A Comparison between the Paper Electrophoresis and the Widal-Agglutination in Chronic Alcoholics[1]

By *Sven Dahl*[2]

Summary

312 male chronic alcoholists without clinical symptons of cirrhosis hepatis are inoculated with heat-killed typhus-paratyphus bacilli. The result of the inoculations is compared with the serum proteins of the patients. A connection between the O-antigens and the γ-globulins is established, and also between specific and unspecific H-antigens and the (α-) β-globulins.

Zusammenfassung

312 männliche chronische Alkoholiker ohne klinische Anzeichen einer Leberzirrhose wurden mit durch Hitze abgetöteten Typhus-Paratyphus-Bazillen geimpft. Das Resultat der Impfungen wurde mit den Serum-Proteinen der Patienten in Beziehung gesetzt. Es ergab sich eine Beziehung zwischen den O-Antigenen und den γ-Globulinen sowie zwischen den spezifischen und unspezifischen H-Antigenen und den (α-) β-Globulinen.

It has been shown in earlier works that the production of anti-substances in chronic alcoholics is not affected by damages to the liver (parenchym-cells), and that patients with anaemia have fewer circulating anti-substances than patients with a normal haemoglobin percentage. Further, patients with a higher fasting blood sugar value than 130 mg% seem to have a reduction of anti-substances in the blood.

Chronic alcoholics, who have not developed cirrhosis hepatis, show distinct changes in the protein fractions of the serum in the period of abstinence. Generally speaking, it can be seen a reduction of most of the globulin fractions. As the anti-substances mostly are "bound" to the γ-globulins and to some of the β-globulins, I have tried, in my investigations, to acquire a knowledge about the relations between the serum globulins and the production of anti-substance after the inoculation with a mixed typhus-paratyphus vaccine. A description of the procedure of the examinations, the vaccine, and the patient material will be found in other works (1, 2).

It should be mentioned here, that the vaccine contains about 2000 million bacilli killed by heat, and that 50% are s.typhi, 25% s.paratyphi A, 12,5% s.paratyphi B, and 12,5% s.Newport. The patients were typical alcoholics in the ages between 20 and 70 years, and the average age was 44½ years. Once a week they were given injections in the following doses: 0,25 ml, 0,25 ml, and 0,50 ml. Widal and serum for protein analyses were taken before the inoculations and 10 days after the last injection.

[1] Second part of "Clinical Laboratory Investigations on Male Chronic Alcoholics".

[2] Chief physician, MD, Hällnäs Vårdhem för kroniske Alkoholister, Fack 16, Hällnäs 2, Sweden.

Results

The patients are divided into small groups according to the concentrations of globulin in the serum. α_1-, α_2-, and the β-globulins were put together in a pool, which was again subdivided into 3 groups. The γ-globulins were subdivided into 2 groups, where 1,0% was the dividing line.

An examination of the numbers of positive and negative agglutinations for the specific H-antigen in Table 1 will show that there is hardly any difference

Table 1

Globulin fractions	Number of titres	Salmonella typhi			Salmonella paratyphi-A			Salmonella paratyphi-B			Salmonella Newport		
		unsp. H	H	O	unsp. H	H	O	unsp. H	H	O	unsp. H	H	O
a^1–a^2–, and β-globulins totalling between 0,1 and 0,5 g%	positive	28	0	3	7	0	0	14	1	2	4	14	0
	negative	15	43	40	36	43	43	29	42	41	39	29	43
The same totalling between 0,6 and 1,0 g%	positive	109	2	12	37	1	0	47	1	9	19	63	0
	negative	44	151	141	116	152	153	106	152	144	134	90	153
The same totalling above 1,1 g% . . .	positive	36	2	9	9	0	0	15	1	1	6	23	0
	negative	14	48	41	41	50	50	35	49	49	44	27	50
γ-globulins 1,0 g% and under	positive	132	2	14	48	1	0	57	2	8	21	71	0
	negative	46	176	164	130	177	178	121	176	170	157	107	178
γ-globulins 1,1 g% and over	positive	38	2	11	9	0	0	21	1	4	7	25	0
	negative	20	56	47	49	58	58	37	57	54	51	33	58

