THE BACTERIOLOGICAL CLASSIFICATION OF THE PRINCIPAL CULTURES USED IN RAT AND MOUSE CONTROL IN GREAT BRITAIN

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1. Introduction

During the early months of the war the author had an opportunity of examining the six principal viruses which are at present used for rat and mouse control in Great Britain. The trade names of these preparations are as follows: Liverpool Virus, Institut Pasteur Virus, Ready Rat Relief Virus, Danysz Virus, London Virus and Ratin. One sample of each was examined, the samples being supplied by the manufacturers or agents concerned, except in the case of Liverpool Virus which was purchased locally at a chemist's shop. There may be a few more strains which have not been obtained, since it is open to any small firm to culture similar bacteria for service work or sale. However, the six which are dealt with here include all the types used by the larger firms undertaking rat control service and the largest retail firm concerned in anti-rodent control by means of virus.

The origin of most of these cultures is now obscure. Danysz's original strain was isolated by him from a natural epidemic among wild voles and at first proved to have only a low-grade power of infecting rats. This was afterwards increased by intraperitoneal passage in collodion sacs (Danysz, 1900). Possibly one or two of the other viruses may be subcultures from this parent strain. The Ratin culture was originally isolated from the urine of a sick child by Neumann in 1902 (Salmonella Subcommittee, 1934).

The word 'virus' is now used with a particular meaning in medicine and bacteriology. But, since this term has been long applied to the bacterial preparations used in anti-rodent control, and since there is no possibility of any mistake in identity arising between them and the true viruses, it has been retained here in order to distinguish these commercial *Salmonella* strains from others of a similar antigenic structure. The names *S. enteritidis* Gaertner, or sometimes simply either Gaertner or *enteritidis*, are used throughout this paper for strains having the antigenic structure, IX:gom:—, in the Kauffmann-White schema (Salmonella Subcommittee, 1934). The var. *danysz* subgroup of *S. enteritidis* is often referred to in the Scandinavian literature as Ratin-type Gaertner; and, in the case of some German authors, it is not always clear whether the term 'Ratinbazillen' refers specifically to strains having the same biochemical properties as *S. enteritidis* var. *danysz*, or whether it simply means a Gaertner culture isolated from

Ratin or some other commercial preparation, and typed as such merely by agglutination tests.

2. METHODS OF EXAMINATION

Platings of the material were made on agar and examined for colony types, possible contaminants, degree of smoothness or roughness, etc. No difficulty was experienced from contaminants; with the exception of the sample of the Danysz Virus, all these platings gave pure cultures. By means of slide agglutination with various pure 'H' and 'O' serums, using the growth on these primary platings, all these strains were provisionally typed as *Salmonella enteritidis* (Gaertner). For the purposes of a more complete analysis a single colony, in appearance as smooth as possible for each plating, was picked for subculture, and the strain of each virus thus derived was used in all the subsequent serological and biochemical work.

The results of the primary platings can be given briefly in the following notes.

Liverpool Virus. Sample of grain bait, labelled 'must be used before 27. v. 40'. Platings on 21. xi. 39 from a broth tube inoculated with the liquid material in which the grain was soaked, gave two types of colony, both smooth in surface, but differing in their appearance with transmitted, artificial light. From their growth in broth these two colony types were provisionally classed as smooth and rough. An estimate by eye of the proportion of the two types gave from 30 to 50 % as being rough and suspensions in broth of the latter type flocculated to titre with a Standards Laboratory Gaertner serum, thus confirming the presence of the *enteritidis* type of 'H' antigen.

Institut Pasteur Virus. Sample labelled 'Virus for Rat and Mouse. *Bac. typhi murium* prepared by the Pasteur Institut, Paris. Not to be used after 1. ix. 40.' The bait was grain. Platings on 24. xi. 39 gave a division into two colony types as in the Liverpool Virus. No estimate was made of the relative numbers. Suspensions of the rough or partially rough colony type agglutinated to titre with an *enteritidis* 'H' serum.

Ready Rat Relief Virus. Sample received in the form of a bacterial culture on a gelatin slope, labelled 'Not to be used after 11. iv. 40'. A plating on 15. i. 40 from this material gave a good growth, all the colonies apparently being smooth. But from the behaviour of a colony picked into broth and the results of the salt-agglutination test (see below) it is suspected that some degree of roughness is also present in this culture.

