VI and from the block diagram fig. 5 that the titers are here much more uniform and do not suggest a division into several groups. It is particularly to be noted that no relation at all would seem to obtain between the water-soluble and the alcohol-soluble antigens with regard to their concentrations. As in the case of the saliva samples here also it may be maintained that all the extracts contain a certain amount of A-antigen, seeing that titer 0 stands for all concentrations below titer 1. It was stated that the alcoholic extracts were made from the organ mass after the production of the aqueous extracts. This would seem the right thing to do in so far as it rendered possible a comparison of two extracts from the same sample. Otherwise the objection might be urged that some of the alcohol-soluble antigens may be added to the aqueous extracts as a result of the boiling, or that a certain amount of water-soluble antigens left in the organ mass may be extracted by the 99 % alcohol. It was, however, established beyond doubt that these two possibilities can be neglected. Three samples of duodenum with high antigen concentrations were boiled 6 to 7 times with fresh water until all traces of water-soluble antigens were removed. After this procedure alcoholic extracts were made from the remaining organ mass. These organ extracts turned out to be just as powerful

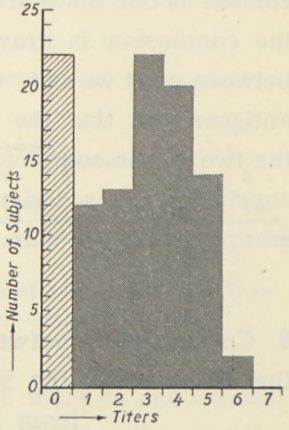

Fig. 5. Distribution Diagram for the Concentration of A-Antigen in alcoholic Extracts of the Duodenum from a Random Group of 105 Cows.

as, or even a little more so than, extracts produced from fresh organ samples. Again, the inverse experiment was performed. After the production of an alcoholic extract the mass left was used for the production of an aqueous extract. This extract likewise showed the same antigen content as one made from fresh organ. From all these facts the conclusion is drawn that a sharp distinction obtains between what we call "water-soluble" and "alcohol-soluble" antigens and that the two methods of extraction separate the two forms completely. The author was led to the same conclusions in a series of investigations on the content of group antigen in man.

6. Comparisons between the Antigen Concentrations in the Duodenum, the Submaxillary Gland and the Saliva from the same Subjects.

It may be asked whether there is any correlation between the antigen concentrations in the duodenum and the saliva—in spite of the distribution curves being rather different. In order to settle this question the duodenum and a saliva sample from each of 50 subjects were compared. The result of the comparison is represented in fig. 6.

The samples are arranged according to their "duodenum titer", so that the highest values are plotted to the left above the axis of abscissa. It appears that no very distinct correlation obtains between the titer values for the two substances. It would, however, seem safe to say that the average antigen concentration in saliva from subjects with high concentrations in the duodenum is distinctly higher than the corresponding value from subjects with a low concentration in the duodenum. If titer 9 is taken as the border

titer between high and low values for the antigen concentration in the duodenum, the average titer for saliva within

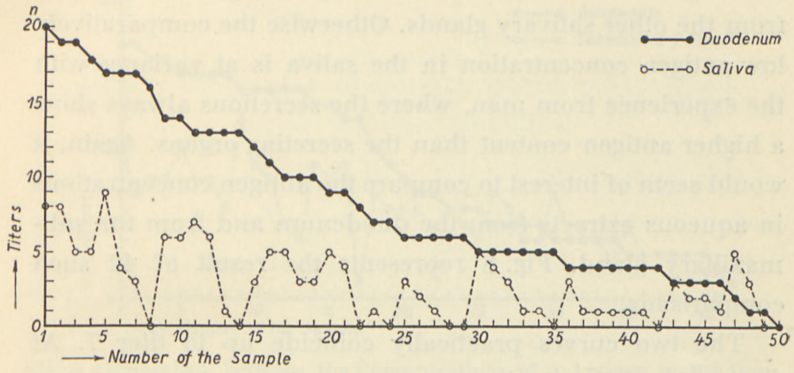

Fig. 6. Comparison between the Concentrations of A-Antigen in the Duodenum and in the Saliva from a Random Group of 50 Cows.

the "strong" group is found to be 4.6 titers as against 1.6 titers in the "weak" group. It was thought that the correlation might prove more distinct in the case of a comparison between saliva and an extract from a salivary gland, e. g. the submaxillary gland. So such a comparison was made and the results plotted, fig. 7, in a similar way to that shown in fig. 6.

