ANTAGONISMS IN THE GENUS ESCHERICHIA IN RELATION TO ANTIGENIC COMPOSITION

by

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Introduction.

The genus Escherichia is a member of the family Enterobacteriaceae Rahn, a complex group of plant and animal parasites and saprophytes. Three of the five tribes in the family (Escherichieae, Proteae and Salmonelleae) may be found in the gastro-intestinal tract of man and occasionally of other vertebrates. The other two tribes (Erwineae and Serrateae) are plant parasites and saprophytes respectively. The organisms included in this family, though somewhat unrelated in habitat and host relationship, are grouped together because they are all gram-negative straight rods, attack glucose forming acid, or acid and gas, and produce nitrites from nitrates.

The genus Escherichia, as defined in Bergey's "Manual of Determinative Bacteriology" (1), consists of gram-negative, non spore-forming straight rods which will ferment lactose with the formation of acid and gas within 24 hours at 37° C., do not produce acetylmethylcarbinol, give a positive methyl red test, and may, or may not, utilize salts of citric acid as their sole

source of carbon. At the present time three species are recognized as belonging to this genus. These species are differentiated on their ability to utilize citrate and to produce hydrogen sulfide. The type species, Escherichia coli, with which this paper is principally concerned, cannot utilize citrate and does not produce hydrogen sulfide. The other two species, Escherichia intermedium and Escherichia freundii, utilize citrate and the latter is also able to produce hydrogen sulfide.

Escherichia coli is a normal inhabitant of the gastro-intestinal tract of man and other vertebrates, establishing its residency shortly after birth and occupying the intestine until death. Because E. coli is exclusively an animal intestinal parasite, it is used by most sanitarians as an indicator of fecal pollution.

E. coli, though apparently non-pathogenic in the intestine of adult animals, has great disease producing potentialities. It has been isolated as the etiological agent of infectious diarrhea of new born, and of diarrheic diseases of the young of lower animals. It is one of the most common agents producing urinary tract infections in man and has been incriminated in cases of meningitis, arthritis, sepsis neonatorum and occasionally in other types of infections.

Many attempts have been made to develop a satisfactory method of classification of the group of organisms to which the genus Escherichia belongs. Most schemes have been based on the biochemical properties of these organisms. MacConkey (2) was the first to outline a useful method. He established four main groups based on the fermentation of sucrose and dulcitol. Classification into subgroups was made on the basis of motility, gelatin liquifaction, indole production and the Voges-Proskauer reaction. The biochemical method of classification has been revised and refined by many investigators so that at the present time four tests are most commonly used. (3) These tests, indole production, methyl red test, Voges-Proskauer reaction (production of acetylmethylcarbinol), and citrate utilization, are known as the "IMViC reactions". These, with the fermentation of cellobiose, have been used by Stuart, Griffin, and Baker (4) to classify a large number of coliform bacteria, with interesting but not particularly useful results, because of the nutritional and biochemical instability of these organisms. Stuart (5) has stated, "The types within the group and even the groups are not wholly constant in their biochemical reactions. Shifts in type occur in orderly sequence within any group, with the occasional shifts from one group to another." Parr

(6) has also investigated the problem of biochemical stability in these organisms principally in relation to citrate utilization. He noted citrate mutants in 1934 and has been able to induce their production by special cultural methods.

The successful use of antigenic structure in classification of the Salmonellas (7) has stimulated similar studies of the Escherichias. Stuart and his co-workers (5) made such a study but concluded that attempts to differentiate antigenically beyond the basic type in a group would be defeated by a vast number of intergrades. The discovery and study of the "K" antigens by Kauffmann (8), Knipschildt (9), and Vahlne (10) however, introduced a new element into the antigenic picture. These "K" antigens, a group of capsular and surface, or envelope, antigens, had previously confused the problem because organisms possessing these factors are inagglutinable in anti-O serums. Two of the three antigens in the K group (L and B antigens) are thermolabile and can be destroyed by heating the culture to 100° C., The other antigen (A) is relatively thermostable. Knowledge of the K antigens has made possible an exact determination of the O antigens (body antigens) in the coli group of organisms. Kauffmann(8), Knipschildt (9), and Vahlne (10) have made such a study and have set up an antigenic

schema, making use of the O, H, and K antigens, by means of which coli strains from any source may be classified into O groups and types within the groups. A total of 112 different O groups have been recognized, but except for groups 1-25, subclassification into types has not been accomplished. The schema promises eventually to be very extensive and complicated.

The phenomenon of antagonism is common among bacteria and higher forms and may vary in its mechanism from space antagonism, where the proximity of one organism is injurious to the growth of the other, to the production and liberation of specific substances which have a bacteriostatic or bactericidal effect on another organism. (11)

That some strains of E. coli have antagonistic activity has been known for 50 years or more. Lewek (12) in 1889, published a paper in which he recorded experiments showing that a gram negative intestinal bacillus inhibited the growth of B. anthracis. The organism was probably E. coli.

In the next decade many investigators reported on the antagonistic activity of E. coli against certain other organisms, principally S. typhi.

Eijkman (13), in 1904, showed that V. cholera would not grow on a solid medium upon which E. coli had

previously been grown, though the reverse was not the case. The work of Eijkman was confirmed in 1905 by Conradie and Kurpjuweit (14) who went on to claim that B. coli produced in broth an"antiseptic" substance which could not be filtered and was heat labile. They stated that their cultures were inhibitory in dilutions as high as 1:3200, a statement that Eijkman questioned.