Danysz Virus. Sample received in the form of a liquid, presumably broth, in which to soak bread. No limiting date for use was given. Platings gave an almost pure growth of flat, spreading, rough-type colonies. Slightly contaminated with a Gram-positive coccus. A small portion of the growth on this primary plating appeared to be of a more smooth type, which was then replated from broth. Although these colonies had the appearance of being smooth, their subsequent behaviour again suggested that they should be classified as being partially rough.

London Virus. Sample received as a grain bait, labelled 'Not to be used after 30. vi. 40'. Plating on 26. ii. 40 showed a mixture of different colony forms, though none of these could be termed as being of the classic rough type.

Ratin. Received in the form of a broth culture labelled 'effective till 3. iii. 40'. Plating on 17. i. 40 gave a good growth, all the colonies apparently being smooth.

3. Antigenic analysis

The agglutination tests were carried out by means of Dreyer's technique, 'H' agglutination being read after 2 hr. and 'O' agglutination after $4\frac{1}{2}$ hr. in the water bath at 52° C. Formolized broth cultures were used as 'H' suspensions. For 'O' suspensions the growth on agar was suspended in a small amount of saline to which about four volumes of absolute alcohol were added, and after $\frac{1}{2}$–1 hr. at room temperature, centrifuged and resuspended in N saline, chloroform being used as a preservative. Agglutinating serums for each strain were made by two injections (0·5 and 1·0 c.c.) of formolized broth culture

Table 1

S. enteritidis (Dennis) serum absorbed with the different virus cultures and tested against S. enteritidis (Dennis) 'H' and 'O' suspensions

	'H' titre	'O' titre	Remarks
Unabsorbed	4,500	700	Average of six tests
Absorbed with:			
Liverpool Virus	<100	260	Tested v. Liverpool suspension 'H' and 'O' titres both <100
Institut Pasteur Virus	<100	<100	
Ready Rat Relief Virus	<100	<100	
Danysz Virus	<100	<100	
London Virus	<100	? 100–200	See notes in text
Ratin	<100	<100	

Control tests with suspensions of the absorbing strains showed in all cases a reduction of the heterologous 'H' and 'O' titres to <100.

Virus serums absorbed with S. enteritidis *(Dennis) culture and tested against the homologous 'H' and 'O' suspensions*

Serum		Unabsorbed	Absorbed	Remarks
Liverpool Virus:	H	20,000 +	<100	
	O	1,200	<100	Poor suspension
Institut Pasteur Virus:	H	6,000	<100	
	O	650	<100	
Ready Rat Relief Virus:	H	20,000 +	? 100	Poor suspension
	O	—	—	Bad suspension
Danysz Virus:	H	6,000	<100	
	O	(2,000)	<100	Approx. reading of unabsorbed serum, poor suspension
London Virus:	H	12,500	<100	
	O	500	<100	
Ratin:	H	12,500	<100	
	O	2,250	<100	

Control tests with suspensions of the absorbing strain showed in all cases a reduction of the heterologous 'H' and 'O' titres to <100.

into the ear vein of rabbits, with an interval of a week between the two doses, and the animals bled 1 week after the last injection. In the absorption tests the 24 hr. growth on from four to six agar plates, depending on the luxuriance of the growth, was removed by means of a platinum scraper and suspended in 2·0 c.c. of a 1/40 dilution of the serum to be tested. The suspensions, together with a control tube of the same serum dilution, were placed in the incubator at 37° C. for 2–3 hr., left overnight at room temperature and centrifuged in the morning.

 The control *S. enteritidis* (Gaertner) culture was called 'Dennis' and is the one at present used by the Standards Laboratory, Medical Research Council, for the preparation

of agglutinating suspensions and serums. Its date of isolation is not known, but it has certainly been subcultured on artificial laboratory media for a number of years.

The results of the reciprocal absorption tests are shown in Table 1, where all the readings are expressed in terms of 'Standard' agglutination, i.e. the calculated dilution of serum which would produce a standard degree of agglutination within 2 hr. in the case of 'H' and $4\frac{1}{2}$ hr. in the case of 'O' agglutinins. It will be seen that, in the case of the 'H' antigen, all the virus cultures absorb the 'H' agglutinins of *S. enteritidis* Dennis serum to below the level of the lowest dilution tested, namely 1/100. Conversely, the Dennis culture absorbs the 'H' agglutinins from each of the virus serums. Thus there appears to be complete identity between the flagellar antigenic complex of *S. enteritidis* (Gaertner) and all these six virus cultures.

