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NATURALLY OCCURRING ANTIBODIES AGAINST COLICINES IN HUMAN SERA

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DURING the last 15 yr, several studies have been made of the antigenicity of colicines. Hamon (1957) immunised rabbits with the supernatants of chloroform-killed colicinogenic cultures, obtained sera with relatively low titres of activity against colicines, and considered bacteriocines to be weak antigens. Sera from rabbits that were immunised with chloroform-killed *Escherichia coli* K₂₃₅L₊O had very high titres for colicine-neutralising antibody (Amano, Goebel and Smidh, 1958; Goebel and Barry, 1958), but sera from horses (Goebel and Staub, 1965) had low titres. Some sera from rabbits (Hamon) and serum from one horse (Goebel and Staub) showed some neutralising activity for heterologous colicines. Senior and Emslie-Smith (1969) found homologous agglutinins and precipitins in the sera of rabbits that had been immunised with "free-colicine" preparations and with O-suspensions of strains producing group B colicines. More recently, Dandeu (1971) immunised rabbits with crude preparations of strains producing E, K, A, and Q colicines.

These reports indicate that antibodies can be formed against colicines under experimental conditions. The purpose of our study was to find out whether they occur naturally in sera from persons of various ages and particularly from patients with bacteriuria, because long-standing infection seems to be the most likely stimulus for the formation of these antibodies. We examined the sera of 20 young adults and 21 elderly persons for colicine-neutralising antibodies and for precipitins.

MATERIALS AND METHODS

Sera

Two groups of human sera were studied.

Group 1. These were 20 unheated sera from healthy adults of both sexes aged 19–26 yr.

Group 2. These were 21 unheated sera from persons of both sexes aged 67–89 yr, of whom

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11 were bacteriuric and 10 were non-bacteriuric. All the bacteriuric subjects had had positive urine cultures for at least 3 yr and had impaired renal function as defined by Dontas *et al.* (1966, 1968).

Bacterial strains

Colicinogenic strains. We used strains producing colicines A, B, C, D, E, F, G, H, I, J+I, K, S₃+I, S₄, S₅ (Fredericq, 1948), L (Hauduroy and Papavassiliou, 1962), X (Papavassiliou, 1961a), V (Gratia, 1925) and Va (Mandel, personal communication). *Escherichia coli* strain Row was used as the indicator strain for the colicine assays (Papavassiliou, 1961a).

Strains isolated from faeces and urine. One hundred and fifty-one strains of coliform bacilli were isolated from faeces and from quantitative cultures of urine. All were examined for colicine production (Papavassiliou, 1961b) by the double-layer method (Fredericq, 1948, 1957). The colicines were typed by their action on a set of resistant mutants (Papavassiliou, 1960) and by an agar-diffusion method (Tzannetis, 1965).

Colicine neutralisation test

A sterile strip of filter-paper (Eaton no. 613, Dickman Co.) 12.5 × 2.0 cm was put on the surface of dried soft nutrient agar in a petri dish. The nutrient agar contained Difco Nutrient Broth (B3), 8 g per l, and Oxoid Ionagar no. 2, 8 g per l. For each serum to be tested, 1.5 ml was carefully spread on one paper strip; overflow was avoided. After the serum had been completely absorbed, 24-hr cultures of the set of colicinogenic strains in Difco Nutrient Broth were stabbed into the agar with a straight wire, each 2.5–3.0 cm from the side of the paper strip and a similar distance from adjacent stabs. Plates were incubated for 48 hr at 37°C to allow diffusion and interaction of serum and colicines. The growth that had developed by this time was killed by exposure to chloroform vapour for 45 min., and the plates were left open on the bench at room temperature for another 45 min. to allow the vapour to disperse. The medium was then overlaid with 7.0 ml of molten soft agar seeded with 1 ml of a Difco Nutrient Broth culture of the indicator strain in the logarithmic phase of growth (Fredericq, 1948, 1957; Papavassiliou, 1961b). Plates were incubated overnight, and the diameters of inhibition areas in the growth of the indicator strain were measured. Any reduction in diameter more than 25 per cent. of that in the controls was regarded as indicating neutralisation of colicine (Goebel and Staub).

A control was included in each colicine neutralisation test, with sterile saline 0.85 per cent. w/v instead of serum in the filter-paper. The influence on the indicator strain of heated sera (56°C for ½ hr) from 15 healthy persons compared with the same sera, unheated, was also examined (Michael and Braun, 1964). This preliminary test served as the indicator-strain control and was performed as follows: 3-mm holes in agar plates were filled with two-fold dilutions (1 in 2–1 in 8) of the sera tested. After the diluted sera were absorbed, the indicator strain was overlaid in molten soft agar, as in colicine neutralisation tests, and the plates were read after overnight incubation at 37°C. Only one of the 15 unheated human sera slightly inhibited the growth of the indicator strain; we therefore did not heat the sera in the rest of our experiments and considered any inhibition of growth of the indicator strain to be due to colicines.