Quite obviously the agreement is much more pronounced than in the case of the duodenum and the saliva.

As a rule saliva would seem to exhibit a lower antigen content than the corresponding gland. This may

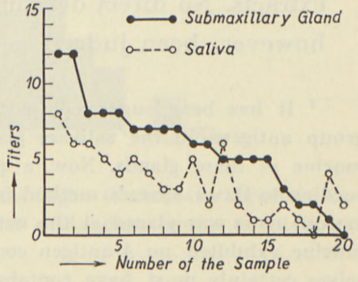

Fig. 7. Comparison between the Concentrations of A-Antigen in the Submaxillary Gland and in the Saliva from a Random Group of 20 Cows.

be due to the complex character of the saliva which undoubtedly contains secretions both from the parotid gland—which gland has a rather low antigen concentration—and from the other salivary glands. Otherwise the comparatively low antigen concentration in the saliva is at variance with the experience from man, where the secretions always show a higher antigen content than the secreting organs. Again, it would seem of interest to compare the antigen concentrations in aqueous extracts from the duodenum and from the submaxillary gland. Fig. 8 represents the result of 42 such comparisons.

The two curves practically coincide up to titer 7. At higher titers they deviate from each other owing to the fact that the submaxillary gland is characterised by a much more uniform antigen concentration than the duodenum. In accordance herewith a distribution curve with one maximum only is found for the submaxillary gland, fig. 9[1].

In our discussion of the indefiniteness and uncertainty, (comp. paragraph 2), we had no occasion to consider the uncertainty due to the production of the extracts. No direct determination of this uncertainty has, however, been judged necessary, because investigations

[1] It has been suggested, particularly by Tasiro, that the human group antigens in the salivary glands might form a constituent of the mucine of these glands. Now, a quantity of pure mucine produced according to Hammarsten's method from 2 kg of submaxillary glands from various cows was placed at the author's disposal. It turned out that the mucine exhibited no A-antigen content at all, though the glands themselves certainly must have contained an ample amount of this antigen. In view of the agreement between the human group antigen A and the A-antigens in cows this experience would seem to be of considerable interest. In this connection it should be emphasised that it is precluded that the application of the Hammarsten method can have had any destructive effect on the A-antigens.

on human organs showed that this uncertainty is of minor importance or practically negligible.

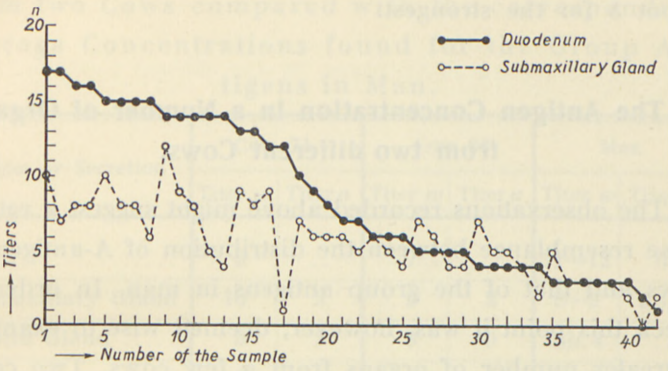

Fig. 8. Comparison between the Concentrations of A-Antigen in the Duodenum and in the Submaxillary Gland from a Random Group of 42 Cows.