With regard to the 'O' antigens there are the following points to be noted. The strains Institut Pasteur Virus, Ready Rat Relief, Danysz and Ratin absorb the 'O' agglutinins from Dennis serum. Liverpool Virus only reduces the homologous titre of this serum from 700 to 260, while it removes the heterologous agglutinins. In the case of the London Virus the reading ?100–200 is entered in the table. The Dennis serum absorbed with

Table 2. *Percentage concentrations of* NaCl

Strain tested	2·0	1·4	1·0	0·8	0·6	0·4	0·2	Control
Liverpool Virus	T	T	T	T –	tr	tr –	0	0
Institut Pasteur Virus	0	0	0	0	0	0	0	0
Ready Rat Relief Virus	T	T	T	T	T	T	T	0
Danysz Virus	T	T	T	T	T	tr –	0	0
London Virus	0	0	0	0	0	0	0	0
Ratin	0	0	0	0	0	0	0	0
S. enteritidis Dennis	slD	slD	?slD	0	0	0	0	0

T = complete agglutination; tr = fine agglutination visible with a hand lens;
slD = some deposit and slight clearing of the supernatant.

this strain and tested against the homologous 'O' suspension showed, after $4\frac{1}{2}$ hr. in the water bath, a very coarse, cotton-wool type of agglutination in the first two tubes. A repetition of the absorption test gave a similar result. The Dennis 'O' suspension was perfectly satisfactory in all the other tests and the Dennis 'H' suspension, in these two tests in which London Virus was the absorbing strain, showed no signs of either 'H' or 'O' type agglutination with the absorbed serum. There is no ready explanation for this result; although entered as ?100–200 in the table, it is not considered to be an incomplete absorption of the 'O' agglutinins by this strain. Turning to the results of the virus serums absorbed with Dennis suspension, it will be seen that the latter removed the 'O' agglutinins from all the serums tested. The Ready Rat Relief serum apparently contained either no homologous 'O' agglutinins or had developed these to only a very low titre. This was difficult to decide owing to the instability of the Ready Rat Relief 'O' suspension. Tested against 'O' suspensions of the other strains, this serum agglutinated London Virus in a dilution of 1/100 and the others either not at all or at most a trace at 1/50.

Since certain of these strains appeared to give unstable suspensions, a salt-agglutination test was carried out (Table 2). A suspension of each strain was made in distilled water and tested against varying concentrations of sodium chloride. The results were read after 21 hr. in the water bath at 52° C.

Taking the results of this test in conjunction with those of the 'O' absorption tests, it would appear that we are dealing with a number of strains showing various gradations between smoothness and roughness, and it is possible that the *S. enteritidis* Dennis control strain may have been itself in some intermediate phase.

Thus, it seems clear that

(1) All these six virus strains can be taken as serologically identical with *S. enteritidis* (Gaertner).

(2) In each sample, with the possible exception of Ratin, various gradations between smoothness and roughness existed. It is well known that among members of the *Salmonella* group this change is usually associated with a progressive diminution or loss of virulence.

These serological findings in regard to Ratin, Danysz Virus and Liverpool Virus agree with those of Bainbridge (1909), Savage & White (1925) and Smith & Scott (1930). Bainbridge also examined Ready Rat Relief Virus with similar results.

4. FERMENTATION TESTS

A detailed examination of the *S. enteritidis* group of organisms has been carried out by Kauffmann (1935), and by means of various fermentation tests the serologically identical types of *S. enteritidis* (Gaertner) were divided into four main subgroups: (1) the classic *S. enteritidis* type; (2) var. *danysz* to which, according to Kauffmann, both the Danysz and Ratin Viruses belong; (3) var. *chaco* which was isolated from cases of continued fever during the Chaco war; and (4) var. *essen* which has been isolated in cases of gastroenteritis in man, and from ducks and duck eggs.