Other investigators, notably Oebius (15) and Manteufel(16), though they obtained the same results as Conradie and Kurpjuweit, were of the opinion that exhaustion of the medium, not the presence of an "auto-toxin", was responsible for the effects noted. Eijkman (17) replied with an experiment which conclusively demonstrated that the effects obtained were not due to exhaustion of the medium.

Moro and Murath (18), in 1906, observed that feces from babies fed on mothers milk did not putrify even after several days and the addition of B. putrificus. They concluded that strong antibacterial substances were responsible for this effect, and that B. coli appeared to play the major role in the elaboration of these substances.

Nissle (19), in 1916, attempted to make a quantitative study of the antagonism exerted by certain strains of B. coli on the growth of S. typhi in broth culture.

He determined what he called the "antagonistic index", or "coli index", by making colony counts of mixed cultures of S. typhi and the strain of E. coli being investigated. The ratio of S. typhi colonies to E. coli colonies he took to be an indication of the antagonistic power of the E. coli strain. His ratio range was great, leading him to conclude that different strains of one species of organism vary markedly in their antagonistic power. Nissle believed that antagonistic strains of E. coli might be valuable therapeutically. The therapeutic value of these organisms is questionable because it has recently been shown by Sears, Brownlie and Uchiyama (20) that the ingestion of large numbers of E. coli of known antigenic type may fail to establish that strain as a "resident" in the gastro-intestinal tract.

McLeod and Govenlock (21), in 1921, reported that broth filtrates in which E. coli had been grown would not then support growth of E. coli nor certain other organisms, but if the broth were diluted it would again support growth, showing that the failure of the filtrate to promote bacterial reproduction was not due to exhaustion of the medium. This effect may, however, have been due, as Heatley (22) thinks, to the production of hydrogen peroxide rather than to a specific antibiotic substance.

Gratia (23) was the first to make a systematic study of the antibiotic substance produced by E. coli. In 1925 he noted that a strain of E. coli (coli V) produced a substance which had an inhibitory effect on another E. coli strain (coli ∅); even in a dilution as high as 1:1,000. The active substance was diffusible through cellophane, thermostable, and could be precipitated by acetone. In 1932 he published a paper (24) describing the properties of the substance he called "principle V". This freely diffusible substance did not reproduce itself like bacteriophage, and was highly thermostable, being able to withstand boiling for one hour without loss of activity. The activity was destroyed by acid solutions and by alcohol, and by prolonged contact with coli V bacteria. Gratia was also the first to note that resistant colonies grew up in the inhibited zone and that, though the development of a sensitive organism in broth might be delayed, full growth eventually occurred.

Fredricq (25), in 1946, showed that variations in the antibiotic activity or sensitivity could be induced by selection of colonies and that this variation was independent of morphological or biochemical characteristics and even of other antibiotic relations.

Gratia and Fredricq (26) and (27), in 1946 and 1947, performed experiments which led them to conclude that there

were several different active substances, or "colicines" (as Gratia has named the active principle), and several different types of sensitivity; so that the antibiotic character of an organism could be determined by its pattern of sensitivity and activity. Fredricq postulated the existance of several "receptor points" in a sensitive organism through which different colicines might act. They listed the characteristics by which the colicines may be differentiated as:

1. The range of susceptible organisms (which may include Shigella and Salmonella).
2. The appearance of specifically resistant strains.
3. The diffusion in agar.
4. The production in broth.
5. Thermostability at 100 C., or relative thermolability (destruction at 70C.).

Heatley and Florey (22) made a detailed study of a colicine produced by a strain of E. coli (CF 1) isolated from cat feces. The colicine was readily produced on solid media but intense aeration was necessary for its production in broth. The inhibitor was extracted in crude form by shaking the broth with charcoal then eluting the active substance with glacial acetic acid. The active material was then precipitated from the acetic acid solution by the addition of alcohol. The substance thus obtained was freely soluble in water at any pH but

insoluble in all of the usual organic solvents except acetic acid. It was odorless but on heating gave an odor typical of burning protein. It was destroyed rapidly by pepsin and trypsin but could withstand 100 degrees centigrade for one hour. This material, even in an impure state, had only a mild toxicity to mice and a very low toxicity to leucocytes. It was non-haemolytic.

Halbert and Magnuson (28) have studied the properties of an antibiotic produced by one strain of E. coli and have found that this substance is very similar to that described by Heatley and Florey, differing only in that it cannot diffuse through cellophane and was not produced in significant amounts in liquid media. These workers think that the antibiotic is a molecule of large size, probably a polypeptide.

Study of the results obtained by the authors whose work has been reviewed indicates beyond a reasonable doubt that some strains of E. coli produce an antibiotic substance which is active against certain other strains of E. coli and against a few other organisms of different species. Most investigators have concluded that the active substance is probably a peptide. It is thermostable and water soluble. That several different colicines exist is likely.

EXPERIMENTAL WORK

Objectives:

Much has been written about the antagonistic properties of E. coli and there has also been extensive research concerning the nature of the antibiotic substance; but there has been no systematic study of a group of serologically classified strains. Such a study must, of necessity, await the formulation of the group. In the Kauffmann type strains for the 112 "O" groups, we have such a series. The main objectives of this study have been to investigate the antibiotic properties of the Kauffmann "O" group strains and to attempt to correlate antibiotic activity with antigenic composition. We have included in this study some observations on the susceptibility of some Salmonella and Shigella strains and some strains of the intermediate group.

Sources of Material:

The strains of E. coli which were first investigated for antagonism were received from Kauffmann, who, with Knipschildt, isolated and classified serologically 112 strains. These strains are the type strains for the Kauffmann "O" groups.