Alcoholic extracts from 36 of the submaxillary glands considered above were examined together with the aqueous extracts. The results were of much the same character as those found for the duodenum extracts. In particular it

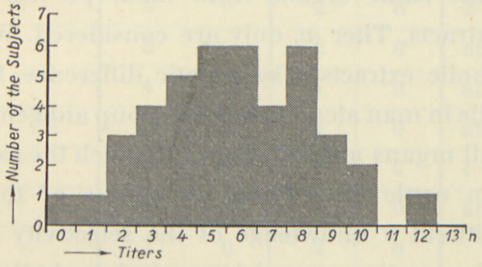

Fig. 9. Distribution Diagram for the A-Antigen Content in aqueous Extracts of the Submaxillary Gland from a Random Group of 42 Cows.

should be noted that no correlation whatever could be traced between the concentrations of water-soluble and of alcohol-soluble antigens. Further it may be remarked that 12 extracts, i. e. about 33 % of the material, showed no

inhibition of the hemolysis. The other extracts exhibited inhibition though of various degrees, the titer readings being about 5 for the strongest.

7. The Antigen Concentration in a Number of Organs from two different Cows.

The observations recorded above might suggest a rather close resemblance between the distribution of A-antigen in cows and that of the group antigens in man. In order to check this point it was, however, deemed wise to examine a greater number of organs from a few cows. Two cows were chosen which, judging from the amount of A-antigen in their salivas, might be expected also to exhibit a measurable amount in the organs; Table VII contains the results.

It appears that a remarkably good agreement obtains between the content of A-antigen in the various organs of the cows and the average concentrations of group antigens found in the same organs from man, provided that the aqueous extracts, Titer *w*, only are considered. With regard to the alcoholic extracts a systematic difference, however, is found. While in man alcohol-soluble group antigens are found in nearly all organs and developed to much the same degree, they are in cattle only found in organs of the digestive system. Without a knowledge of the selectivity of the two methods of extraction one might a priori doubt the reliability of the observations and suspect a transmission of water-soluble antigens to the alcoholic extracts, seeing that the organs of the digestive system are particularly rich in the water-soluble antigen. Now, however, the investigations described in paragraph 5 showed rather clearly that the water-soluble antigens were not transmitted to the alcoholic

Table VII.

The Concentrations of A-Antigen in various Organs from two Cows compared with the corresponding average Concentrations found for the Group Antigens in Man.

Organ or Secretion	Cow 71		Cow 83		Man	
	Titer *w*	Titer *a*	Titer *w*	Titer *a*	Titer *w*	Titer *a*
Saliva	9	..	7	..	11—12	0
Submaxillary Gland	10	3	9	2	8—9	ab. 3
Parotid Gland	5	3	3	0	ab. 4	..
Pancreas	7	2	8	1	8—7	ab. 3
Rumen	5	3	3	3	..	..
Reticulum	8	3	0	2	..	..
Abomasus	16	4	12	4	11—12	4—5
Duodenum	17	3	13	2	10—11	ab. 4
Liver	3	0	3	0	ab. 4	3—4
Gall Bladder	6	0	5	0	8—9	ab. 3
Colon	3	0	2	0	2—3	..
Lung	5	0	2	0	ab. 3	ab. 4
Kidney	4	0	1	0	ab. 4	4—5
Myocardium	0	0	2	0	ab. 4	ab. 4
Muscle	3	0	3	0	4—5	ab. 3
Fat	4	0	3	0	ab. 4	..
Thymus	2	0	2	0	..	..
Spleen	3	0	1	0	ab. 2	4—5
Blood	0	0	0	0	0	4—5

extracts. So there is in fact no reason for doubting the reliability of the observations considered above. As to the organs outside the digestive system no trace at all of a content of alcohol-soluble antigens could be established. This

holds good also for the extracts of the blood corpuscles, a peculiar fact to which we shall return in the next paragraph.

