THE EXPERIMENTAL INFECTION OF CALVES WITH BACTERIAEMIA-PRODUCING STRAINS OF *ESCHERICHIA COLI*: THE INFLUENCE OF COLOSTRUM

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PLATE IV

AT least two forms of clinical infection with *Escherichia coli* occur in calves, an intestinal form and a generalised form. In the intestinal form the bacteria proliferate in the small intestine and produce enterotoxin, which is responsible for the severe diarrhoea so characteristic of this form of the disease; they are not usually invasive. In the generalised form the bacteria are found distributed throughout the body. Ability to produce enterotoxin is not a feature of the strains of *E. coli* incriminated in the generalised form of infection (Smith and Halls, 1967).

The incidence of the different serotypes of *E. coli* isolated from calves suffering from generalised infection has been well documented (see Gay, 1965; Sojka, 1965). Very little information, however, is available on the immune-globulin content of the blood of these calves. This aspect of *E. coli* infection has assumed great importance recently because the blood of an unexpectedly high proportion of calves kept under ordinary commercial conditions has been shown to be partly or completely deficient in immune globulins, and some animals have died from an *E. coli* bacteraemia as a result (Fey and Margadant, 1962; Smith, 1962; Gay *et al.*, 1965; Gay, Fisher and McEwan, 1965; Smith, O'Neil and Simmons, 1967). The calves in some of these studies were known to have had access to, or had been given, colostrum very soon after birth. It is known, too, that bacteraemia can be consistently produced in calves whose blood is devoid of immune globulins (IG—), but not in calves whose blood contains immune globulins (IG+), by the oral administration of certain strains of *E. coli*, including representatives of some of the serotypes found in the surveys referred to previously. Some natural infections with *E. coli* may arise by routes other than the alimentary one. Dam (1960), for example, considered that an infected umbilicus was the portal of entry in 160 of 430 cases of generalised *E. coli* infection in calves. It is conceivable that some such cases might well occur in IG+ calves as well as in IG— calves, particularly if the numbers of *E. coli* entering the body by this route were large.

It was decided, therefore, to study the sequence of events that follows the oral and intravenous administration of *E. coli* to IG+ and IG— calves. One strain of *E. coli* of antigenic structure O78: K80 was used in most of the experiments because this serotype is the one found most commonly in calves suffering from generalised infection. Smith found that strains of this serotype and all other strains isolated from cases of bacteraemia in IG— calves were able to multiply *in vitro* in the sera of IG— calves. Many of them, however, were killed in serum samples of IG+ calves indicating that bactericidal antibody might be

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important in protecting calves against *E. coli* bacteraemia. This view was weakened by the observation that two strains of antigenic structure O20: K?: H— and O20: K?: H9 grew in all the serum samples tested yet they were innocuous to IG+ calves, even when given by the intravenous route. Some strains examined by Smith were killed *in vitro* by sera of both IG— and IG+ calves. These strains did not produce bacteraemia in IG— calves after oral inoculation. Strains that give rise to the intestinal form of *E. coli* infection usually belong to this category (Smith and Halls). One such strain, of antigenic formula O101: K?, as well as the O78: K80, O20: K?: H— and O20: K?: H9 strains, were used in the present investigation. All four strains were smooth.

MATERIALS AND METHODS

Calves. All calves were males obtained from four Jersey herds kept on farms near the laboratory. They were classified provisionally as colostrum-deprived or colostrum-fed according to the owners' reports. On arrival at the laboratory, immediately before experiment, and when they were killed, their sera were examined by the zinc sulphate flocculation test (Aschaffenburg, 1949) and, according to the results, were classified as immune-globulin-negative or immune-globulin-positive (IG— or IG+). Unless otherwise stated, the calves were 6–24-hr-old when they were given an inoculation of *E. coli*. They were fed with 1 litre of cow's milk twice daily.

Method of inoculation. Nutrient agar cultures incubated at 37°C for 8 hr were suspended in phosphate buffer, pH 7.0. The numbers of viable organisms present were estimated by the method of Miles and Misra (Miles, Misra and Irwin, 1938). Intravenous inoculation was performed via a jugular vein and oral inoculation by permitting calves to drink 0.5 litres of cow's milk to which the organisms had been added. This was the first feed the IG— calves received.

Examination procedure. At the chosen times calves were killed by intravenous injection of sufficient pentobarbitone sodium to produce deep anaesthesia and then by severance of the carotid arteries. Portions of c. 2 g of different organs were immediately removed with sterile instruments, dipped in 90 per cent. ethanol, flamed, ground with 1 g of sterile sand in a sterile pestle and mortar for 3 min., and diluted to 20 ml with phosphate buffer. The portion of muscle examined was taken from a region of the biceps femoris drained by the popliteal lymph-gland. In some calves six mesenteric lymph-glands were examined. These were situated at approximately equal distances apart along the course of the small intestine. The first was in the duodenal region; the sixth was the ileocaecal. When only one was examined it was taken from the mesentery of the middle of the small intestine. Body fluids were removed with sterile pipettes and diluted in the same manner as the ground-up organs. The numbers of *E. coli* bacteria in both kinds of materials were then counted by a modification of the method of Miles and Misra, the culture medium employed being MacConkey's agar; as few as 50 viable bacteria per g could be estimated. The identity of the colonies growing on the culture medium was confirmed, when necessary, by submitting a few of them to slide agglutination tests with the appropriate antisera. The O101: K? strain was streptomycin-resistant. When mixed infections of this strain and the O78: K80 strain were studied, MacConkey's medium with and without streptomycin sulphate, 25 µg per ml, was used in the counting procedure.