between the 5 groups of patients for s.paratyphi B and s.paratyphi Newport. For s.paratyphi B there are about twice as many negative titres as positive, whether there is a low concentration of the various globulins or a high concentration. For s.Newport the ratio between positive and negative titres is about 1:7, although the number of negative agglutinations is increased if the α-β-globulins have extremely low values (between 0,1 and 0,5 g%). For s.Newport many unspecific H-antigen agglutinations will be found. The ratio between the number of negative and positive titres seems here independent of the concentration of the γ-globulins, but dependent on the α-β-globulin concentration. If this concentration is high, the ratio positive:negative titre is 1:1, but if the concentration is very low, the ratio is 1:2. This fact with regard to s.Newport might indicate that the anti-substances against unspecific H-antigen are to be found among the β-globulins, as for the time being I leave out of account the α-globulins as carriers of anti-substances.

If the negative Widal reactions are left out of account, and if only the positive titres against the H-antigens (specific as well as unspecific) are taken

Table 2

		Comparison between the Widal and the α_1- + α_2- + β-globulins						Comparison between the Widal and γ-globulins			
		$\alpha_1+\alpha_2+\beta$ total 0,1–0,5 g%		$\alpha_1+\alpha_2+\beta$ total 0,6–1,0 g%		$\alpha_1+\alpha_2+\beta$ total 1,1 g% and over		γ-globulins 1,0 g % and under		γ-globulins 1,1 g% and over	
		number	per cent	number	per cent	number	per cent	number	per cent	number	per cent
table 2A	Number of positive H-titres	53	72,6	212	70,8	72	65,5	258	72,5	75	63,5
	Number of positive O-titres	5	6,8	21	7,0	10	9,1	22	6,2	15	12,7
	Number of positive unspecific H-titres	15	20,6	67	22,2	28	25,4	76	21,3	28	23,8
	Total	73	100,0	300	100,0	110	100,0	356	100,0	118	100,0
table 2B	negative in all 4 "types"	11	25,6	30	19,6	3	6,0	32	17,9	8	13,8
	positive in 1 type	14	32,7	53	35,8	18	36,0	60	33,7	21	36,2
	positive in 2 types	6	13,9	27	18,5	12	24,0	30	16,8	14	24,1
	positive in 3 types	9	20,9	20	12,1	14	28,0	30	16,8	11	19,0
	positive in 4 types	3	6,9	23	14,0	3	6,0	26	14,8	4	6,9
	Total	43	100,0	153	100,0	50	100,0	178	100,0	58	100,0
table 2C	0 positive titres	11	25,6	30	19,6	3	6,0	32	17,9	8	13,8
	1 positive titre	13	30,3	51	33,5	18	36,0	59	38,9	19	32,7
	2 positive titres	5	11,6	20	13,1	7	14,0	23	11,2	8	13,8
	3 positive titres	8	18,6	21	13,7	13	26,0	28	13,5	11	19,0
	4 positive titres	5	11,6	16	10,4	6	12,0	21	10,1	8	13,8
	5 positive titres			11	7,2	3	6,0	10	5,6	4	6,9
	6 positive titres	1	2,3	1	0,6			2	1,1		
	7 positive titres			3	1,9			3	1,7		
	Total	43	100,0	153	100,0	50	100,0	178	100,0	58	100,0
table 2D	Number of titres of $^1/_{40}$	17	23,3	83	27,7	26	23,6	96	26,9	26	22,0
	Number of titres of $^1/_{80}$	22	30,1	78	26,0	31	28,2	96	26,9	34	29,0
	Number of titres of $^1/_{160}$	14	19,2	48	16,0	18	16,4	60	17,8	19	16,1
	Number of titres of $^1/_{320}$	14	19,2	38	12,7	20	18,2	53	13,1	16	13,5
	Number of titres of $^1/_{640}$	4	5,5	29	9,7	4	3,6	25	8,0	12	10,2
	Number of titres of $^1/_{1280}$	2	2,7	12	4,0	6	5,5	15	4,2	5	4,2
	Number of titres of $^1/_{2560}$			7	2,3	3	2,7	6	1,7	4	3,4
	Number of titres of $^1/_{5120}$			4	1,3	1	0,9	4	1,1	1	0,8
	Number of titres of $^1/_{10240}$			1	0,3	1	0,9	1	0,3	1	0,8
	Total	73	100,0	300	100,0	110	100,0	356	100,0	118	100,0

into consideration it will be seen that there is no significant difference between the patients who have low globulin titres, and those who have high ones. It seems as if the patients with low globulin concentrations have larger amounts of circulating anti-substances against the H-antigens (Table 2A).