8. Research on A-Antigens in the Blood Corpuscles of Cattle.

In 1927 Witebsky published some investigations on A-antigens in blood from cattle. He found that about $^1/_4$ of all cows exhibited a certain amount of A-antigen in their blood corpuscles. He arrived at this result by showing that the blood corpuscles from these cows were hemolysed by the addition of complement and immune anti-A serum produced by immunising rabbits with human blood corpuscles of group A, i. e. a serum similar to that used in our investigations on alcoholic extracts. He likewise found that alcoholic extracts of blood from "A-cows" were active in a complement fixation test. This observation was not borne out by our experience, at least not in the case of the two cows examined. The disagreement might be accidental, though it would seem rather strange that cows exhibiting an ample amount of A-antigen, should not also have A-antigen in their blood. In order to settle the question 9 fresh alcoholic extracts of blood from different cows were produced. However, these extracts, too, gave no reaction in a complement fixation test. Four different immune sera were employed in the experiments. Hereafter natural blood corpuscles (about a score of samples) were examined in the way indicated by Witebsky. These too failed to give any reaction, at least when they were quite fresh. After having been kept for one or two days in the icebox they sometimes gave a faint hemolysis, but this reaction was not distinct and was certainly of a "non-specific" character.

Finally some experiments were made on the ability of the blood corpuscles to inhibit the agglutination of human A-blood corpuscles by anti-A sera. Washed blood corpuscles (samples from 8 cows) were added to two different sera, an immune serum and an iso-serum, the blood volumes being $^1/_2$, 1, $^3/_2$ and twice that of the serum in question. Even in the case of absorption with two volumes of blood corpuscles no trace of A-antigen could be detected in any of the blood samples. So it would seem safe to say that alcohol-soluble A-antigen is not present in cattle outside the digestive system.

9. A Correlation in Cattle between the Anti-A in the Serum and A-Antigen in the Saliva.

The paper by WITEBSKY mentioned above also included some observations on serum antibody in cattle. WITEBSKY found that serum from cows, diagnosticated as belonging to group O, agglutinated A-blood corpuscles from man to a higher degree than blood corpuscles of other groups, in contradistinction to the serum from cows belonging to group A. At first sight it would seem strange that such a discontinuity should prevail in the amount of anti-A in the serum, while the amount of A-antigen in the organism shows a continuous variation in strength within a random group of cows. FRIEDENREICH has subjected the question as to the serum antibodies to a thorough examination. By the courtesy of Dr. FRIEDENREICH these investigations, so far unpublished, have been placed at my disposal. FRIEDENREICH examined corresponding samples of saliva and serum from 69 cows with regard to their content of A-antigen and anti-A respectively. The amount of A-antigen was determined

in the way indicated above, so that these titers could be directly compared to those found by the author. The serum anti-A was determined in the following way. After absorption with blood corpuscles of group O and B in the ratios $^1/_3$—$^1/_2$, the serum was examined for its abilitity to agglutinate blood corpuscles of group A_1. This ability was read on a scale of serum concentrations $^1/_2{}^0$—$^1/_2{}^1$—$^1/_2{}^2$—$^1/_2{}^3$ - - - - $^1/_2{}^n$. The strength or ability was indicated as n, when n was the last tube in which agglutination of the blood corpuscles still took place. The readings were made both at 20° C. and at 37° C. Also the absorptions were generally carried out at both of these temperatures. The readings were as a rule the same at the two temperatures, in four cases only was a reduced titer found at 37° C. (indicated by crosses in the table). This anomaly was, however, undoubtedly due to the fact that in these four cases the sera were rather old. The complete material from Friedenreich's experiments is given in Table VIII.

In order to see whether a more or less simple correlation obtains between the amounts of antigen and antibody a diagram, fig. 10, comprising all the corresponding samples was drawn in the same manner as those of figs. 6—8. Quite obviously there is a tendency for the antibody to increase at decreasing content of the antigen. Otherwise it appears that rather large fluctuations obtain. Thus for instance a subject exhibiting no antigen in its saliva may at the same time show a complete lack of anti-A in its serum (compare subjects Nos. 32 and 33).

Disregarding for a moment these anomalies we may try to produce a curve showing the average variation of the content of antigen in the saliva with the concentration of anti-A in the serum. The experimental material (saliva)

Table VIII.

Comparisons between the Concentrations of A-Antigen in Saliva from a Random Group of Cows and the Concentrations of Anti-A in the Serum from the same Subjects. (Friedenreich).