Bactericidal activity of serum and blood. Serum obtained from blood that had been allowed to clot naturally after removal from the jugular vein was either tested immediately or stored at –20°C until required. To determine whether or not a specimen of serum was bactericidal for a particular strain of *E. coli*, 0.3 ml of the serum was placed in a small screw-capped bottle and 0.02 ml of an 18-hr broth culture diluted in phosphate buffer, pH 7.0, to contain 50,000 viable bacteria per ml, was added. A control consisting of phosphate buffer instead of serum was kept. The bottles were placed in a waterbath at 37°C for 3 hr and then

0.02 ml of the contents was spread evenly over half of the surface of a dried petri plate of MacConkey's medium. The plates were incubated at 37°C for 18 hr and read. If a serum sample was bactericidal, either no viable bacteria were detected, or the numbers present were much lower than at the commencement of the experiment.

In some studies the bactericidal power of serum was compared with that of blood. Clotting was prevented by collecting the blood in sodium citrate (1.0 per cent.) or heparin (20 mg per 100 ml) or by defibrinating it by shaking with sterile beads.

RESULTS

Evaluation of the technique for estimating the numbers of Escherichia coli bacteria in tissues

It was considered initially that inefficiency of the grinding procedure might seriously complicate our attempts to obtain accurate estimates of the numbers of *Escherichia coli* bacteria in the tissues of the calves. However, the results of performing counts on liver and spleen ground for different periods of time and by different methods (table I) did not indicate that this would be so.

TABLE I

The effect of grinding and macerating on the liberation of Escherichia coli bacteria from specimens of organs of calves into which E. coli O78: K80 had been inoculated intravenously

Treatment of specimen	Duration of treatment (min.)	Log ₁₀ number of viable <i>E. coli</i> bacteria per g in	
		spleen of IG— calf	liver of IG+ calf
Grinding with sand in a pestle and mortar	1	6.2	6.9
	2	6.4	6.9
	4	6.5	6.8
	8	6.4	6.9
	16	6.2	6.9
Grinding for 16 min. and then macerating* for the stated period	4	...	7.2
	14	...	7.0

* Maceration was done in an MSE macerator.

IG+ and IG— = Immune-globulin-positive and immune-globulin-negative.

Bacterial content of the tissues of IG— calves at different times after oral inoculation of E. coli

The bacterial content of the tissues of twelve immune-globulin-negative (IG—) calves at different times after oral inoculation of antilog₁₀ 9.7 viable bacteria of *E. coli* O78: K80 is illustrated in table II. Another two calves were not submitted to a detailed bacteriological examination because when they were found dead 36 hr after inoculation it was obvious that they had been dead for several hours.

All 14 calves appeared well for the first 15 hr after inoculation. Then they became progressively more dull and depressed, and soon collapsed. A burst

TABLE II

The distribution of E. coli O78: K80 bacteria in the tissues of immune-globulin-negative calves at different times after oral inoculation of antilog₁₀ 9.7 viable bacteria

Calf no.	Time after inoculation (hr)	Health status	Log ₁₀ numbers of viable bacteria per g in																			
			blood	liver	bile	spleen	lung	kidney	urine	brain	CSF	muscle	lymph-gland									
													P	SM	RP	AB	M1	M2	M3	M4	M5	M6
1	2½	Normal	N	N	N	N	N	N	N	...	...	N	N	N	2·6	N	2·7	2·9	1·7	N	N	N
2	4	Normal	N	N	N	N	N	N	N	...	...	N	N	3·7	...	4·3	2·7	3·0	3·0	3·0	3·3	1·7
3	5	Normal	N	2·7	N	3·3	2·7	3·0	N	...	...	...	...	2·6	3·2	N	3·4	3·4	3·2	3·2	5·3	3·4
4	8	Normal	3·9	4·2	N	5·0	5·7	5·5	N	...	...	2·4	4·2	N	2·7	2·0	4·8	4·3	4·2	3·7	3·6	3·5
5	15	Normal	5·4	4·2	N	5·4	3·7	5·7	N	...	...	3·7	5·0	4·0	4·5	4·0	4·0	4·2	4·7	4·6	4·6	3·9
6	18	Nearly dead	6·7	5·2	4·5	5·3	5·5	6·5	1·7	4·7	5·0	4·6	...	...	...	...	...	5·0	...	...	...	...
7	18	Just dead	7·5	6·2	3·8	5·4	4·9	7·2	6·0	5·2	2·6	5·2	6·9	...	6·9	...	6·6	...	7·2	...	...	7·0
8	19	At death	7·8	6·7	6·5	6·7	6·2	7·5	3·5	5·9	4·5	5·7	6·6	...	...	...	...	...	6·5	...	...	...
9	19	Just dead	8·2	7·2	5·3	6·7	6·7	7·8	3·6	...	...	5·6	7·0	5·9	6·0	5·9	6·8	6·5	6·5	6·6	6·5	7·0
10	20	At death	8·5	7·7	...	7·4	7·6	7·9	6·2	...	...	...	...	...	...	...	...	...	...	...	...	...
11	20	Collapsed	8·0	7·2	5·0	7·6	6·4	7·8	N	5·6	...	6·2	...	...	...	...	...	...	7·3	...	...	...
12	23	Nearing collapse	6·0	4·8	N	5·5	4·6	6·7	N	4·3	2·0	4·3	4·9	...	...	...	...	...	6·2	...	...	...

CSF = Cerebrospinal fluid; P = popliteal lymph-gland; SM = submaxillary lymph-gland; RP = retropharyngeal lymph-gland; AB = abomasal lymph-gland; M1-M6 = mesenteric lymph-glands, M1 being that nearest the stomach and M6 that nearest the caecum.

N = No viable bacteria in 0.02 g of tissue.

The numbers of *E. coli* O78: K80 bacteria in the contents of the small intestine in the twelve calves ranged from antilog₁₀ 6.9 to 8.7 per g.

ESCHERICHIA COLI BACTERIAEMIA IN CALVES

FIGURE.—Enlarged, diseased kidney (right) of a calf into which *Escherichia coli* O78: K80 bacteria had been inoculated intravenously 168 hr before it was killed, compared with a normal kidney (left) from a healthy calf. $\times 0.75$.

of diarrhoea usually occurred in the terminal stages of the disease, always after the onset of collapse. The interval between the first sign of ill-health and impending death was less than 3 hr.