Also included in this study are 268 strains of enteric bacteria which have been isolated in this

laboratory, mainly from the urine of patients suffering from urinary tract infections, though a few of the strains were isolated from patients with a bacteremia or septicemia. These include strains of E. freundii and of E. intermedium as well as strains of E. coli.

The 27 Salmonella strains investigated were obtained from several sources: Kauffmann sent us 19 strains, 6 strains were received from the University of California, and 2 strains were isolated locally.

The Shigella strains investigated are stock strains available here. They have been gathered from many sources over a period of several years.

Methods:

Our investigation of the antibiotic properties of the Kauffmann strains of E. coli, and of a few other strains of E. coli and other enteric bacteria available here, has been made in two phases. The organisms to be tested were first screened for antibiotic activity and sensitivity by a plate technique; then each organism which gave evidence of activity on the plate, was grown in broth and attempts made to secure a sterile solution containing the active substance elaborated by the organism.

The culture medium which we found most useful for the production of the antibiotic substance is one devised

by Halbert and Magnuson (25) with the following composition:

Proteose peptone No. 3 - - - -	20.0 Gm.
Glucose- - - - - - - - - - - -	0.5 Gm.
Sodium chloride- - - - - - - -	5.0 Gm.
Sodium Phosphate - - - - - - -	5.0 Gm.
Water- - - - - - - - - - - - -	1000.0 C.C.

To this liquid medium 5.0 Gms. of agar were added when a solid medium was desired.

The organisms to be tested were inoculated into a tube of broth and allowed to grow overnight in the incubator (16-20 hours at 37° C.). The next day a loopful of the culture to be tested for sensitivity was stirred into the melted, cooled agar medium which was then poured into a petri dish with good agitation so that the organisms were well dispersed throughout the medium. After the medium had cooled and solidified, the plates were placed in the incubator with the lids off for one hour. This procedure dries the surface of the medium. A small drop of a broth culture of the organism to be tested for antibiotic activity was then placed on the surface of the solid medium and the plate incubated for 16-20 hours before the results were recorded. It was found feasible to test the activity of 6-8 different strains on the same plate.

After incubation, many minute distinct colonies could be seen throughout the medium; these were colonies

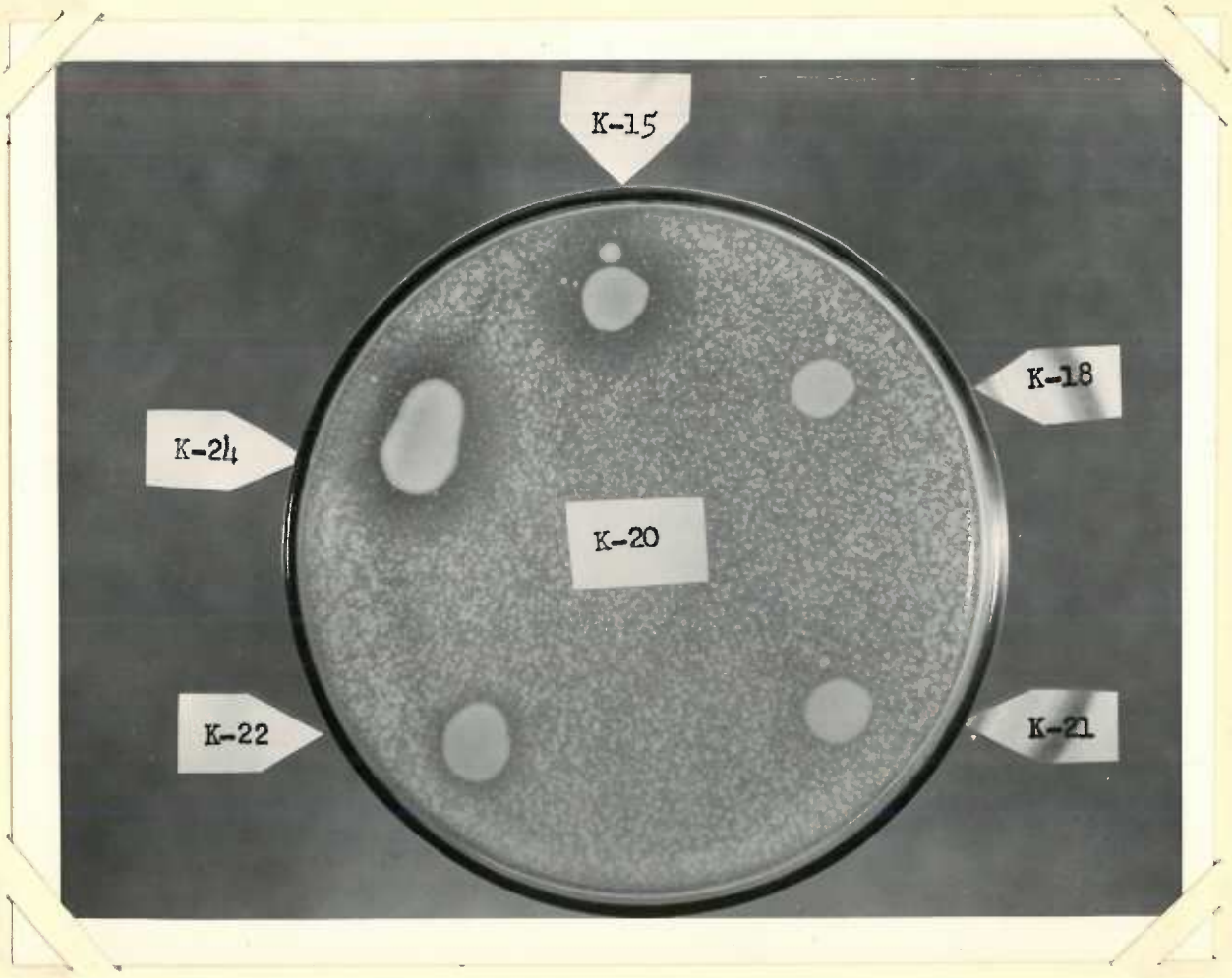
which had grown from the organism being tested for sensitivity. On the surface were the larger colonies of the organisms being tested for activity. Around the colonies of organisms which produce the antagonistic substance, a clear zone appeared in which the background growth was suppressed. The width of this zone varied, some antagonistic strains consistently producing zones 3-6 mm. wide; others produced much smaller zones. The width of the zone is not necessarily a measure of the antibiotic power of the organism, as some colicines may be more diffusible than others. Plate #1 shows a typical completed test of the kind just discussed.