Number of the Cow	Saliva Titer *n*	Serum Anti-A Content	Number of the Cow	Saliva Titer *n*	Serum Anti-A Content
11	0	3	50	6	0
12	0	5	51	0	3
13	0	7	52	0	4
14	6	1	53	0	5
15 a	0	1	54	0	5
15 b	7	3	55	5	1
16	9	0	56	0	6
17	6	0	57	0	5
18	0	7	58	0	3
19	0	4	59	0	5
(x) 20	5	4	60	0	5
21	0	2	61	0	7
22	10	0	62	6	2
(x) 23	4	5	63	2	7
24	0	2	71	9	1
25	5	0	72	2	1
(x) 26	2	4	73	0	0
(x) 27	0	4	74	0	2
28	0	6	75	10	0
29	0	6	76	0	1
30	6	1	77	0	0
31	7	3	78	4	2
32	0	4	79	0	5
33	0	7	80	9	1
34	0	3	82	0	2
41	2	3	83	8	0
42	3	0	84	2	6
43	4	2	85	0	6
44	7	1	86	5	1
45	9	2	87	0	7
46	5	2	88	0	2
47	0	7	89	6	3
48	0	2	90	0	8
49	11	2	91	0	7

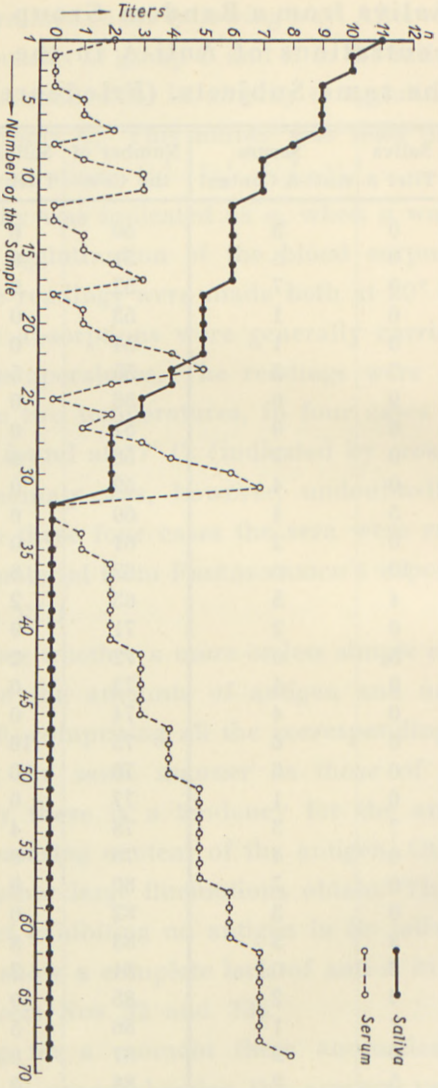

Fig. 10. Comparisons between the Concentrations of A-Antigen in Saliva from a Random Group of Cows and the Concentrations of Anti-A in the Serum from the same Subjects. (FRIEDENREICH).

was for this purpose divided into groups each covering two titers. Of each group the average antibody concentration was calculated. The results are stated in Table IX and further represented in fig. 11 where the abscissa is the average concentration of the antibody and the ordinate the average concentration of the corresponding antigen.

In spite of the scanty material within the groups containing the higher antigen concentrations the curve is rather regular, showing a smooth decrease of the concentration of A-antigen with increasing concentration of the anti-A. In fact the curve may with fairly great exactitude be represented by a hyperbola. From the results now stated it is seen that the presence of A-antigen in cows does not at all, as in man, preclude the presence of anti-A in the serum. Only it would seem that a large content of A-antigen in some degree hampers the development of anti-A.