E. coli O78:K80 bacteria were first found in the lymph-glands of the alimentary tract. By 5 hr after inoculation, when the numbers in these glands were still low, they had spread to organs remote from the alimentary tract. Ultimately, all tissues became infected. *E. coli* was first isolated from the blood at 8 hr (calf no. 4). In calves examined at times later than 8 hr the blood always contained large numbers of bacteria. In the ill calves the blood was the most heavily infected tissue, often containing concentrations of bacteria ten times higher than those in organs rich in reticulo-endothelial cells, such as spleen, liver and lungs. The concentrations in kidney tissue, too, were higher than the concentrations in these organs. Except in calves in the early stages of infection, the numbers of *E. coli* in the popliteal lymph-glands were similar to those in the lymph-glands of the alimentary tract. They were much greater than the numbers in the muscle they drained. The bile and urine were not found to be infected before 18 hr; the numbers of *E. coli* bacteria present in these fluids were usually considerably lower than those in the liver and kidney.

An additional IG— calf was given by mouth antilog₁₀ 9·7 viable bacteria of each of *E. coli* strains O78:K80 and O101:K?. Strain O101:K? differed from strain O78:K80 in being susceptible *in vitro* to the bactericidal action of the serum of IG— calves. The animal was examined when it was near to death. The number of bacteria of each strain of *E. coli* in the contents of the small intestine, was *c.* antilog₁₀ 7·7 per g. The figures for the O78:K80 strain in the blood, liver, spleen, kidney, lung and mesenteric lymph-gland were respectively, 5·5, 5·2, 5·6, 6·3, 4·3 and 5·6. By contrast, bacteria of the O101:K? strain were found only in the mesenteric lymph-gland, in numbers of 100 per g.

Bacterial content of the tissues of IG+ calves at different times after oral inoculation of E. coli

Ten immune-globulin-positive (IG+) calves were given by mouth, antilog₁₀ 9·7 viable bacteria of the O78:K80 strain of *E. coli*. All remained healthy during the observation period. Six were killed at different times between 6 and 96 hr after inoculation and examined bacteriologically; the results are shown in table III. *E. coli* O78:K80 bacteria were found in moderately large numbers in the small-intestinal contents, but, in sharp contrast to the results for the IG— calves, they were not found in any of the tissues except the lymph-glands of the alimentary tract. In these glands they were found only inconsistently and in small numbers.

Bacterial content of the tissues of IG— calves at different times after intravenous inoculation of E. coli

The results of the bacteriological examinations on five IG— calves into which the O78:K80 strain of *E. coli* was inoculated intravenously are illustrated in table IV. Those given antilog₁₀ 9·7 bacteria underwent a period of partial

TABLE III

The distribution of E. coli O78: K80 bacteria in the tissues of immune-globulin-positive calves at different times after oral inoculation of antilog₁₀ 9.7 viable bacteria

Calf no.	Time after inoculation (hr)	Log ₁₀ numbers of viable bacteria per g in										
		body fluids *	organs †	lymph-gland								
				SM	RP	AB	M1	M2	M3	M4	M5	M6
15	6	N	N	N	N	N	N	2.5	N	2.3	N	N
16	12	N	N	N	N	3.8	2.9	3.5	3.8	3.0	1.7	N
17	24	N	N	N	N	N	1.7	N	N	N	N	2.0
18	48	N	N	N	N	N	N	N	N	N	N	N
19	72	N	N	N	N	N	3.0	N	2.4	2.0	N	2.4
20	96	N	N	3.2	3.9	2.2	2.4	2.2	3.2	3.2	3.2	3.3

* = Blood, urine and bile.

† = Liver, spleen, lung, kidney, muscle and popliteal lymph-gland.

SM = Submaxillary lymph-gland; RP = retropharyngeal lymph-gland; AB = abomasal lymph-gland; M1-M6 = mesenteric lymph-glands, M1 being that nearest the stomach and M6 that nearest the caecum.

N = No viable bacteria in 0.02 g of tissue.

The numbers of *E. coli* O78: K80 bacteria in the contents of the small intestine in the six calves ranged from antilog₁₀ 4.4 to 7.6 per g.

TABLE IV

The distribution of E. coli bacteria in the tissues of immune-globulin-negative calves at different times after intravenous inoculation of antilog₁₀ 9·7 viable bacteria

Calf no.	Strain inoculated	Time after inoculation (hr)	Health status	Log ₁₀ numbers of viable bacteria of inoculated strain per g in											
				blood	liver	bile	spleen	lung	kidney	urine	brain	CSF	muscle	PLG	MLG
24	O78: K80	$\frac{2}{3}$	Normal	6·3	5·9	2·5	6·9	5·8	6·2	2·2	4·2	5·8	4·7	4·9	4·9
25		1½	Normal	6·0	5·9	5·0	6·2	6·3	6·6	3·3	4·4	5·3	5·0	5·0	5·0
26 *		3½	Normal	5·7 *	5·7	...	5·2	4·8	6·4	2·7	4·6	3·9	4·6	5·0	5·2
27		8	Collapsed	8·2	7·0	5·5	7·3	6·0	7·8	4·2	...	8·3	5·7	6·7	7·2
28		9	Collapsed	8·2	7·5	...	7·4	...	7·7	...	...	...	6·5	7·2	...
29	O101: K?	21	Normal	N	5·4	N	7·0	4·0	4·0	N	N	N	N	3·2	4·3
256		72	Normal	N	3·3	7·0	3·3	2·0	N	N	N	N	2·7	N	2·7

* The inoculum for calf no. 26 was only one-tenth as great as that for the others.
 CSF = Cerebrospinal fluid; PLG = popliteal lymph-gland; MLG = mesenteric lymph-gland.
 N = No viable bacteria in 0·02 g of tissue.

collapse lasting for a few hours in the period 1–5 hr after inoculation. The calves examined at 8 and 9 hr had recovered completely. Shortly before they were killed they experienced a more severe collapse which undoubtedly would have been followed swiftly by death had they not been killed.