Any organism which gave evidence of antagonism on the screening test was then inoculated into a tube of broth and allowed to grow overnight. The broth was then centrifuged at high speed to throw down the bacteria and the supernatant fluid collected. The clear fluid thus obtained was heated at 60° C. for one hour to kill any bacteria which might remain in the fluid. Having obtained a sterile, nearly cell free liquid, containing the substances elaborated by the bacteria, the next step was to determine whether the fluid had any antagonistic effect on the growth of other bacteria. This problem was solved by a procedure much like the screening test. An organism which had been susceptible to the antagonistic substance

PLATE I

This plate demonstrates the antagonistic properties of five typical Kauffmann "O" group type strains. K-15 and K-24 are markedly antagonistic to the background strain, K-20. K-22 has only slight activity. K-18 which, like K-20, is a sensitive strain has no activity against K-20. K-21 has no activity and, in other tests, was found to be resistant to antagonistic activity.

PLATE I



during the screening test was seeded throughout the agar medium and the plate was allowed to dry as before; then with a sterile cork borer, a plug of the solid medium 6 mm. in diameter was removed to form a well. Into this well a few drops of the sterile fluid containing the bacterial products were then introduced and the plate incubated overnight.

The results obtained from this procedure were similar to those of the screening test; about the well containing fluid from an antagonistic organism could be seen a clear zone in which no colonies of the sensitive organism were visible. The width of the zone was variable as before, but fairly constant for any particular organism.

Antibiotic Properties of Kauffmann O Group Type Strains:

Our investigation of the antagonistic properties of the Kauffmann strains of E. coli was started with the ambitious aim of testing each of the 112 strains against each of the others; determining both sensitivity and antagonistic activity for each organism. This project was abandoned after some two thousand tests, when it was found that the pattern of activity and sensitivity was well established. Several strains were found to be highly susceptible to antagonistic activity. Three of

the more sensitive strains O-18, O-20 and O-39, were used as test organisms to determine the activity of the other strains.

The results of this series of tests can be summarized as follows:

1. 30.35% of the Kauffmann strains exhibited some antagonistic activity against the most sensitive strain, O-39.

2. 20.45% of the Kauffmann strains proved resistant to the activity of all antagonistic strains tested.

3. The antagonistic strains were, in general, resistant to the activity of other antagonistic strains. This resistance, however, was not a property of the antagonistic strains alone. Many strains without antagonistic activity were completely resistant.

4. Sensitive strains showed little or no antagonistic activity.

A representative portion of the chart illustrating some of the results of this series of tests may be seen in table 1. It can be seen that O-15 and O-24 are antagonistic organisms, exhibiting activity against nine of the twelve organisms listed. Neither was active against the other and neither was active against O-21, which was not antagonistic to any of the other strains.

TABLE 1

Antibiotic activity and sensitivity of the Kauffmann strains of E. coli

		Antagonists												
		O-	13	14	15	16	17	18	19	20	21	22	23	24
Susceptibles	O-13	-	-	1*	-	-	-	-	-	-	-	-		2
	O-14	-	-	4	-	-	-	-	-	-	2	-		5
	O-15	-	-	-	-	-	-	-	-	-	-	-		-
	O-16	-	-	3	-	-	-	-	-	-	-	-		3
	O-17	-	-	3	-	-	-	-	-	-	-	-		4
	O-18	-	-	5	-	-	-	-	-	-	1	-		5
	O-19	-	-	3	-	-	-	-	-	-	-	-		5
	O-20	-	-	3	1	-	-	-	-	-	1	-		4
	O-21	-	-	-	-	-	-	-	-	-	-	-		-
	O-22	-	-	3	-	-	-	-	-	-	-	-		3
	O-23	-	-	3	-	-	-	-	-	-	-	-		2
	O-24	-	-	-	-	-	-	-	-	-	-	-		-

*Numbers indicate the width of the clear zone, in millimeters, about colonies of active organisms.

Pathogenic group classified E. coli strains:

We have in this laboratory, 173 E. coli strains which have been isolated here mailly from the urine of patients suffering from urinary tract infections. These organisms have been typed serologically and placed in their proper "O" groups, twenty of which are represented in this study. We have tested these organisms for antibiotic activity by the plate technique previously described, using O-18 as the susceptible organism. Theobjective of this series of tests was to investigate the correlation of antibiotic activity with O antigenic composition. The results obtained cannot be considered conclusive because of the small number of organisms tested in any group. The results of this series of tests are shown in Table 2, and can be summarized as follows:

1. 26% of these organisms exhibited some antagonism to O-18.

2. The data obtained in this series of tests does not indicate any correlation between antagonistic activity and O antigenic composition.