In order to determine whether all these six virus strains should be classified as var. *danysz*, two media were used: Bitter's rhamnose (Bitter, Weigmann & Habs, 1926) and Stern's 'Glycerinfuchsinbouillon' (Stern, 1916). The reactions of the four different subgroups in these two media are given by Kauffmann as:

Subgroup		Bitter's rhamnose	Stern's medium
S. enteritidis		±	+
,,	var. *danysz*	−	−
,,	var. *chaco*	+	±
,,	var. *essen*	+	+

The Bitter's rhamnose medium is incubated from 16 to 20 hr. at 37° C. and a few drops of an alcoholic solution of methyl red are then added to the tubes: a positive reaction is shown by a red colour, a negative by a yellow. The power to ferment glycerol in Stern's medium is shown after 20 hr. incubation by the development of a deep red colour, which within 2 days passes through the stages, deep purple to deep lilac. The golden yellow colour of the medium has not been changed during this time by a negative strain. Kauffmann further subdivides these negative strains (± and −) according to the reactions of the medium after a fortnight's incubation. In the notation used here, + signifies a strain which turns the medium deep red within 17 hr. and deep lilac within 2 days, − signifies a strain with which the yellow colour of the medium is unchanged at the end of 3 days. The tests were not observed for longer than a week, by which time various shades of rosy red colour had developed in the negative and sterile control tubes.

The results are given in Table 3.

The inoculations of the Bitter's medium were made with one large loop of a 17 hr. old broth culture and the results read after 16 hr. incubation. A repetition of the test, using saline suspensions of the organisms as the inoculum (Kauffmann's recommendation), and incubated for 21 hr., gave precisely similar results, *enteritidis* Dennis, London Virus and Institut Pasteur Virus being rhamnose-positive, the remaining four negative. Controls, consisting of three *Salmonella* (*typhi murium*, *paratyphi* B and English hog cholera), were also put up in both media.

Tested in Bitter's Dulcite, which serves to differentiate the var. *essen* subgroup, both London Virus and the Institut Pasteur Virus were positive after 16 hr. incubation. Thus, according to Kauffmann's classification, both these strains would be assigned to the classic *S. enteritidis* subgroup, and not to the var. *essen*.

Table 3

| Strain | Bitter's rhamnose | | Stern's glycerol |
	Reaction	Approx. pH	
Liverpool Virus	−	>6·0	−
Institut Pasteur Virus	+	5·2–5·4	+
Ready Rat Relief Virus	−	>6·0	−
Danysz Virus	−	>6·0	−
London Virus	+	5·2–5·4	+
Ratin	−	>6·0	−
S. enteritidis Dennis	+	4·8–5·0	+

The results of these fermentation tests may be summarized as follows:

(1) The Liverpool, Ready Rat Relief, Danysz and Ratin Viruses belong to the var. *danysz* subgroup.

(2) The Institut Pasteur and London Viruses could not be differentiated from the classic type of *S. enteritidis*.

Although such reactions as have been described in this section are an important aid to the bacteriologist in identification, they are in themselves no guarantee of any specific racial differences in pathogenicity for rodents as against man. Moreover, it is still unknown whether these relatively minor differences in fermentative power, such as the ability or otherwise to ferment rhamnose in a special medium, remain a permanent feature of some particular strain.

5. PATHOGENICITY FOR MAN AND DOMESTIC ANIMALS

Owing to the antigenic identity which has long been known to exist between many of these virus strains and *enteritidis* Gaertner, numerous warnings have been given by reputable authorities, both in England and on the Continent, of their potential danger to man. In particular it has been emphasized that great care is necessary in using them in places where contamination of food or drink might occur, either with the virus material itself or by the excreta of rats or mice infected with it (Ministry of Health, 1933, p. 159). There are, too, a number of cases reported in the literature where outbreaks of gastro-enteritis in man have been either directly traced, or attributed on reasonable evidence, to the use of some virus preparation of the Gaertner type, e.g. Kristensen & Bojlén (1931), Boecker & Kauffmann (1930), Spray (1926), Wreschner (1921), and Willführ & Wendtlandt (1921). Among these references special mention will be made here of

Kristensen & Bojlén's work, since their cases are perhaps less well known than some of the others.

These authors describe ten outbreaks of acute gastro-enteritis, involving fifty-two persons in Denmark between 1928 and 1930 in which *S. enteritidis* var. *danysz* was iso-lated from at least one case in each outbreak. The bacteriological typing was done by agglutination and fermentation tests, and, among the latter, both Bitter's rhamnose and Stern's Glycerinfuchsinbouillon were used in order to differentiate these var. *danysz* strains from the classic Gaertner type. In addition to the laboratory investigations a personal visit was paid to the scene of each outbreak in order to verify the field evidence. In two of these outbreaks, consisting of one fatal case and three severe cases respectively, Kristensen & Bojlén considered that the evidence pointed to the Ratin Virus as having been almost certainly the source of infection. The details of these two cases are briefly as follows, the case numbers being the same as those used in the original paper.