The bacteriological picture in these calves closely resembled that in the IG— calves that had been given the O78: K80 strain by mouth and examined in the later stages of the disease process. All the tissues examined were infected, and once again the most heavily infected tissue was the blood and the next most heavily, the kidney.

The results of giving two IG— calves the O101: K? strain of *E. coli* by intravenous injection are also shown in table IV. In contrast to the results obtained with the O78: K80 strain, both calves remained well and no bacteria were found either in their blood or in any of several other tissues. Large numbers of bacteria, however, were found in the liver and spleen of the calf killed at 21 hr. Much smaller numbers were present in these organs in the calf killed at 72 hr, but the bile of this calf was heavily infected.

Bacterial content of the tissues of IG+ calves at different times after intravenous inoculation of E. coli

Twenty-five IG+ calves were given an intravenous injection of antilog₁₀ 9·7 viable bacteria of the O78: K80 strain of *E. coli* (table V). Shortly after inoculation the calves collapsed in a manner similar to that shown by the IG— calves. After recovery, they remained normal until about 14 hr post-inoculation. Six then exhibited progressive signs of ill-health leading to complete collapse. These six were either dead or would have been so by 24 hr had they not been killed; two were unsuitable for bacteriological examination because they had been dead too long. Apart from one calf that died 48 hr after inoculation, the remaining 9 remained healthy until 60 hr, when the condition of 7 of the 9 calves still alive commenced to deteriorate slowly. The principal clinical signs they exhibited were weakness and loss in weight with, in some cases, lameness and involvement of the central nervous system, characterised by varying degrees of incoordination. These signs were progressive and it was judged that the calves would have died within a few days had they not been killed.

No macroscopical lesions were seen in the calves examined before 120 hr. The principal lesion in 6 of the 7 animals examined after this time was a severe proliferative bilateral nephritis. In some kidneys only a few lobes were affected. The diseased regions were swollen and contained a number of grey irregular foci of varying size. The capsule covering the diseased tissue was roughened and dull in appearance. In some kidneys the grey foci had coalesced and little or no normal tissue remained. A severely diseased kidney is illustrated in the figure. Its weight was 112 g; that of a kidney of a healthy calf was 48 g. The hilus of the kidneys contained a large amount of pus. So did the urine of the six calves, which, as a consequence, was opaque. The bladder wall was normal. In one of the calves (no. 49, table V) the cerebrospinal fluid was opaque, owing

The distribution of E. coli bacteria in the tissues of immune-globulin-positive calves at different times after intravenous inoculation of antilog₁₀ 9.7 viable bacteria

Calf no.	Strain inoculated	Time after inoculation (hr)	Health status	Log ₁₀ numbers of viable bacteria of inoculated strain per g in											
				blood	liver	bile	spleen	lung	kidney	urine	brain	CSF	muscle	PLG	MLG
31	O78: K80	$\frac{1}{20}$	Normal . . .	5.2	6.2	N	6.3	5.5	5.6	N	...	...	3.2	4.5	5.3
32		$\frac{3}{20}$	Normal . . .	3.0	5.9	N	6.2	6.3	5.7	N	3.0	2.9	2.3	4.7	5.2
33		$\frac{3}{20}$	Partial collapse . . .	N	6.4	N	5.4	6.3	4.3	N	N	N	N	3.4	N
34		3	Almost recovered from collapse	N	5.5	N	5.3	6.3	4.3	N	N	N	3.7	3.7	3.4
35		5	Normal . . .	N	5.5	N	5.5	5.7	4.3	N	N	N	N	2.9	3.4
36		6	Normal . . .	N	6.0	N	5.6	5.8	5.3	N	N	N	N	3.7	5.0
37		12	Normal . . .	3.0	5.7	N	5.6	5.4	4.9	N	1.7	2.5	1.7	3.7	3.5
38		16	Just dead . . .	3.2	7.2	N	7.8	7.4	4.4	N	...	...	N	2.4	4.6
39		18	Near death . . .	3.4	6.0	8.3	6.6	5.0	4.2	N	N	N	2.7	3.4	N
40		22	Collapse . . .	5.5	5.4	N	6.2	5.3	6.2	8.3	4.7	6.6	3.4	4.6	4.7
41		23	Near death . . .	3.3	7.3	4.2	6.5	6.5	4.6	N	4.5	N	2.5	3.5	5.3
42		24	Normal . . .	N	5.4	7.7	5.6	5.8	3.4	N	N	N	N	3.1	3.9
43		48	Normal . . .	2.7	5.2	7.9	4.4	4.7	3.6	N	N	N	N	2.5	3.5
44		48	Just dead . . .	2.3	6.5	7.8	6.5	5.2	5.7	7.0	4.5	...	3.4	4.3	4.3
45		72	Incoordinated . . .	2.0	3.2	N	2.8	2.4	N	N	5.2	6.5	N	N	3.8
46		96	Normal . . .	N	2.3	7.5	N	2.0	N	N	N	4.4	N	N	3.0
47		120	Weak, nephritis . . .	2.6	4.2	7.9	3.3	3.7	8.0	7.7	N	N	N	3.7	3.7
48		168	Very weak, nephritis . . .	N	2.6	7.5	1.7	2.4	4.9	7.0	2.5	3.4	N	N	1.7
49		168	Weak, incoordinated, nephritis	N	3.2	7.6	2.3	3.0	7.3	7.7	5.8	6.7	N	N	3.0
50		192	Normal . . .	N	N	N	N	N	N	N	N	N	N	N	N
51		192	Weak, lame, polyarthrits, nephritis	N	N	7.5	N	3.0	5.9	7.7	N	N	N	2.5	N
52		216	Very weak, nephritis . . .	N	3.4	7.9	2.7	2.7	7.2	8.2	N	N	N	N	N
53		312	Weak, nephritis . . .	N	N	N	N	N	6.9	5.0	N	N	N	N	N
54	O20: K?:H—	2	Partial collapse . . .	2.0	6.0	N	5.9	6.0	3.9	N	N	N	2.9	4.0	3.9
55	O20: K?:H9	5½	Collapse . . .	3.0	6.3	N	6.3	5.3	4.9	N	N	N	2.7	3.9	5.0
56	O101: K?	24	Normal . . .	1.7	5.2	2.0	4.2	7.0	2.7	N	N	N	N	2.3	2.6
58	O78: K80 ($\frac{1}{100}$ th dose)	1	Normal . . .	N	4.4	N	4.6	4.0	3.6	N	N	N	N	N	N
59		24	Normal . . .	N	N	N	N	N	N	N	N	N	N	N	N
60	O78: K80 ($\frac{1}{100}$ th dose)	1	Normal . . .	N	4.0	N	4.0	3.1	2.0	N	N	N	N	N	2.7
61		1	Normal . . .	2.4	3.9	N	4.0	3.9	3.4	N	N	N	N	N	N
62		24	Normal . . .	N	N	N	N	N	N	N	N	N	N	N	N
63		24	Normal . . .	N	N	N	N	N	N	N	N	N	N	N	N