The same tendency is seen for s.typhi and s.paratyphi A, that is that the α-β-globulin concentration is of importance for the amount of anti-substances against the H-antigens, while it is doubtful whether the γ-globulins have any importance as carriers of anti-substances against these antigens.

It is rather difficult to form an opinion about the place among the globulins where the anti-substances against the O-antigens are to be found. An examination of s.typhi would make it possible that both the β- and the γ-globulins could come into consideration here, as high concentrations of these proteins give slightly fewer negative titres (Table 1). If, however, the positive agglutinations against the O-antigens are considered by themselves (Table 2A), it is not possible to see any certain connection with the β-globulins, while the connection between the O-antigens and γ-globulins is significant.

The majority of the patients inoculated give positive reactions against only one type of salmonella, while relatively few give positive Widal to all 4 types (Table 2B). The number of negative reactions to all 4 types is 25%, when the concentration of α-β-globulins is extremely low, while the number of negative reactions is reduced to 6% when the concentration of α-β-globulins is more than 1,1%. The numbers of negative Widal reactions to all 4 types by low and high concentration of the γ-globulin are respectively 17,9 and 13,8%, that is, on the border of what is certain. A glance at the positive Widal reactions to the individual types of salmonella will show that the difference in the number at low and high concentrations of γ-globulins is on the border of the significant, while there is a clear difference whether the concentration of the α-β-globulins is low or high (Table 2B).

If a patient has a positive titre with one of the 4 types of salmonella, he reacts in most cases only against the H-antigen. In theory it should be possible to obtain 12 antigen-reactions when inoculating with 4 salmonella bacilli, but in practice it is very few of them who get 6 or 7 positive agglutinations. Among the nearly 600 patients I have been able to follow, the maximum positive agglutinations in one patient have not exceeded 6 or 7.

A study of the material given here after a division of the number of positive titres will show that the anti-substances are rather to be found among the α-β-globulins than among the γ-globulins (Table 2C). The material is further subdivided according to the dilutions of the anti-substances which gave positive deposits (Table 2D). An increase of the titres from $^1/_{320}$ to $^1/_{10240}$ will be found at higher concentrations of the α- and β-globulins, and of the γ-globulins, which indicates an increase of the circulating anti-substances. This leads to the supposition that the production of anti-substances is some-

what more "vigorous" than in patients with lower globulin concentrations in the serum.

The investigations of serum-protein in the chronic alcoholics offered several surprises (also described in other works [4, 5, 6]), as there were found considerable changes both in the albumins and in the globulins. For this reason I have thought that I had to compare the results of the inoculations with the ratio albumin/globulin (in the following written alb/glob). This index is normally between 1,5 and 2,0 (Hammersten), but it will be seen from Table 3 that very few of the alcoholics I examined had an index under 2,0. Out of the 360 patients in the age between 20 and 70 years there were 27 patients who had so low values. This is due to the fact that the amount of globulin had decreased at the same time as the albumin values were rather high. In my material I have taken for granted that the alb/glob index may be 3,0 without having to be considered as abnormal, which again means that 104 patients out of 360 had normal relations between the serum protein fractions.

If the alb/glob ratio is normal, positive Widals are more often found for s.typhi and for s.paratyphi (Table 4). If the number of negative agglutinations

Table 3 (Maximum albumins/globulins was 12,8; minimum 1,0)

Age	Index: albumins / globulins										
	1,0–1,9		2,0–2,9		3,0–4,9		5,0–9,9		10,0 and over		total
	number	per cent	number	per cent	number	per cent	number	per cent	number	per cent	
20–30	3	7,3	7	17,1	22	53,6	9	22,0			41
31–40	6	5,6	24	22,4	48	44,6	27	25,5	2	1,9	107
41–50	11	10,2	22	20,4	53	49,0	19	17,6	3	2,8	108
51–60	5	6,6	13	17,1	48	63,2	7	9,2	3	3,9	76
61–70	2	7,2	11	39,4	10	35,8	4	14,1	1	3,5	28
total	27		77		181		66		9		360