TABLE 2

Data showing the antagonism of E. coli strains classified into O antigenic groups to susceptible strain O-18

Strains of O Group #1

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-1	3	U-259	-
U-24	-	U-328	-
U-123	-	U-367	-
U-127	-	U-379	-
U-132	-	U-421	-
U-172	-	AB-4	-
U-184	-	Un.-2	-

Strains of O Group #2

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-2	2	U-150	-
U-8	-	U-275	-
U-21	-	U-335	-
U-22	-	U-351	-
U-23	4	U-372	-
U-30	-	U-386	-
U-35	-	U-390	4
U-37	5	Un.-9	-
U-38	4	Un.-13	1
U-50	4	Un.-39	-
U-113	3	Bl.-16	4
U-114	4	R.K.-5	1
U-120	3	P-2	-
U-130	-	L.K.-1	-
U-140	2		

Strains of O Group #4

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
U-25B	2	U-273	2
U-25C	1	U-245	-
U-31	2	U-249	-
U-33	1	U-338	1
U-55	-	U-364	-
U-87	2	U-370	-
U-201	-	U-388	-
U-212	-	U-413	2

TABLE 2 (Continued)

Strains of O Group #4 (Continued)

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
U-416	-	Bl.-9	3
U-425	-	Bl.-27	-
U-427	-	Bl.-39	2
Un.-1	-	Bl.-45	-
Un.-5	1	Bl.-47	-
Un.-21	1	R.K.-17	-
Un.-25B	2	L.K.-24	-
Un.-31	-	Sw.-3B	-

Strains of O Group #6

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-6	1	U-214	-
U-28	-	U-231	-
U-92	-	U-235	-
U-97	-	U-238	-
U-133	-	U-295	-
U-137	-	U-308	-
U-144	1	U-389	1
U-167	1	Un.-17B	-
U-182	-	AB-5	-

Strains of O Group #7

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-7	-	U-303	2
U-12	-	U-321	-
U-71	-	U-352	2
U-103	-	U-393	1
U-121	-	U-399	2
U-134	-	U-423	-
U-145	-	U-426	-
U-166	-	U-428	2
U-241	-	Un.-61	1
U-288	-	R.K.-14A	-
U-301	-		

TABLE 2 (Continued)

Strains of O Group #6

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-8	-	AB-1	3
U-115	-		

Strains of O Group #9

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-9	4	U-194	-
U-62	-	U-329	3
U-284	-		

Strains of O Group #15

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-15	5	U-407	-
U-342	-	Bl.-49	-

Strains of O Group #17

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-17	-	U-324	-
U-165	-	U-422	-
U-192	-	Un.-38	-

Strains of O Group #19

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-19	-	Bl.-43	-
U-65	-	Un.-66	-
U-349	-	L.K.-4	-
Bl.-3	-	L.K.-22	-
Bl.-4A	-	R.K.-15	-

Strains of O Group #24

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-24	5	U-110	-
U-4	2	Bl.-8	-
U-39	-		

TABLE 2 (Continued)

Strains of O Group #26

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-26	3	U-406	1
U-120	-	Un.-6	-
U-124	-	Un.-10	-
U-131	-	Bl.-20	1
U-173	2		

Strains of O Group #75

Organisms	Antagonism to O-18	Organisms	Antagonism to O-18
O-75	2	U-174	-
U-63	-	Sw.-2	2
U-69	-	S.P.-1	1
U-78	2	S.U.-11	-
U-148	-	Bl.-10	2
U-153	-	N.-1	-
U-155	-	H.-1	2
U-157	-	H.-6	-
U-160	-	H.-9	-
U-161	-		

O Groups #11, 12, 14, 18, 20, 22 and 23 were represented by only 1 or 2 organisms each; none of which had any antagonistic activity to O-18.

Strains of the Intermediate Group:

Seventy-four strains of the intermediate group have been investigated for antagonism and for susceptibility to the antagonistic substance produced by antagonistic strains of E. coli. These organisms are gram negative rods and have irregular IMViC reactions; that is, they do not give ++-- reactions. Many of these strains are members of the species E. Freundii and E. Intermedium.

These organisms have been tested by the procedures previously described. The results of these tests may be seen in Table 3 and may be summarized as follows:

1. Only one of the 74 strains tested was antagonistic to a sensitive strain of E. coli. This strain, U-163, produced good clear zones repeatedly when tested against susceptible strains 0-18 and 0-20.

2. Nine of the 74 strains were susceptible to the antagonistic activity of 0-15 and 0-24.

TABLE 3

Data showing susceptibility and antagonism of strains of the intermediate group

Organisms	Sensitivity to 0-15	Sensitivity to 0-24	Antagonism to 0-18
U-5	-	-	-
U-13	-	-	-
U-18	-	-	-
U-19	-	-	-
U-20	3	5	-
U-48	-	-	-
U-51	-	-	-
U-54	-	-	-
U-60	-	-	-
U-79	-	-	-
U-80	-	-	-
U-84	-	-	-
U-88	-	-	-
U-98	-	-	-
U-119	-	-	-
U-135	-	-	-
U-136A	-	-	-
U-139	2	2	-
U-142	-	-	-
U-147	-	-	-
U-149	-	-	-
U-159A	-	-	-
U-159B	-	-	-
U-162	-	-	-
U-163	-	-	3*
U-169	-	-	-
U-177	-	-	-
U-178	2	2	-
U-179	-	-	-
U-191	-	-	-
U-193	-	-	-
U-195	2	3	-
U-197	3	3	-
U-198	-	-	-
U-204	-	-	-
U-206	-	-	-
U-207	-	-	-
U-213	-	-	-
U-215	-	-	-

*This strain was antagonistic to O-18 (exp. repeated several times).