CSF = Cerebrospinal fluid; PLG = popliteal lymph-gland; MLG = mesenteric lymph-gland.

N = No viable bacteria in 0.02 g of tissue.

to the presence of leucocytes. Leucocytes were also present in the cerebrospinal fluid of another incoordinated calf (no. 45) and in several leg joints of a calf (no. 51) suffering from polyarthritis.

The kidneys of four of the calves suffering from nephritis were examined histologically by Mr J. E. T. Jones of the Royal Veterinary College, London. His report was as follows. The cellular reaction seen in the lesions is the same in all four calves, but it varies in distribution and severity. In two calves (no. 48 and 49) the lesions are distributed as focal spherical aggregations of inflammatory cells, especially prominent in the cortex and corticomedullary junction. The cellular aggregations are composed of polymorphonuclear leucocytes, lymphocytes and histiocytes. They are not encapsulated, but are sharply demarcated from the surrounding normal tubules. At the periphery of the lesions the infiltrating cells are intact, but in the centre many are degenerate and necrotic with their nuclei showing signs of karyorrhexis and pyknosis. Remnants of glomeruli and tubular epithelial cells are also present in these cellular aggregates. In the other two calves (no. 47 and 51) the lesions are distributed as diffuse linear streaks tending to be wedge-shaped with the long axis parallel to the long axis of the kidney tubules. Although a few focal lesions are present in the cortex, the inflammatory reaction in these two calves is diffusely distributed, involving large areas of cortex and medulla. The cellular components of the infiltrate are the same as in the case of calves no. 48 and 49. In the kidneys of all four calves many of the distal convoluted tubules are filled with polymorphonuclear leucocytes. There is considerable loss of normal tissue elements and there are signs of early replacement fibrosis. In some sections several capillaries are filled with fibrin plugs containing many leucocytes.

The results of the bacteriological examinations of 23 of the 25 calves referred to above are also illustrated in table V. The distribution of *E. coli* bacteria in these calves differed markedly from that in the IG— calves that had received similar inocula (table IV). The greatest difference was in the blood bacterial counts, which were either negative or very low in the IG+ calves; the low counts usually were found in the calves examined between 12 and 48 hr, and many of the calves examined at this time were nearly dead. The highest concentrations of *E. coli* in the calves killed in the first 48 hr were found in the liver, spleen and lungs, organs rich in reticulo-endothelial cells. Much lower numbers were present in these organs after 48 hr. Again, in contrast to the IG— calves, the kidney counts were low in the calves killed in the early stages of infection. Later, they were high in the calves that developed nephritis, a situation that was reflected in the urine counts; the specimens of urine examined from most of the other calves were sterile. The numbers of *E. coli* in the muscle and the popliteal and mesenteric lymph-glands were almost invariably low. Rather similar results were obtained in the examinations of brain and cerebrospinal fluid, except that moderately high numbers of bacteria were found in these tissues in the two calves that exhibited clinical and histological signs of disease of the central nervous system (no. 45 and 49). Although *E. coli* bacteria were not found in the bile of the 8 calves examined within 18 hr of inoculation, they were present in large numbers in 10 of the 15 animals examined after this

time. The synovial fluid of three leg joints of the calf with polyarthritis contained *c.* 1000 viable bacteria per ml.

The distribution of the bacteria in the tissues of three calves into which antilog₁₀ 9·7 viable bacteria of *E. coli* of strain O101: K?, O20: K?: H— or O20: K?: H9 had been inoculated intravenously, is also shown in table V; the two latter strains were the ones that had previously been found to grow in the serum of IG+ calves (Smith). The distribution of the bacteria resembled that in calves given a similar amount of *E. coli* O78: K80 bacteria.

An additional six IG+ calves were given intravenous inoculations of *E. coli* O78: K80 bacteria in a dose reduced to a tenth or a hundredth of the usual amount. Moderate numbers of *E. coli* were found, principally in the liver, spleen, lung and kidney, in the three animals examined 1 hr after inoculation. No *E. coli* bacteria were found in any tissue of the three animals examined at 24 hr (table V).

An additional two IG+ calves were given mixed intravenous inoculations of antilog₁₀ 9·7 viable bacteria of each of the *E. coli* strains O78: K80 and O101: K?. One was killed 1 hr later and the other 24 hr later. The distribution of the two organisms was similar. In the calf examined at 1 hr the numbers, log₁₀ per g, of the O78: K80 and O101: K? bacteria in the blood, liver, spleen, lung, kidney, mesenteric lymph-gland, and popliteal gland were, respectively, 2·0 and 0, 5·8 and 5·7, 5·9 and 5·8, 5·9 and 6·0, 5·0 and 5·0, 3·9 and 3·5, and 4·7 and 4·7. The corresponding figures for the calf examined at 24 hr were 0 and 0, 5·2 and 5·0, 4·6 and 4·0, 3·4 and 3·5, 2·9 and 2·0, 3·4 and 3·5 and 2·4 and 2·2. No *E. coli* bacteria were found in the muscle, brain, cerebrospinal fluid, urine or bile of either calf.