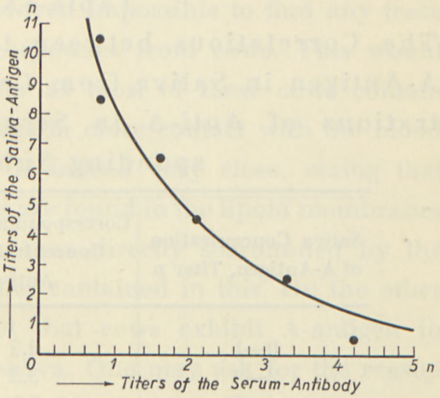

Fig. 11. The Correlation between the Concentration of A-Antigen in Saliva from Cattle and the Concentrations of Anti-A in Serum from the corresponding Subjects.

Now this result seemed so remarkable that it was deemed necessary to subject the antibody in question to a closer investigation. As appears from figs. 10 and 11, a considerable number of cows exhibited both a relatively large content of A-antigen in the saliva and a measurable content of antibody in the serum. Now the investigation referred to gave the highly interesting result that this antibody

actually reacted with the saliva antigen from the same animal in an agglutination-inhibition test. Again the reactions with salivas from cow and man of 1) a strong anti-A from a cow and 2) the ordinary iso-anti-A were compared. An absorbed anti-A serum from a cow in a dilution of $^1/_8$

Table IX.

The Correlations between the Concentration of A-Antigen in Saliva from Cattle and the Concentrations of Anti-A in Serum from the Corresponding Subjects.

Saliva Concentration of A-Antigen, Titer *n*	Corresponding Serum Concentration of Anti-A	Number of Observations
0—1	4.2	38
2—3	3.3	7
4—5	2.1	7
6—7	1.6	9
8—9	0.8	5
10—11	0.8	3

showed the same power to agglutinate human A_1 blood corpuscles as the iso-anti-A, Ulla $^1/_{16}$, employed in the present research. The titers of 8 saliva samples from different individuals and 13 saliva samples from different cows were read on two titer scales, employing the two sera respectively. In Table X a—b the results of the comparison are given. It is seen that no difference whatever could be traced between the quantitative effects of the two antibodies. So, from this twin experiment at least, no difference between man and cattle could be established either with regard to the anti-A or with regard to the antigen in the saliva.

Now if it be true that the same organism may contain a preformed antibody together with the corresponding preformed antigen, this fact will throw light on some peculiarities found in the distribution of the A-antigen within this organism. In order to understand what is meant the following points must be recalled. In the first place it will be remembered that it proved impossible to find any trace of A-antigen in blood corpuscles from cows. This would seem intelligible in so far as most of these cows contain anti-A in their serum, thus in close contact with the blood corpuscles. The contact is indeed very close, seeing that in man the group antigens are found in the lipoid membranes of the blood corpuscles, thus directly surrounded by the plasma and the anti-bodies contained in this. On the other hand, it was established that cows exhibit A-antigen in their organs and in the saliva. One may ask for the reason of this difference between blood corpuscles and organs (saliva). Most probably the explanation is to be found in the fact that the antigens in organs (saliva) are isolated from the corresponding antibodies by a barrier or wall built of various cells. This wall should not of course be considered as an absolute barrier between the antigen and the antibody. On the contrary the relation found between the concentrations of antigen and antibody in the same organism shows that some interaction must take place between the two. The ability of the serum antibodies to pass cell walls is perhaps best known from the maternal iso-antibodies which will pass in small quantities through the single layer of cells separating the maternal and the fetal circulations in the placenta. A similar limited permeability may exist, within the cells of the organism proper.

Table

Comparisons between the quantitative Effects of a) Cow Cow Serum, and of b) Human Saliva on Human Iso-anti-A of the Ag-

a.