Table 4

Albumin / globulin	Number of	Salmonella typhi			Salmonella paratyphi-A			Salmonella paratyphi-B			Salmonella Newport		
		unsp. H	H	O	unsp. H	H	O	unsp. H	H	O	unsp. H	H	O
1,0–1,9	positive	17	1	5	5	0	0	8	1	1	3	13	0
	negative	10	26	22	22	27	27	19	26	26	24	14	27
2,0–2,9	positive	31	1	7	10	0	1	15	0	3	8	27	0
	negative	27	57	51	48	58	57	43	58	55	50	31	58
3,0–4,9	positive	107	2	13	36	1	0	46	1	8	16	50	0
	negative	50	155	144	121	156	157	111	156	149	141	107	157
5,0–9,9	positive	30	0	2	9	1	0	14	1	1	3	15	0
	negative	31	61	59	52	60	61	47	60	60	58	46	61
10,0 and over	positive	4	0	1	2	0	0	0	0	0	0	4	0
	negative	5	9	8	7	9	9	9	9	9	9	5	9

Table 5

		Albumine/globulin ratio											
		1,0–1,9		2,0–2,9		3,0–4,9		5,0–9,9		10,0 and over		1,0–2,9		3,0–12,8	
		number	per cent	number	per cent	number	per cent	number	per cent	number	per cent	number	per cent	number	per cent
table 5A	negative in all "types"	6	22,2	16	27,6	44	28,0	27	44,4	3	33,3	22	25,9	74	32,6
	positive in 1 type	8	29,7	14	24,1	47	29,9	16	26,2	4	44,5	22	25,9	67	29,5
	positive in 2 types	5	18,5	13	22,4	23	14,6	6	9,8			18	21,2	29	12,8
	positive in 3 types	6	22,2	12	20,7	21	13,5	7	11,4	2	22,2	18	21,2	30	13,2
	positive in 4 types	2	7,4	3	5,2	22	14,0	5	8,2			5	5,8	27	11,9
	Total	27	100,0	58	100,0	157	100,0	61	100,0	9	100,0	85	100,0	227	100,0
table 5B	number of positive H-titres	33	61,0	64	63,0	205	73,2	56	73,7	6	54,5	97	60,8	267	72,8
	number of positive O-titres	6	11,1	11	9,8	21	7,5	3	3,9	1	9,1	17	10,8	25	6,8
	number of positive unspecific H-titres	15	27,9	28	27,2	54	19,3	17	22,4	4	36,4	43	28,4	75	20,4
	Total	54	100,0	103	100,0	280	100,0	76	100,0	11	100,0	157	100,0	367	100,0
table 5C	negative in all titres	6	22,2	16	27,7	44	28,0	27	44,4	3	33,3	22	25,9	74	32,6
	1 positive titre	8	29,7	14	24,1	44	28,0	15	24,6	4	44,5	22	25,9	63	27,5
	2 positive titres	2	7,4	8	13,8	20	13,0	6	9,8			10	11,6	26	11,0
	3 positive titres	6	22,2	10	17,2	20	13,0	6	9,8	1	11,1	16	19,5	27	11,8
	4 positive titres	1	3,7	9	15,5	15	9,1	5	8,2	1	11,1	10	11,6	21	9,2
	5 positive titres	4	14,8			11	7,1	1	1,6			4	4,4	12	5,3
	6 positive titres					1	0,6	1	1,6					2	0,9
	7 positive titres					2	1,2							2	0,9
	8–12 positive titres														
	Total	27	100,0	58	100,0	157	100,0	61	100,0	9	100,0	85	100,0	227	100,0
table 5D	number of titres of $^1/_{40}$	9	16,7	30	29,1	84	31,0	17	22,4	5	45,4	39	24,9	106	29,0
	number of titres of $^1/_{80}$	16	29,5	34	33,2	65	22,8	23	30,3	3	27,3	50	31,8	91	24,8
	number of titres of $^1/_{160}$	9	16,7	13	12,6	45	15,7	16	21,0			22	14,0	61	16,6
	number of titres of $^1/_{320}$	6	11,1	16	15,5	41	14,6	8	10,5	2	18,2	22	14,0	51	13,9
	number of titres of $^1/_{640}$	5	9,3	5	4,9	24	8,6	7	9,2	1	9,1	10	6,4	32	8,7
	number of titres of $^1/_{1280}$	4	7,4	3	2,9	10	3,5	4	5,3			7	4,5	14	3,8
	number of titres of $^1/_{2560}$	3	5,7	1	0,9	5	1,7	1	1,3			4	2,6	6	1,6
	number of titres of $^1/_{5120}$	1	1,8			6	2,1					1	0,6	6	1,6
	number of titres of $^1/_{10240}$	1	1,8	1	0,9							2	1,2		
	Total	54	100,0	103	100,0	280	100,0	76	100,0	11	100,0	157	100,0	367	100,0