Fate of E. coli in blood in vivo and in vitro

Counts of viable *E. coli* bacteria in the blood were performed on more than one occasion on many of the calves used in these experiments. The *E. coli* bacteria usually disappeared very rapidly from the blood of IG+ calves after intravenous inoculation, very few being found after 30 min. The subject was investigated more fully in eight IG+ and four IG— calves. A sample of 5 ml of blood was removed from each of these calves 2½ min. after intravenous inoculation of antilog₁₀ 9·7 viable bacteria, and was heparinised and held at 37°C. *E. coli* counts were performed on these specimens and on freshly drawn blood at a number of different times afterwards so that the fate of the inoculated *E. coli* bacteria could be compared *in vitro* and *in vivo*. Similar counts were performed on blood removed immediately before inoculation, and heparinised and held at 37°C after the addition of bacteria from the same culture as the one to be inoculated into the calves. As the results were similar to those for the specimens removed from the calves 2½ min. after inoculation, they are not referred to below.

In four out of five IG+ calves given the O78: K80 strain of *E. coli* the numbers of viable bacteria decreased very much more rapidly *in vivo* than *in vitro*; the numbers were greatly reduced *in vitro* only after 2 hr. The results

TABLE VI
The fate of E. coli bacteria in the blood of calves in vivo and in vitro

Strain of <i>E. coli</i>	Blood			Log ₁₀ numbers of viable bacteria per ml blood, at time after inoculation (hr)										
	Source (calf no.)	Immune- globulin status	Site during experiment	$\frac{1}{24}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	1	2	3	4	5	6	7
O78: K80	255	+	<i>in vivo</i>	6.3	4.3	3.3	2.3	2.0	N	N	N	N	...	...
				6.3	6.3	6.3	6.0	5.7	4.7	3.4	2.7	2.7	...	...
O78: K80	27	—	<i>in vivo</i>	5.2	5.5	5.4	5.2	5.7	5.7	6.5	6.8	6.9	7.6	7.8
				5.2	5.6	5.4	5.3	5.8	6.3	7.4	8.0	8.2	8.5	8.9
O101: K?	256	—	<i>in vivo</i>	5.9	5.3	4.9	3.9	2.7	N	N	N	N	N	N
				5.9	6.2	5.7	5.9	6.2	6.0	5.6	4.9	4.7	4.6	...
O20: K?: H -	180	+	<i>in vivo</i>	6.0	4.3	3.5	3.4	3.6	3.7	3.3	3.2	3.0	...	...
				6.0	6.2	6.3	6.2	6.2	6.7	7.5	7.9	8.0	8.5	9.0
O20: K?: H9	151	+	<i>in vivo</i>	6.1	4.0	2.0	N	2.9	3.3	3.0	3.2	...	...	...
				6.1	6.1	6.3	6.2	6.2	5.6	5.9	5.9	6.2	6.7	7.3

Calf 27 had been given an intravenous inoculation of antilog₁₀ 8.7 viable bacteria; the other calves had been given antilog₁₀ 9.7 viable bacteria. Blood removed 2½ min. ($\frac{1}{24}$ th hr) after inoculation, heparinised and held at 37°C constituted the in-vitro specimen in each case.

N = No viable bacteria in 0.02 ml of blood.

for one of these calves, a typical example, are illustrated in table VI. The rate of decrease *in vivo* was much slower in the fifth calf; the immune-globulin content of the blood of this calf was very low. The number of viable bacteria in the blood of three IG— calves into which the O78: K80 strain was inoculated increased with time, both *in vivo* and *in vitro*, the rate of increase being greater *in vitro* than *in vivo*; the results for one of these calves are shown in table VI. By contrast, the bacteria disappeared from the blood of an IG— calf given an inoculation of the O101: K? strain (table VI), the rate of decrease being much more rapid *in vivo* than *in vitro*. Table VI also illustrates the results of inoculating the O20: K?: H— and O20: K?: H9 strains into two IG+ calves. The bacteria of these strains disappeared rapidly *in vivo*, but they actually proliferated in the in-vitro specimens. Rapid disappearance *in vivo* also occurred in another IG+ calf given an inoculation of O20: K?: H9 strain. *In vitro*, the number of bacteria showed some decrease at 3 hr, after which they increased.

In view of the difference in the rates of disappearance of *E. coli* from blood *in vivo* and *in vitro*, some IG+ blood samples into which O78: K80 bacteria had been inoculated were agitated continuously in a shaking machine to simulate in an exaggerated manner the movement of blood *in vivo*. This procedure, however, had no effect on the rate of reduction of the numbers of viable bacteria. Neither did reducing the number of bacteria inoculated into an IG+ blood sample have a marked effect. For example, a sample taken from calf 255 (table VI) into which only 250 viable bacteria of the O78: K80 strain were inoculated per ml did not show a definite reduction in numbers until it had been incubated for 1 hr.

Fate of E. coli O78: K80 in IG+ calf blood treated in different ways

The results of estimating the numbers of viable bacteria in blood taken from an IG+ calf and treated in different ways before *E. coli* strain O78: K80 was inoculated into it are illustrated in table VII. Similar results were obtained when the experiment was repeated with blood from another two calves. Blood in which coagulation was prevented by the addition of 1 per cent. sodium citrate was included in these experiments because of the inhibiting effect sodium citrate has on phagocytosis (Victor *et al.*, 1952). Except for heat-treated serum, in which multiplication took place, the bacteria were killed at very much the same rate in each of the differently treated blood specimens; only small numbers were still alive after incubation for 2 hr. Small numbers of bacteria continued to be isolated at most of the subsequent examinations. No explanation was forthcoming for this; cultures prepared from bacteria still alive at the end of the experiments possessed the same susceptibility to the bactericidal action of blood as those of the parent O78: K80 strain itself.