TABLE 3 (Continued)

Organisms	Sensitivity to 0-15	Sensitivity to 0-24	Antagonism to 0-18
U-218	-	-	-
U-225	-	-	-
U-230	-	-	-
U-236	-	-	-
U-243	-	-	-
U-246	3	5	-
U-248	-	-	-
U-280	-	-	-
U-285	-	-	-
U-291	-	-	-
U-292	-	-	-
U-296	-	-	-
U-297	-	-	-
U-312	-	-	-
U-316	-	-	-
U-322	-	-	-
U-325	-	-	-
U-328	-	-	-
U-346	-	-	-
U-369	-	-	-
Un.-18	9	5	-
Un.-44	-	-	-
Un.-46	-	-	-
Un.-60	-	-	-
Bl.-5	-	-	-
Bl.-7	-	-	-
Bl.-28	-	-	-
Bl.-36	-	-	-
Bl.-44	7	6	-
Bl.-51	-	-	-
Bl.-53	-	-	-
L.K.-11	7	8	-
L.K.-12	-	-	-
Sw.-4A	-	-	-

Salmonella and Shigella Strains:

In order to determine some of the range of activity of the colicine produced by our active strains, we have tested twenty-seven Salmonella strains and sixteen Shigella strains for susceptibility to the antagonistic principle. The results of these tests are shown in Tables 4 and 5, and may be summarized as follows:

1. Twenty-five of the twenty-seven Salmonella strains tested (92.6%) were susceptible to the activity of antagonistic E. coli strains, 0-15 and 0-25.

2. Four of the sixteen Shigella strains tested were susceptible to the activity of strains 0-15 and 0-25.

3. None of the Salmonella strains tested had any antagonistic activity against a sensitive E. coli strain, 0-18.

TABLE 4

Data showing sensitivity of some Salmonella strains to antagonistic E. coli strains O-15 and O-24

Organisms	Sensitivity to O-15	Sensitivity to O-24	Antagonism to O-15
K-PA	2	2	-
P-A(class strain)	-	-	-
K-P-B	1	1	-
P-B(Lowery)	1	2	-
K-T.M.	2	2	-
S-T.M.(Eugene)	2	2	-
K-Chol.Suis (Davis)	2	3	-
S. Shotmulerie	2	2	-
K-Rat.(Danyz)	5	5	-
S. Bareilly	1	2	-
S. Anatum	1	2	-
K. Aberdeen	1	2	-
K. Kunzendorf	1	2	-
K. London	-	-	-
K. Newport	2	3	-
K. Poona	3	3	-
K. Thompson	1	1	-
K. Stanley	1	2	-
K. Moscow	3	4	-
K. Rubislaw	1	2	-
K. Oranienburg	5	5	-
K. Rostock	2	3	-
K. Virchow	1	2	-
K. Dar-es-Salaam	1	2	-
S. Panama	3	4	-
K. Dublin	3	3	-
S. San Diego	2	2	-

TABLE 5

Data showing the sensitivity of some Shigella strains to antagonistic E. coli strains 0-15 and 0-24

	Organisms	Sensitivity to 0-15	0-24
1.	Sh. alkalescens V.P.D.	-	-
2.	Yee Fong urine str. alk.	-	-
3.	Yee Fong urine str. alk.	-	-
4.	Ivorsen alk.	-	-
5.	Newcastle bacillus	-	-
6.	Class str. Shiga F.M.S.	5	5
7.	Sh. Schmitz Haines Flynn	-	-
8.	Flexner V Oxford Flynn	1	1
9.	Weaver Flexner W	-	-
10.	Flexner X Toner Flynn	-	-
11.	Class str. Flexner Y Ledingham Flynn	-	-
12.	Flexner Z Whittington Flynn	2	2
13.	Flexner Z Zook	-	-
14.	Vinton Flexner Boyd 103-X	-	-
15.	Arnold Thomas Flexner X-103	2	3
16.	London X	-	-

Production of Colicine in broth cultures:

After the pattern of activity and sensitivity had been determined, the second phase of our investigation was begun. Our object now was to obtain a cell free, sterile fluid containing the inhibitory substance which is produced by "antagonistic" organisms. We first attempted to produce such a fluid by a technique which had been devised by Heatley and Florey (22). This method consists of growing the "active" organisms on the outer surface of a cellophane sac which is filled with nutrient broth and suspended in a wide glass tube plugged with cotton wool at top and bottom. After incubation for 24-36 hours the broth, which has not come into direct physical contact with the bacteria, is drawn off and tested for activity. Heatley and Florey produced fluids which contained the antagonistic principle but we were not able to do so, although the experiment was repeated several times with three of our most antagonistic strains O-13, O-24 and O-47. The organisms grew well on the cellophone sac but we could not demonstrate any activity in the broth. We concluded, therefore, that the antagonistic substance either was not produced under these conditions or could not pass through the cellophane membrane into the broth.

Our next, more successful, attempt to produce a sterile "active" fluid utilized the freeze-thaw technique of Halbert and Magnuson (28). Organisms were grown on a semi-solid medium made from brain-heart infusion. After 24 hours of incubation the culture was quickly frozen in carbon dioxide and then allowed to thaw. The cloudy fluid obtained by this procedure was strained through cheese cloth to remove bits of agar, then centrifuged to throw down the bacteria and the particles of solid medium remaining in the fluid. The supernatant was heated to 60° C. for one hour to sterilize the fluid and was then tested for antagonistic activity by the plate technique previously discussed.