Saliva from Cow...	2	3	4	5	6	7	8	9
Calf Terp	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Cow Calf	0	0	0	0	0	(+)	+	+(+)
Terp 1	0	0	0	0	(+)	+	+(+)	++
Cow 117	0	(+)	+	+(+)	++	+++	+++	+++
	0	0	(+)	+	+(+)	++	+++	+++
Cow Calf	0	0	(+)	+	+	+(+)	++	+++
Terp 97	0	0	0	(+)	+	+(+)	++	++
Calf.....	0	0	(+)	+	+(+)	++	+++	+++
Terp 149.....	0	0	0	(+)	+	+(+)	+(+)	++
Calf 1 C. W.	0	0	0	(+)	+	+(+)	+(+)	++
	0	0	0	0	0	(+)	+(+)	++
Calf 12 H. A.....	0	0	0	0	0	(+)	+	+(+)
	0	0	0	0	+	+	++	+++
Cow 97	0	0	(+)	+	+(+)	++	+++	+++
	0	0	0	(+)	+(+)	++	+++	+++
Bull Terp.....	0	(+)	+	++	+++	+++	+++	+++
	0	0	0	(+)	+	+(+)	++	+++
Cow Calf	0	0	(+)	+	+(+)	++	+++	+++
Terp 164	0	0	0	(+)	+	+(+)	++	++
Calf 66 B. L.....	0	0	0	0	(+)	+	++	+++
	0	0	0	0	+	+(+)	++	+++
Bull	0	0	0	0	(+)	+	++	+++
Kallehave.....	0	0	0	(+)	+	+(+)	+++	+++
Cow 75	0	0	0	(+)	+	+(+)	++	+++
	0	0	0	(+)	+	+(+)	++	+++

X a.

Saliva on Human Iso-anti-A and on Anti-A preformed in and on Anti-A preformed in Cow Serum. Method: Inhibition glutination.

Titer n											Serum
10	11	12	13	14	15	16	17	18	19	20	
0	0	0	0	0	0	0	0	(+)	+	+(+)	Ulla $\frac{1}{16}$
0	0	0	0	0	0	0	(+)	(+)	+	+(+)	Cow2 $\frac{1}{8}$
++	+++	+++	..	..	..	..	..	..	..	..	Ulla
+++	+++	+++	..	..	..	..	..	..	..	..	Cow 2
..	..	..	..	..	..	..	..	..	..	..	Ulla
..	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	+++	..	..	..	..	..	..	..	..	..	Ulla
+++	+++	..	..	..	..	..	..	..	..	..	Cow 2
+++	+++	..	..	..	..	..	..	..	..	..	Ulla
+++	+++	..	..	..	..	..	..	..	..	..	Cow 2
++	+++	+++	..	..	..	..	..	..	..	..	Ulla
+++	+++	+++	..	..	..	..	..	..	..	..	Cow 2
..	..	..	..	..	..	..	..	..	..	..	Ulla
..	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	+++	..	..	..	..	..	..	..	..	..	Ulla
+++	+++	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2

b. Table

Human Saliva from:	5	6	7	8	9	10	11
S. Group A_1	0	0	0	0	0	0	(+)
	0	0	0	0	0	(+)	(+)
K. Group A_1.....	0	0	0	(+)	+	+(+)	++
	0	0	0	(+)	+	+(+)	(+)
L. Group A_1.....	0	0	0	0	0	(+)	+
	0	0	0	0	0	+	+(+)
K. Group A_1.....	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
M. S. Group A_1	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
H. Group A_1	0	0	0	0	0	0	0
	0	0	0	0	0	0	(+)
V. F. Group A_1	0	0	0	0	0	0	(+)
	0	0	0	0	0	0	+
K. B. Group A_1	0	0	0	0	0	0	0
	0	0	0	0	0	0	(+)

Otherwise, the assumption of a barrier between the two substances is perhaps not necessary at all. In fact, HEIDELBERGER and coworkers, working with the purified specific polysaccharide of Type III pneumococcus as antigen, found that when certain assumptions were made the law of mass action might be applied to the antigen-antibody reaction in a precipitation test. Here it should be noted that in our case as in that of HEIDELBERGER the antigen forms a constituent of a solution so that ionic processes are not precluded. Otherwise there is no reason for believing that the antigen-antibody reaction should take place in an essentially different way in vivo than in the precipitation. Unfortunately, however, it is not feasible with the methods

X b.