Bactericidal activity of calf sera against E. coli

The results of testing the sera of 55 of the calves for bactericidal activity against *E. coli* strains O78: K80, O101: K?, O20: K?: H— and O20: K?: H9 are summarised in table VIII. Although practically all the serum samples were

recorded as being non-bactericidal for strains O20: K?: H— and O20: K?: H9, it was noted that these organisms multiplied considerably during the test period in some samples whereas in others their numbers were but little altered. The results of estimating the numbers of viable bacteria in raw and heated samples

TABLE VII

The fate of E. coli O78: K80 bacteria in a specimen of immune-globulin-positive calf blood that had been treated in different ways before inoculation of the bacteria and incubation at 37°C

Time after inoculation (hr)	Log ₁₀ numbers of viable bacteria per ml, in						
	heparinised blood	heparinised blood with "buffy" coat removed	blood + 1 per cent. sodium citrate	defibrinated blood	plasma from heparinised blood	serum	serum after holding at 60°C for 1 hr
0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
0.5	5.9	6.2	5.9	5.9	6.0	5.9	6.0
1	5.9	6.0	6.0	6.0	5.8	5.8	6.0
1.5	5.8	5.8	6.1	5.5	5.7	5.3	6.2
2	5.0	5.4	6.0	5.2	5.3	5.0	5.9
3	3.3	3.6	4.0	3.2	3.9	3.3	6.0
4	2.8	3.2	2.5	2.8	2.6	2.6	7.2
5	2.5	3.2	2.5	2.7	2.4	2.0	7.6
6	2.7	3.0	N	2.8	2.3	N	7.7
6	3.4	2.6	3.3	2.5	N	1.7	8.2

The bacteria, as a suspension of an 8-hr nutrient agar culture, were added to the blood immediately before the commencement of the experiment, 1½ hr after the blood had been collected from the calf.

N = No viable bacteria in 0.02 ml.

TABLE VIII

The bactericidal action of samples of serum from different calves in tests against different strains of E. coli

Immune-globulin status of serum	Number of sera tested	Number of sera bactericidal for <i>E. coli</i> strain			
		O78: K80	O101: K?	O20: K?: H—	O20: K?: H9
—	19	0	19	0	0
+	36	34	36	1	2

The serum sample that was bactericidal for strain O20: K?: H— was not bactericidal for strain O20: K?: H9.

of IG+ and IG— serum at different times after they had been given an inoculum of the O20: K?: H— strain are shown in table IX. Although in routine tests these serum samples would have been regarded as being non-bactericidal for strain O20: K?: H—, a comparison of the counts indicated that the IG+ sample had a definite inhibitory effect on the rate of multiplication of this strain.

DISCUSSION

The finding of *Escherichia coli* O78: K80 bacteria in the submaxillary, retropharyngeal, abomasal and small-intestinal lymph-glands of the immune-globulin-negative (IG—) calves within a few hours after oral inoculation illustrated the rapidity with which the alimentary tract of these animals was penetrated and the extensive region over which the penetration took place. The lymph-glands provided very little defence against invasion, since the O78: K80 bacteria were found in organs remote from the alimentary tract 5 hr

TABLE IX

The multiplication of bacteria of E. coli strain O20: K?: H— in the sera of an immune-globulin-positive (IG+) and an immune-globulin-negative (IG—) calf during incubation at 37°C

Time after inoculation (hr)	Log ₁₀ number of viable bacteria per ml in			
	IG— serum		IG+ serum	
	raw	heated *	raw	heated *
0	3.5	3.5	3.5	3.5
1	3.6	3.6	3.5	3.6
2	4.3	4.2	3.6	4.0
3	4.8	4.7	4.2	4.7
4	5.3	5.3	4.5	5.7
5	5.5	5.5	4.6	6.3
6	7.0	6.7	5.9	7.9

* The serum was heated at 60°C for 1 hr before inoculation of the bacteria.
A 24-hr broth culture suitably diluted was used for inoculating the sera.

after inoculation. Neither did the reticulo-endothelial system nor any other defence mechanisms function with any degree of efficiency, for the bacteria were soon found in high concentrations in practically all the tissue examined, particularly in the blood and kidneys. *E. coli* O78: K80 bacteria were also found, after oral inoculation, in the lymph-glands of the alimentary tract in the immune-globulin-positive (IG+) calves. The defence mechanisms of these IG+ calves, however, effectively prevented any extension of the invasion. After intravenous inoculation, too, the bacteria did not rapidly proliferate throughout the body in the IG+ calves, though they did so in the IG— ones. Shortly after inoculation into the IG+ calves they were found in high concentrations only in those organs, such as liver, spleen and lungs, that were rich in reticulo-endothelial cells. They were either absent or present only in small numbers in the other tissues examined at that time. The finding of considerable numbers of bacteria in kidney, cerebrospinal fluid, bile and synovia in some of the calves killed at periods later than 5 hr probably reflects the escape of small numbers of bacteria to sites where the defence mechanisms were unable to operate efficiently.