The results obtained were encouraging but posed another problem, for only half of the organisms which produced zones of inhibition when grown on the solid medium produced fluids which had antagonistic activity. No organism which was inactive when grown on the solid medium produced an active fluid. These reactions may be seen in Table 6. While we were testing these organisms for antagonistic activity we noted that sterile broth in which antagonistic organisms had been grown had antagonistic activity. By simply growing active organisms in broth for 24 hours, then centrifuging the fluid and sterilizing the supernatant by heating at 60° C. for one hour, we were able

to obtain fluids with antibiotic activity from each organism which had antagonistic activity when grown upon the solid medium. As controls we used non-antagonistic Kauffmann strains of E. coli, several other species of organisms, and sterile broth in which no organisms had been grown. None of the controls produced fluids with antagonistic activity.

TABLE 6

Data showing reactions of fluids obtained by freeze-thaw technique of Halbert and Magnuson

Fluid from organisms	Susceptible organism O-18
O-1	3
O-2	-
O-3	-
O-9	-
O-15	3
O-24	3
O-26	-
O-29	-
O-47	2
O-65	2
O-77	3
O-91	-
O-105	4
O-18	-
O-20	-
Sterile Broth	-

Numbers indicate the width of the clear zone, in millimeters about colonies of active organisms.

Characteristics of the antibiotic substance:

Since it has been repeatedly observed that some strains of E. coli produce hydrogen peroxide, which is antagonistic to the growth of some other organisms, we felt that we should investigate the possibility of the antagonism which we had observed being due to this substance. This problem was simply resolved by repeating the screening test just as before except that we added 2 cc. of fresh human blood to each plate. The catalase in the blood would inactivate any hydrogen peroxide produced by the organisms. The results obtained were identical to those of the screening tests; antagonistic strains produced good zones of inhibition, and non-antagonistic strains produced no clear zones. This series of tests demonstrated, to our satisfaction, that the antagonistic strains is not due to hydrogen peroxide.

The antibiotic substance which we have been investigating is very thermostable, being able to withstand autoclaving at 20 pounds pressure for 15 minutes without demonstrable loss of activity. This substance also retains its activity after rapid freezing in carbon dioxide ice.

Activity in broth:

Attempts were made to determine the activity of the antagonistic substance in inhibiting growth in broth

cultures. The procedure consisted of making serial dilutions of the active fluid in sterile broth, inoculating each tube with a loopful of a young culture of a susceptible strain(C-20) and making plate counts at intervals of the number of bacteria in each tube. The results of this test were:

1. All tubes with a dilution of 1:20 or below showed a marked reduction in the number of organisms after two hours incubation, the number being reduced to about 1/10th of that noted at the start of the experiment. Tubes with dilutions above 1:20 had plate counts, at two hours, which were at least twice as high as the original count.

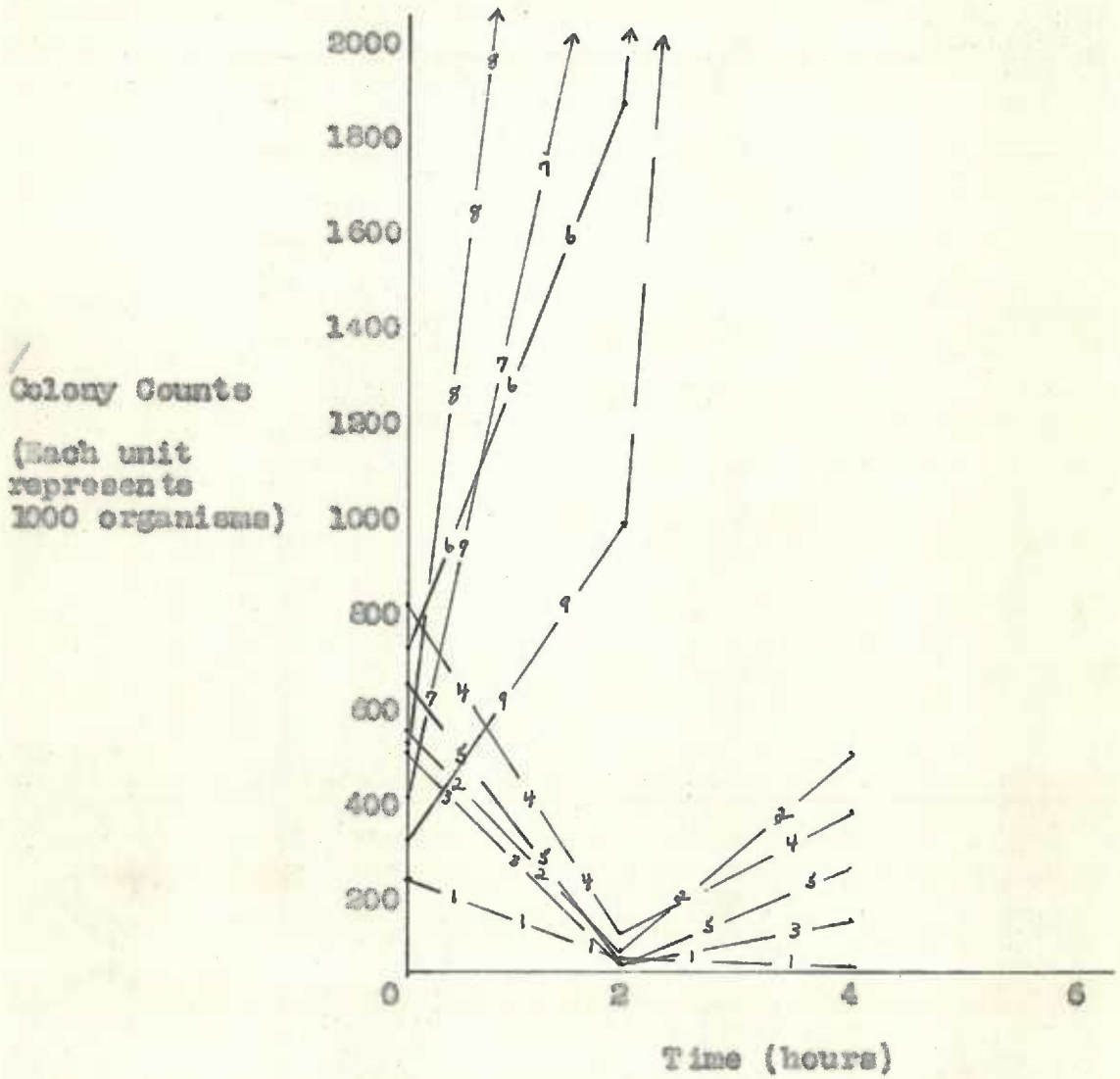
2. After 4 hours the no. of bacteria in all of the tubes except no. 1 (which was undiluted active fluid) was climbing rapidly, but, in dilutions below 1:100, had not reached the original number.