Case (1) occurred in December 1928 at Vester Thorsted, Jutland. A household, con-sisting of a man and his wife together with four children, had been greatly troubled by mice. On 8. xii baits (bread soaked in Ratin culture) were laid out in the house and, among other places, ten of these baits were put on a newspaper in the family bedroom. Next morning between 8 and 9 a.m. one of the children, a boy aged one year four months, was observed holding a piece of the bread in his hand and there was only one bait left on the newspaper. It was never found out whether the remaining eight had been eaten by mice or by the child. The boy's face and hands were not washed after this discovery. During the afternoon of the same day he began to be troubled with diarrhoea, and by 10 p.m. that night a severe attack of gastro-enteritis had developed. He died at 1 p.m. on 10. xii, i.e. some 28–29 hr. after contact with the Ratin. At the post-mortem no other signs of illness were found except those of an acute intestinal infection; and portions of the small intestine, with an adhering lymph gland, and of the large intestine were sent in to the laboratory for examination, together with the remainder of the Ratin bottle. The organism was isolated from all three sources and there was complete agree-ment between the strains as regards agglutination and fermentation tests. Moreover, the lymph gland and virus strains were similar in their virulence for white rats, 4 out of 10 and 5 out of 10 animals respectively dying within 3 weeks after the same individual doses of broth culture by the mouth. Except for this child, no other member of the family was ill.

Case (8) occurred at Uldum in June 1930. Between 4. vi and 14. vi Ratin baits were laid down daily in the town by a man aged 55 years (A.N.). The preparation of the baits took place on his dining-room table and, in this part of the work, he was assisted by his 12-year-old daughter (O.N.). A 2-year-old boy (T.H.) was also an interested spectator. On 14. vi the Ratin had spurted up in T.H.'s face, and during the afternoon A.N. had given him a piece of bread and butter while in the middle of preparing the bait. On the morning of 14. vi A.N. had felt ill with qualms and vomiting; and by the next day he was down with the typical symptoms of gastro-enteritis. In the afternoon the daughter started with colic pains and diarrhoea, and in the evening of the same day the child also. The patients were admitted to hospital and all three recovered after an illness of some 3–4 days' duration. An *enteritidis* var. *danysz* organism was isolated from A.N.'s faeces on 15. vi and 21. vi, after which date the results were negative. No pathogenic organisms were isolated from the daughter or the child, but the earliest examination was on 21. vi by which time the patients were better. Agglutination tests done on 22. vi and 29. vi

with the patient's serums and a suspension of the virus organism gave the following results: O.N. positive 1/100 and 1/100; T.H. positive 1/50 and 1/100 on the respective dates.

In regard to the remaining eight of their outbreaks Kristensen & Bojlén point out that the following conditions were present: (1) the isolation of Ratin-type Gaertner organisms from the faeces of patients suffering from acute gastro-enteritis; (2) Ratin had been laid out a short time previously; (3) there was no certain evidence of any other source of infection. However, they concluded that in these eight cases there was no convincing proof of the aetiological role of the virus, although as a possibility it could not be entirely excluded, and even if Ratin were assumed to be in fact the causal agent, it could not be stated in what way the infection had been transmitted to the patients. One further case, no. (11), mentioned in parenthesis in their paper, is of some interest. It related to a woman who became ill after drinking a bottle of Ratin, and from personal information received from Dr Kristensen in 1936 it seems that the woman, aged 28, had attempted to commit suicide by drinking 175 c.c. of culture.