is taken into consideration it will be found that the number rises the more the index deviates from the normal (Tables 5A and 5C). At the same time it will be found that the number of positive titres against the O-antigen and the unspecific H-antigen are reduced, and that the number of positive titres against a specific H-antigen is increased (Tables 5B and 5C). This seems to indicate that the protein fractions which are carriers of anti-substances against unspecific H-antigens, and against O-antigens, are decreased in chronic alcoholics, while the protein fraction which carries the anti-substance against specific H-antigen, shows a tendency to increase.

The titre-values are not affected by the changes in the protein fractions, as the number of titres $^1/_{40} - {}^1/_{80} - {}^1/_{160}$ is exactly the same in patients with a normal alb/glob index, and in those who have pathological high index values (Table 5D).

Discussion

The investigation has shown that the anti-substances, which are produced by the inoculation against typhus-paratyphus of chronic alcoholics must be bound to different fractions of the serum proteins. There are many indications that the anti-substances against the O-antigens are to be found among the γ-globulins. Other of my series of investigations have made it probable that it is a fraction among the β_{2M}-globulins that is the carrier of the anti-substances against the specific H-antigen. Dr. P. Grabar has told me that he has found that β_{2M}-globulin was the specific anti-substance, which agreed well with my immuno-electrophoresis investigations, which often showed an increase of just this β-globulin fraction, where the other fractions had decreased in quantity. All things taken into consideration, there is much that indicates that the chief part of the anti-substances produced by means of an inoculation with heat-killed typhus-paratyphus bacilli, is to be found among the α-β-globulins, and that only the anti-substances against the O-antigens are to be found among the γ-globulins.

The question is whether the α-globulins can at all be carriers of anti-substances. In this connection I have started a series of investigations where I try to absorb the anti-substances from the serum, after which the remaining proteins in the "serum" exposed to an electrophoresis. These investigations are as yet only in their early beginning, but there are things that indicate that parts of the α-globulins are removed at the absorption. This may be due to faulty analysis, but it may also mean that there are specific anti-substances in this globulin fraction.

The investigations has shown that the amount of circulating anti-substances is not diminished because of alcoholism. On the contrary, series of investigations as yet unfinished, show that the amount of circulating anti-substances against the H-antigens is increased in these patients, compared with a group of people who never "touch" alcohol. I have had occasion to examine very few alcoholics

with cirrhosis hepatis. Here the amount of albumin in the serum is rather low, about 2,5–3,5 g%. These patients do not seem to differ from the other alcoholics with regard to circulating anti-substances. Everything thus indicates that the function of the liver-parenchym plays no part for the capacity of an organism to avert infections. In a way, this was to be expected, because the production of the anti-substances takes place, first and foremost, if not completely, in the cells which are grouped under the nomenclature of RES (the reticulo-endothelial system).

As the amount of macroglobulins is often increased in the serum of alcoholics, at the same time as the other globulin fractions on the whole show a diminished concentration (with certain reservations, however; for instance, the glyco-proteins show movements in both directions), it is permissible to think that the RES is directly affected by the abuse of alcohol. The increase of β_{2M} in particular might indicate a change of the permeability of the cells. Here it is possible to make certain parallel comparisons with the transaminases.

I cannot yet decide whether it is permissible to reason as Dr. Grabar does – that the increase of β_{2M} (that is, an increase of the H-agglutination) – is a measure for the alcoholism of the patient – but it is not impossible.

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