Precipitin reactions

These were performed by the gel-precipitin method (Elek, 1948; Ouchterlony, 1948) in plates of soft agar. The colicinogenic strains were stabbed into the agar and the plates were incubated for 48 hr at 37°C. Holes were then cut in the agar 1 cm from each other in a circle of radius 3 cm around the stab inocula. Sera were placed in the holes; after they had been absorbed the plates were incubated for 24 hr at 37°C. Results were recorded at that time and after a further 24 hr at 4°C. All the sera were examined for activity against the 18 colicinogenic strains.

RESULTS

Sera from elderly bacteriuric subjects

Sera from subjects 6 and 7 exerted a very strong anti-colicine activity (table I). Neutralisation of 14 and 11 types of colicine respectively by these sera ranged from 25 to 100 per cent. Two other sera (nos. 8 and 3) neutralised four and three colicines respectively, while serum no. 9 neutralised only one. The other six sera did not neutralise any colicine. Colicines D and K were neutralised by more sera than the others, while J+I and S₃+I were not neutralised by any of the sera of this group.

Of the 35 coliform strains isolated from 11 cultures of urine from these subjects, only one was colicinogenic, and it produced a non-typable colicine. The

TABLE I

Neutralisation of type-colicines by 11 human sera obtained from elderly bacteriuric subjects

Serum from subject no.	Percentage reduction of diameter of inhibition zone from colicine type																	
	A	B	C	D	E	F	G	H	I	J+I	K	L	S ₃ +I	S ₄	S ₅	X	V	Va
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	100	0	30	0	0	0	0	33	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	76	74	45	100	59	0	100	100	0	0	40	38	0	55	57	100	100	58
7	0	74	0	100	0	0	100	100	62	0	25	45	0	65	65	0	42	100
8	0	0	27	36	41	0	0	0	0	0	100	0	0	0	0	0	0	0
9	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	<25	<25	0	0	0	0	0	0	0	0	0	<25	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<25	0	0

corresponding serum showed no activity against any of the colicines. Of the 69 coliform strains isolated from 11 cultures of faeces, 44 were colicinogenic and produced various typable, mixtures of typable, and untypable colicines (table II).

The isolation of colicinogenic strains from the faeces was usually associated with neutralising activity of the sera. In only three instances (subjects 1, 2 and 10) was this not so. Sera from subjects 6 and 7 formed an intense precipitation line with most of the colicinogenic strains against which they were tested. These sera were those that had shown the greatest activity in the neutralisation tests.

Sera from elderly non-bacteriuric subjects

One serum, from subject no. 6, exerted high neutralising activity (45–100 per cent.) against all colicines tested (table III). Two other sera (subjects 1 and 10) neutralised 10 and 14 of the colicines respectively. Sera from subject no. 9 neutralised four colicines, serum from subject no. 3 neutralised two, and sera from subjects 4 and 8 neutralised one colicine each (colicine B). Colicines H, L, X, V, B, C, D, E, K, S₄, S₅ and Va were more often neutralised than the others. All the colicines were neutralised by at least one of the sera tested.

Of the 47 coliform strains isolated from ten cultures of faeces, 23 were colicinogenic (table IV). Most of these strains produced untypable colicines and some of them colicines E and K. Neutralising activity of the sera was

TABLE II

Relationship between the presence of colicinogenic strains in the faeces and neutralising activity of the serum from bacteriuric subjects

Subject number	Faeces		Serum	
	Number of coliform strains		Neutralising activity†	
	examined	colicinogenic		
1	5	5	V + B (5)	—
2	5	5	E (5)	—
3	5	3	NT (3)	+
4	5	0	...	—
5	5	0	...	—
6	12	12	E (10); V + E (2)	+
7	12	7	K (4); NT (2); E (1)	+
8	5	5	B (4); V + B (1)	+
9	5	2	NT (2)	+
10	5	5	K (5)	—
11	5	0	...	—
All	69	44	E (16); K (9); NT (7); V + B (6); B (4); V + E (2)	6—; 5 +

* Numbers in brackets indicate the number of colicinogenic strains.

† — = Neutralisation nil or <25 per cent.; + = neutralisation $\geq$ 25 per cent.

NT = Not typable.