Titer *n*							Serum
12	13	14	15	16	17	18	
+	+(+)	++	+++	+++	..	..	Ulla
+	+(+)	++	+++	+++	..	..	Cow 2
+++	+++	+++	..	..	..	..	Ulla
++	+++	+++	..	..	..	..	Cow 2
+(+)	+++	+++	+++	..	..	..	Ulla
+(+)	++	+++	+++	..	..	..	Cow 2
(+)	+	+(+)	++	+++	+++	+++	Ulla
0	(+)	+	+(+)	(+)	++	+++	Cow 2
0	0	0	0	0	(+)	+(+)	Ulla
0	0	0	0	0	0	+	Cow 2
+(+)	+(+)	++	+++	+++	..	..	Ulla
+	+(+)	+++	+++	+++	..	..	Cow 2
+	++	++	+++	+++	..	..	Ulla
+(+)	+(+)	+++	+++	+++	..	..	Cow 2
0	(+)	+	++	+++	+++	..	Ulla
+	+	++	+++	+++	+++	..	Cow 2

available and the unknown quantities of antigen and antibody in the initial materials to settle the question whether the "hyperbolic" relation found between these two substances is derivable from HEIDELBERGER's theory or if other conceptions, such as that of a barrier, must be introduced.

10. Discussion and Summary.

The investigations on the A-antigen in cattle gave as the main result that the antigen was to be considered as a species character of these animals. Further a number of analogies were disclosed between man and cows with regard to the distribution of this antigen. These analogies,

however, exist only in the case of water-soluble antigen, while the distribution of alcohol-soluble antigen exhibited remarkable differences from that found in man. The analogies with regard to the water-soluble A-antigen are:

1) If a cow has in its saliva an amount of A-antigen comparable to that found in the saliva from a human A_1 secreter, then the distribution of the A-antigen in the various organs of this cow is also found to agree quantitatively with the distribution in the same organs from the human A_1 secreter.

2) The concentration of antigen in saliva from calves is higher than that found for the adult animals. This corresponds to the phenomenon found for non-secreter babies of group A and B, who would seem to have, at least as a rule, a certain amount of antigen in their saliva, this antigen, however, disappearing during the first year.

3) From investigations on aqueous extracts of duodenum the impression is gained that the A-antigen in cows consists of two or three quantitatively different types. This is an analogy to the $A_1 - A_2 - A_3 - (A_4)$ system of the human group antigen A.

On the basis of these analogies it may be considered highly probable that the genetics of the water-soluble antigen in cows are essentially the same as those of the A-antigen in man. That is to say, we may expect the development of the A-antigen to depend on multiple allelomorphs similar to those determining the A_1, A_2 and A_3 qualities in man. It should here be noted that this conclusion could not be drawn with certainty on the basis of the genetic investigations on saliva, owing to the complications arising from the particular conditions in calves. That is to say,

the possibility is not precluded that the heredity might depend on a limited number of multiple factors, seeing that such an heredity could certainly give rise to quantitatively distinguishable A-types. We pass on to consider the differences between the A-antigen in man and in cattle. These differences chiefly concern the distribution of the alcohol-soluble antigens throughout the organism. While in man alcohol-soluble antigens are found in most of the organs and particularly in the blood corpuscles, they are in cattle found only in organs belonging to the digestive system and no trace at all can be demonstrated in the blood corpuscles. A similar difference is found with regard to the contents of anti-A in serum from man and cattle. In man either A-antigen or anti-A obtain, the two substances never existing together in the same subject. Otherwise in cattle, where the two substances are found in the same subject. This curious difference was explained by the assumption of a barrier or wall of cells separating the two substances in cattle. It proved impossible to distinguish between the anti-A in cattle and the iso-anti-A in man with regard to their effects on saliva from man and cows. This would seem to be a fact of considerable interest and the more so since a distinct difference obtains in the reactions of cow saliva and human saliva with human immune anti-A. (FRIEDENREICH). While human saliva scarcely reacts with immune serum produced by immunisation of rabbits with human A_1 blood corpuscles, the reaction of cow saliva is as pronounced with such an immune serum as with human iso-sera.

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