Wreschner (1921) reported two outbreaks which were attributed to the use, or rather misuse, of virus preparations. In the first of these two Russian prisoners of war, in spite of being told not to, ate a large number of Ratin potato baits which were being laid out in the camp. They both fell ill in a short time with the usual symptoms and death followed. Post-mortem, bacilli were isolated indistinguishable from the Ratin strain. The second was an outbreak of 35–40 cases occurring in 1918 in a Thüringen town. Two of the patients died, and from one of them a Gaertner strain was isolated post-mortem. The source of the infection was traced to a fancy cake, and it later appeared that a baker had intentionally added Ratin to the cream used in its preparation (for confirmatory evidence see Kristensen & Bojlén, 1931). Wreschner also mentions a third case, but this is clearly the same as that reported more fully by Willführ & Wendtlandt (1921). The outbreak consisted of ninety-five cases in a Brandenburg school and was attributed to a 'Ratten-typhus' virus which had been laid down 3 days before. The chain of evidence, however, is not entirely complete, since the organism was not isolated from any of the patients, the first specimen for examination being received 10 days after the infection started. A Gaertner-type organism was isolated from the remainder of the virus, and fifty-eight out of sixty-nine serums from the patients agglutinated this strain in dilutions mostly of 1/100–1/200, though a few were even higher, ranging up to 1/800. In a small number of patients control tests were done with other Salmonellas (T.A. and B.), and the titres recorded were about a quarter of those against the virus suspension. On the whole this case must be considered as suggestive rather than proved.

Spray (1926) gives a very full account of an outbreak of food poisoning, involving 123 persons at West Virginia University, which, he concluded, was probably due to the contamination by flies or red ants of some milk and cream with an unnamed rat virus. The organism was isolated from fourteen out of thirty-five patients examined 4–6 days after the start of the outbreak; from some virus bait which was found still in situ when the circumstances were investigated; and from a fresh sample of the virus. All these strains proved to be identical and were typed as *enteritidis* Gaertner. One suggestive link in the train of evidence was that, among the many reduction tests employed, all these cultures showed a delayed and slight fermentation of both dextrin and glycerol in egg-water broth, in contrast with a control strain of Gaertner which rapidly fermented

them. Boecker & Kauffmann (1930) reported four cases of food poisoning in the same household where Ratin baits had been laid down 2–3 days before. The organism was isolated from all four patients and from the remains of the bottle of virus. It was suggested that various domestic utensils had been contaminated by a rag which was used to clean the receptacles in which the bait had been prepared. Other recent outbreaks of food poisoning attributed to the use of viruses of the *S. enteritidis* type have been described by Scott (1933); Lentz, cited by Hohn & Herrmann (1935); and Solomin (1935). Kathe (1935) reported a number of human infections arising from the widespread use of a mixture of 'Mäusetyphus' (*typhi murium*) and 'Ratinbazillen' (Gaertner) cultures in order to combat a serious field-mouse plague in south Germany.

There is thus a body of cumulative evidence to show that human cases of gastro-enteritis have been caused by the use of virus preparations. Moreover, the recent Scandinavian work (Kristensen & Bojlén, 1931; Ströman & Örn, 1932) leaves little doubt as to the pathogenicity of the var. *danysz* subgroup for man. It is true that many of these reported outbreaks due to viruses have arisen from an improper or careless use of the preparation in question. From this point of view it is clear that some at least might have been obviated by a greater degree of care and attention in handling the material. There is, however, one result of the application of a bacterial culture in order to exterminate rats or mice which it is impossible to control. A number of animals will undoubtedly survive the dose consumed, and may continue to excrete the organism in their faeces or urine for variable periods of time. Thus Welch, Ostrolenk & Bartram (1941) have shown in the laboratory that white rats, infected with Gaertner by the mouth, may excrete the organism in their faeces up to 148 days afterwards. According to Price-Jones (1927) rats infected with Gaertner carried the organism for some period of time in the liver and spleen; about 10 % of his animals were still positive 2 months after infection, and a minority up to 5 months. Experimentally these apparently healthy survivors were able to initiate an epidemic among fresh rats under laboratory conditions. The potential danger to man arising from rodent carriers of Salmonellas in places where food is stored or prepared has been stressed by many workers. Actual outbreaks of food poisoning attributed to this source of infection have been described by Scott (1933), Staff & Grover (1936) and Jones & Wright (1936). In the first two of these cases *S. enteritidis* Gaertner was the causal organism, and in the last *S. typhi murium*.