3. Six hours after inoculation all of the tubes with dilutions above 1:20 were turbid and plate counts could not be made.

The results of this test are graphically depicted in Plate #2.

PLATE 2

Data showing the activity of the antagonistic substance in broth cultures (O-20 used as the susceptible organism)



1. dilution 1:1 (all active fluid)
2. dilution 1:2
3. dilution 1:4
4. dilution 1:10
5. dilution 1:20
6. dilution 1:100
7. dilution 1:1000
8. dilution 1:10,000
9. dilution 1:∞ (No active fluid)

Discussion

The fact that certain strains of E. coli have antagonistic activity was first noted over 50 years ago. Since then there has been much study concerning the range of activity of these antagonistic strains and systematic studies of the antibiotic substance have been made. It has been well shown by Fredricq (23), and others, that the antibiotic activity was independent of morphological and biochemical characteristics. The objectives of this study have been to investigate the antibiotic properties of the Kauffmann "O" group type strains and to attempt to correlate the antibiotic characteristics of our "O" group strains with their antigenic composition.

Our method of investigation consisted of implanting a colony of the organism being tested for antagonism on the surface of a plate of solid medium which had been seeded with organisms of the strain being tested for sensitivity. About the colonies of antagonistic strains a clear zone could be seen in which no colonies of the seeded, sensitive strain were visable. We had in this laboratory 173 E. coli strains which had been isolated mainly from the urine of patients suffering from urinary tract infections. These strains had been typed serologically and had been placed in their proper "O" groups, twenty of which are represented in this study. These

strains were then tested for antagonistic activity by the plate technique previously described using as the "sensitive" organism Kauffmann's O-18 strain which had been found to be very sensitive to the colicines. There were highly antagonistic strains and strains without activity in the same "O" group; and sensitive strains were susceptible to the activity of antagonistic strains of the same group. No definite conclusions can be drawn from our data because of the limited number of organisms tested in each "O" group, but our data suggests that antagonistic properties are independent of "O" antigenic composition.

Our data does not include direct investigation of the H and K antigens, but the fact that some of our antagonistic strains possessed K antigens while others did not, suggests that antibiotic production is not dependent upon the presence or absence of the K antigen.

The methods by which antagonistic strains inhibit the growth of, or kill, sensitive strains is not known, and the data that we have gives us no clue as to the mode of action of the colicine. We can, however, narrow the field of possible antagonistic methods by eliminating some of the modes of antagonism which obviously are not involved here. The antagonism is not due to exhaustion of the medium, for sensitive organisms will eventually grow in broth in which antagonistic organisms have been

grown. That the antagonism is not due to a non-specific toxic substance is evident from its marked strain specificity. These observations lead us to conclude that the antagonistic substance is a highly specific material which interferes with some essential metabolic or reproductive function of sensitive organisms.

We believe that the active substance must be a molecule of large size because it did not readily pass through a cellophane membrane. It is highly thermostable and is not inactivated by freezing.

Summary

The place of the genus Escherichia among microorganisms is discussed, and the work done by other investigators on the problem of their antagonistic properties is outlined.

The 112 Kauffmann "O" group type strains have been tested for antagonistic activity and for sensitivity, and several striking facts concerning the antagonistic characteristics of these organisms have become apparent:

1. 30.35% of the Kauffmann strains exhibited some antagonistic activity against the most sensitive strain, O-39.

2. 20.45% of the Kauffmann strains proved resistant to the activity of all antagonistic strains tested.

3. The antagonistic strains were, in general, resistant to the activity of other antagonistic strains. This resistance, however, was not a property of the antagonistic strains alone. Many strains without antagonistic activity were completely resistant.

4. Sensitive strains showed little or no antagonistic activity.

We have also tested 173 "O" group classified E. coli strains for antagonistic activity and have attempted to correlate the antagonism with the antigenic composition of the organisms. No relationship between these two properties was apparent.

Seventy-four strains of the intermediate group have been tested. Nine of the strains were sensitive to the antagonistic activity of strains O-15 and O-24. Only one had any antagonistic activity.

Some Salmonella and Shigella strains have been tested for sensitivity to the antagonism of O-15 and O-24. The Salmonella strains were very susceptible (92.6%). The Shigella strains were much less sensitive (4 of the 16 strains were susceptible).

The production of the colicine in broth is discussed and some of the properties of the antagonistic substance have been determined.

1. Colicine was readily produced in broth and was active against broth cultures of sensitive organisms.

2. The antagonistic substance is thermostable, being able to withstand autoclaving at 20 pounds pressure for 30 minutes without loss of activity.

3. The colicine is water soluble and is not inactivated by freezing.

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