TABLE III

Neutralisation of type-colicines by ten human sera obtained from elderly non-bacteriuric subjects

Serum from subject no.	Percentage reduction of diameter of inhibition zone from colicine type:																	
	A	B	C	D	E	F	G	H	I	J+I	K	L	S ₃ +I	S ₄	S ₅	X	V	Va
1	0	0	37	25	27	0	0	0	0	0	0	55	32	25	67	100	100	100
2	<25	0	0	0	0	0	0	0	0	0	0	<25	<25	<25	0	0	0	0
3	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	100	0	0
4	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	<25	<25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	69	58	68	100	41	37	100	100	100	45	90	80	58	90	66	100	100	100
7	<25	<25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	26	0	0	30	45	0	0	0	0	85	0
10	0	0	36	100	55	0	100	100	100	53	100	59	0	50	57	100	29	100

usually associated with the isolation of colicinogenic strains from the faeces. In two instances (subjects 5 and 7) the sera were inactive against any colicine despite the isolation of colicinogenic strains from the faeces; and in two other

instances (subjects 1 and 9) the opposite situation was the case. Sera from subjects 1, 6 and 10 formed intense precipitation lines with most of the colicines tested. These were the sera that had also shown the greatest activity in the neutralisation tests.

Sera from healthy young adults

None of the 20 sera from healthy young adults neutralised any of the 18 type-colicines, nor were any precipitins detected.

TABLE IV

Relation between the presence of colicinogenic strains in the faeces and neutralising activity in the serum of non-bacteriuric subjects

Subject number	Faeces		Serum
	Number of coliform strains		Neutralising activity†
	examined	colicinogenic	
1	0	...	+
2	5	0	—
3	5	1	+
4	5	4	+
5	5	5	—
6	2	2	+
7	5	5	—
8	6	3	+
9	10	0	+
10	4	3	+
All	47	23	NT (16); E (4); K (2); B+K (1)

* Numbers in brackets indicate the number of colicinogenic strains.

† — = Neutralisation nil or <25 per cent.; + = neutralisation $\geq$ 25 per cent.

NT = Not typable.

DISCUSSION

Previous workers (Hamon, 1957; Amano, Goebel and Smidth, 1958; Goebel and Barry, 1958; Goebel and Staub, 1965; Senior and Emslie-Smith, 1969; Dandeu, 1971) reported that colicines may act as antigens stimulating the production of homologous and occasionally of heterologous antibodies in experimental animals. The animals were actively immunised by intravenous injections of pure colicine, or a chloroform-killed culture of a colicinogenic strain.

Examining sera from elderly persons, we found that 12 out of 21 exerted a significantly high neutralising activity against various type-colicines, and five out of the 21 sera contained precipitins. However, 20 sera from healthy young adults contained neither neutralising antibodies nor precipitins against colicines.

The isolation of colicinogenic strains from the infected urine of elderly bacteriuric subjects was rare. This was probably the reason why sera from such people had anticolicine activity no greater than that from non-bacteriuric subjects.

The isolation of colicinogenic strains from the gut of elderly subjects does not in itself explain the neutralising activity of their sera, because sera from healthy adults generally gave negative results, while the occurrence of colicinogenic strains in the gut of young and of older subjects is nearly the same (Papavassiliou, 1960). The activity of the sera from older subjects could perhaps be explained by all or some of the following factors: prolonged time of harbouring colicinogenic strains; changes in the permeability of the gut; differences of immune response. We noticed that most of the sera examined could neutralise more than one colicine. This property was independent of the isolation of colicinogenic strains from the gut that produced the corresponding colicines. These discrepancies might be attributed to the following: failure to isolate colicinogenic strains producing other types of colicines, because usually only a small proportion of the coliforms isolated from the gut produce colicines (Papavassiliou, 1961*b*, 1963); changes of the colicinogenic flora of the gut (Sears *et al.*, 1956; Lemcke and Hurst, 1961; Tadd and Hurst, 1961); production of non-typable colicines or mixtures of colicines; the related chemical or antigenic structure of different colicines (Goebel and Staub; Hamon and Peron, 1966; Dandeu). Quantitative differences of the neutralising activity and the higher frequency of neutralisation of the type-colicines by sera from non-bacteriuric subjects are difficult to explain.

SUMMARY

Of 21 human sera from elderly bacteriuric and non-bacteriuric subjects, 12 exerted a significant neutralising activity against 18 type-colicines. The presence of precipitins was demonstrated in five out of the 21 sera.

Sera from 20 healthy young adults contained neither neutralising antibodies nor precipitins against type-colicines. The mode of immunisation via the gut and the probable factors influencing this mechanism are discussed. There was no evidence of immunisation via the infected urinary tract in older bacteriuric subjects.

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