Finally, there is one further point of importance and that is the question whether these virus strains may not be more dangerous to domestic animals, including poultry, than is generally assumed by their manufacturers. The serological type of *enteritidis* Gaertner has been isolated from a variety of mammals, and there is a certain amount of evidence that the var. *danysz* subgroup has a wider range of pathogenicity than merely for rodents and man. Thus Bahr (1929) states that out of fifty strains of his *enteritidis* type IV, which corresponds to the var. *danysz*, thirty were isolated from various rodents, fourteen from cases of human gastro-enteritis, one from a pig, one from a cow, and four from calves. In this connexion Hohn & Herrmann (1935) lay stress on the possible chain of infection, virus bait, pigs, man; and they refer to two cases of this nature described by Lütje. The original article appears to be unobtainable at present, but according to Hohn & Herrmann one of these outbreaks occurred at Osnabrück in 1926 and affected 180 people, of whom one died. The source of the infection was traced to a pig which had

infected itself with 'Ratinbazillen', and which then underwent emergency slaughter, the carcass being allowed by the veterinary surgeon to be sold for food.

The problem with regard to poultry is important, since so many of these farms are liable to harbour rats and may therefore suffer various methods of anti-rodent control, including the use of viruses. Experimentally, ducks can be easily infected with Gaertner by feeding, and the birds continue to discharge the organism abundantly in their faeces for some time (Ministry of Health, 1929, p. 212). In the same series of experiments it was also shown that duck eggs in contact with their damp, infected faeces can become infected through the shell, in the same way as with *S. typhi murium*. Outbreaks of food poisoning in man caused by the consumption of duck eggs infected with Gaertner have occurred in Western Germany (Hohn & Herrmann, 1935); and Kauffmann (1935), who examined a number of these strains, confirmed the serological typing of *S. enteritidis* and from their fermentation reactions placed them in the var. *essen* subgroup. In England, Gordon (1940) reported the isolation of *enteritidis* from carrier ducks and pointed out that thousands of ducklings are lost as the result of epidemics of Gaertner and other Salmonellas. But, although it is clear that this serological type is pathogenic for ducks, and possibly also for chickens, the var. *danysz* subgroup does not appear to have been isolated so far from poultry. The special types of fermentation tests, by means of which the serologically identical group of *enteritidis* Gaertner is further subdivided, are not, however, in common use as a routine method of analysis. In the present state of our knowledge it would be very dangerous to assume that these somewhat fine differences between strains indicate any clear-cut limitation of their pathogenicity to one species or another.

From the evidence which has been cited in this section it would appear safe to conclude that viruses of the *enteritidis* type can, if virulent to rats and mice, be also virulent to man. Moreover, we have reasonable grounds for believing that their range of pathogenicity is wider than this and includes many of the domestic animals, as well as some poultry. It is only fair to add that the number of reported outbreaks of human illness attributed to viruses is few in comparison with the extensive use which has been made of these preparations, both in England and on the Continent. In this connexion it has been suggested (Ministry of Health, 1933, p. 159) that probably relatively large doses of the organism are necessary to produce disease in man, and that the circumstances in which such doses might be ingested, seldom occur. But in general it may be said that outbreaks of food poisoning, limited to a small number of cases, are frequently overlooked and thus escape a full and detailed examination of the circumstances. The absence of reported cases, therefore, cannot be taken as evidence that the viruses are, to all intents and purposes, non-pathogenic, or that their use is unattended by any risk to the human population. The potential danger to man would appear to lie, not so much in the accidental consumption of the raw virus material itself—although this path of infection cannot be entirely excluded, particularly in the case of children, who are perhaps susceptible to much lower doses than an adult—but in the contamination of foodstuffs, such as milk, cream, custard, cooked meat or pies to be eaten cold, and so forth, all of which form admirable media for the growth of bacteria and in which rapid multiplication may occur under suitable conditions.

SUMMARY

1. The six 'viruses', Liverpool, Danysz, London, Ready Rat Relief, Institut Pasteur and Ratin, which are the principal bacterial cultures at present employed for anti-rodent control in Great Britain, have been examined.

2. By means of reciprocal absorption tests all these six strains were found to be serologically identical with *S. enteritidis* Gaertner, antigenic structure, IX:gom:—.

3. From the results of the fermentation tests, which may be used to subdivide this serological type, Liverpool, Danysz, Ready Rat Relief and Ratin were assigned to the var. *danysz* subgroup; while the London and Institut Pasteur strains could not be distinguished from the classic *S. enteritidis* type.

4. Both of these subgroups are pathogenic for man, and evidence is cited which shows quite clearly that human cases of gastro-enteritis have been caused by the use of virus preparations. There are, also, reasonable grounds for believing that these bacterial types may be pathogenic for a number of domestic animals, including some poultry.

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