In a previous study (Smith, 1962) it was found that strains of *E. coli*, such as the O78: K80 strain, that produced bacteraemia in IG— calves, were distinguishable from those, like the O101: K? strain, that did not produce bacteraemia, by their ability in bactericidal tests to survive and multiply in the sera of IG— calves. They did not produce bacteraemia in IG+ calves and most of them failed to multiply in the sera of such calves in bactericidal tests, a failure that was shown to be due to the bactericidal antibody content of the sera. As far as the O78: K80 and O101: K? strains were concerned, a close correlation was also found in the present studies between the results of bactericidal tests and the results of inoculations in calves. Thus it seemed possible that the bactericidal activity of blood might be important in protecting calves against *E. coli* bacteraemia. However, when the rate of disappearance of strain O78: K80 from IG+ blood *in vivo* was compared with its rate of disappearance from the same blood *in vitro*, it was apparent that the bacteria were removed much too rapidly *in vivo* for the removal to be due wholly or mainly to the bactericidal action of the blood. Similar results were obtained in studies made with strain O101: K? in an IG— calf. Furthermore, the subsequent finding that the O20: K?: H— and O20: K?: H9 strains, which were included in this investigation because they had multiplied in all samples of calf sera previously studied, disappeared rapidly from the blood of IG+ calves *in vivo*, yet multiplied in the blood of the same animals *in vitro*, confirmed the view previously expressed (Smith) that the IG+ calf has a form of protection against *E. coli* bacteraemia that is wider than that measurable by *in-vitro* bactericidal tests with serum and blood. The *E. coli* counts on the tissues of the IG+ calves into which O78: K80, O20: K?: H— and O20: K?: H9 bacteria had been inoculated intravenously and the IG— calves into which O101: K? bacteria had been inoculated intravenously indicated that the reticulo-endothelial system is importantly involved in this protection. The bacteria were rapidly removed from the blood and then slowly killed. It is noteworthy that the rate of killing in the reticulo-endothelial tissues was slow, much slower, for example, than the rate of killing in blood *in vitro*. Considering all the evidence it would appear then that the principal defence mechanism of the IG+ calf against *E. coli* bacteraemia is provided by antibody absorbed from the colostrum. This antibody sensitises any invading *E. coli* to phagocytosis by cells of the reticulo-endothelial system. In the absence of this sensitisation, as in the IG— calf, the phagocytic activity is reduced to a point where the animal has little or no protection against the development of a fatal bacteraemia with strains like those of types O78: K80, O20: K?: H— and O20: K?: H9. The lesser degree of phagocytic activity, however, is sufficient to cope with invasion by strains, such as strain O101: K?, that under natural conditions produce a localised intestinal infection and not bacteraemia.

The intravenous inoculation of $10^{9.7}$ viable bacteria of O78: K80 into IG+ calves produced a disease whose clinical and pathological signs, particularly those involving the kidneys, central nervous system and joints, resembled those sometimes seen in calves in the field. This inoculum was very large, and it is unlikely that such numbers would penetrate the alimentary tract

of healthy IG+ calves kept under natural conditions. The negative results of attempting to infect IG+ calves by mouth in the present work and practically all the published work support this view. Whether the organisms would penetrate the alimentary-tract mucosa in calves whose alimentary tracts were severely damaged by other diseases is another matter. The entry of large numbers of *E. coli* bacteria into the blood of IG+ calves might be more likely to occur as a complication of *E. coli* omphalitis, particularly as the umbilical veins are usually involved in these infections. It is noteworthy in this respect that Dam (1960) considered that an infected umbilicus was the portal of entry in 160 of 430 cases of generalised *E. coli* infection in calves; no information was provided on the immune-globulin status of these calves. In view of the recent reports (Fey and Margadant, 1962; Smith; Gay *et al.*, 1965; Gay, Fisher and McEwan, 1965) that an unexpectedly high proportion of calves have no, or very low levels of immune-globulin in their blood and may die from an *E. coli* bacteraemia as a consequence, there is now an obvious need for further surveys in which the immune-globulin levels of calves suffering from generalised *E. coli* infections are measured. Only by such surveys can we discover whether or not these infections present a problem in the IG+ as distinct from the IG- calf.

SUMMARY

Four selected smooth strains of *Escherichia coli* were inoculated orally and intravenously into calves, aged 6–24 hr, whose blood, judged by the zinc sulphate flocculation test, did or did not contain immune globulins (IG+ or IG-), and the numbers of the bacteria in the tissues were estimated when the animals were killed after different intervals. In bactericidal tests *in vitro*, one of the strains, which belonged to the serotype O78: K80, most commonly found in calves suffering from generalised *E. coli* infections, survived and multiplied in all samples of IG- serum, but not in most samples of IG+ serum. Two strains, O20: K?: H9 and O20: K?: H-, grew in most IG- and IG+ sera, and the fourth strain, O101: K?, which had been isolated from a calf suffering from the localised intestinal form of *E. coli* infection characterised by severe diarrhoea, did not grow in any sample of either IG- or IG+ serum.

After oral inoculation into IG- calves, O78: K80 bacteria rapidly invaded the blood and tissues, and their ultimate concentration in the blood was often ten times greater than that in tissues rich in reticulo-endothelial cells. The calves became ill after about 15 hr and soon collapsed and died. Similar results were obtained when O78: K80 bacteria were inoculated intravenously. The blood counts of IG+ calves into which O78: K80 bacteria had been inoculated intravenously were either negative or very low, the highest concentration of the bacteria being found in tissues rich in reticulo-endothelial cells; some of these calves, into which large doses of the O78: K80 bacteria had been inoculated, developed lesions in the kidneys, central nervous system and joints which closely resembled those seen in some natural cases of generalised *E. coli* infection. The blood and tissues were not invaded after oral inoculation of O101: K? bacteria in IG- calves or of O78: K80 bacteria in IG+ calves; these animals remained

well. The distribution of bacteria in IG+ calves into which strains O101: K?, O20: K?: H9 and O20: K?: H— had been inoculated intravenously was similar to that obtained with the O78: K80 strain.

The rate of disappearance of bacteria of strain O78: K80 from IG+ blood *in vivo* was compared with its rate of disappearance from the same blood *in vitro*. The bacteria were removed much too rapidly *in vivo* for the removal to be due wholly or mainly to the bactericidal action of the blood. Similar results were obtained with the O101: K? strain and IG— blood. The O20: K?: H9 and the O20: K?: H— strains disappeared rapidly from the blood of IG+ calves *in vivo*, yet multiplied in the blood of the same calves *in vitro*. The O78: K80 strain multiplied in IG— blood both *in vivo* and *in vitro*, the rate of multiplication being faster *in vitro*.

It is concluded that the reticulo-endothelial system constitutes the principal defence mechanism against *E. coli* bacteraemia, and that in calves whose blood contains immune globulins as a result of their having been fed with colostrum this protection is of a comprehensive nature.

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