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Studies of the Action of Sodium Fluoride on Human Enamel by Electron Microscopy and Electron Diffraction

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Clinical evidence accumulated over the past few years has indicated that the topical application of a sodium fluoride solution is effective in controlling dental caries (*1*). The mechanism by which the fluoride acts is as yet not known and is a matter of widespread laboratory investigation. Most studies to this end have been in the field of analytical chemistry, and they have shown that fluorine is taken up by powdered enamel suspended in solutions of sodium fluoride (*2*), and that after such treatment the powdered enamel is less soluble in certain dilute acids (*3, 4, 5*). Recent studies have demonstrated that electron microscopy, and X-ray and electron diffraction afford useful physical approaches to the problem. Gerould, by electron microscopy of silica replicas (*6*), has shown a deposit on enamel slabs after immersion for 30 days in 4 percent NaF. His X-ray and electron diffraction studies of fluoride-treated samples of powdered enamel have indicated that the product of the reaction is calcium fluoride. Holager and Syrrist (*7*), and Syrrist (*8*), have also reported that calcium fluoride is formed.

This report describes three sets of experiments designed to study by electron microscopy the visible effect of treating enamel with sodium fluoride, and to study by electron diffraction the crystalline changes on similarly treated enamel surfaces. The first series involves the electron microscopy of enamel slabs treated for varying lengths of time with NaF solutions; the second is devoted to the electron microscopy of enamel slabs etched with acid before and after treatment with NaF. In the third set of experiments, electron diffraction studies have been made of the crystalline composition of the surfaces of enamel slabs before and after similar treatments.

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Previous studies (*9, 10, 11*) have shown that the outer surfaces of the enamel, which are those treated clinically, are rich in structural detail, and that this detail varies according to age of the tooth, the location of the surface on the tooth, and the position of the tooth in the dental arch. Because of these variations and the fact that our knowledge of tooth surfaces is still largely restricted to detail visible at the low magnifications of the optical microscope, it has seemed best not to examine fluoride-treated outer surfaces in these first experiments. Instead all specimens have been slabs of subsurface enamel. The slabs, from noncarious molars, were so cut and polished (*12*) that their faces were approximately parallel to the outer enamel surfaces.

All test and control solutions were filtered and placed in paraffin-lined jars. Eight to twelve slabs were placed, polished face up, on the bottom of a jar containing 200 ml. of solution, and the closed jar was kept at room temperature for the required length of time. The slabs were then removed and rinsed for one minute by agitation in distilled water. At this point, in those experiments that required it, the slabs were etched and rinsed.

The slabs were next dried and collodion replicas made of the polished surfaces according to the usual techniques (*12*). These replicas were then shadowed with chromium at a 3:1 angle and examined and photographed with an RCA type EMU electron microscope. A total of about 1,200 preparations from the replicas were examined; observations were recorded in approximately 3,000 micrographs.

Electron diffraction was conducted on intact slabs prepared and treated in the same manner as for electron microscopy. For examination these slabs were removed from the solutions, rinsed, dried, and placed immediately in the specimen holder of an RCA type EMD diffraction equipment.

Electron Microscopy of Enamel Treated with Sodium Fluoride

In these experiments, enamel slabs from 261 teeth were immersed in sodium fluoride solutions for periods ranging from 5 minutes to 30 days. The number of specimens and length of treatment are shown below.

Immersion time	*Number of specimens immersed in*	
	NaF	H_2O
5 minutes	30	12
16 minutes–2 hours	48	22
16 –24 hours	39	21
2 days	20	10
7 days	12	8
15 days	18	12
30 days	94	68
Total	261	153

Control slabs, distributed as shown in the table, were immersed for the same periods of time in distilled water. All solutions were 2 percent NaF except in the experiment where slabs were immersed for 30 days. In this case, 20 specimens were immersed in 3 percent NaF and a like number in 4 percent NaF. The pH of the solutions was not adjusted, but measurements were made at the beginning and end of each experiment. The initial pH was always between 7 and 7.7; it did not change appreciably during the shorter experiments, but at the end of longer experiments the final pH was generally higher with a maximum of 8.5.

There has been considerable variation in the appearance of specimens subjected to the same treatment. These variations have existed not only between specimens but also between different areas on the same specimen. The controls have been relatively uniform, showing only minute roughness (fig. 1) except in a few instances where this roughness has been more pronounced (fig. 2). In the treated specimens, on the other hand, there have been great differences in appearance. About three-quarters of the specimens immersed in NaF for periods from 5 minutes to 24 hours closely resembled the controls (fig. 3). There were a few small areas on the remainder which, though slightly different in appearance from the controls, did not exhibit the pronounced changes noted in specimens exposed for 15 and 30 days. Half of the 2- and 7-day specimens appeared the same as the controls. About a quarter showed small patches like those seen after the shorter exposures, while the remaining quarter had small areas demonstrating the pronounced changes seen after the long immersions (fig. 4). A widespread, striking alteration in surface appearance was first noted after immersion for 15 days. About half of these specimens showed an inhomogeneous, apparently crystalline deposit (fig. 5). The crystals were distributed over the entire area observed, but were sufficiently separated to leave seemingly unaffected areas. The remaining 15-day specimens showed only small scattered areas of similar deposit. About two-thirds of the slabs immersed for 30 days showed crystalline deposits over the entire areas observed. The amount of deposit varied from that noted in the 15-day specimens to layers so heavy that no unaffected areas could be seen (fig. 6). Most of the remaining specimens showed only small areas of deposit; a few were negative and resembled the controls.

To determine whether stronger NaF solutions would produce a more plentiful deposit, 20 more specimens were immersed for 30 days in 3 percent NaF, and 20 in 4 percent NaF. The same variations in deposit were observed with these stronger solutions as with the 2 percent solution, and the effects did not appear more pronounced.

These experiments bring out the fact that prolonged treatments

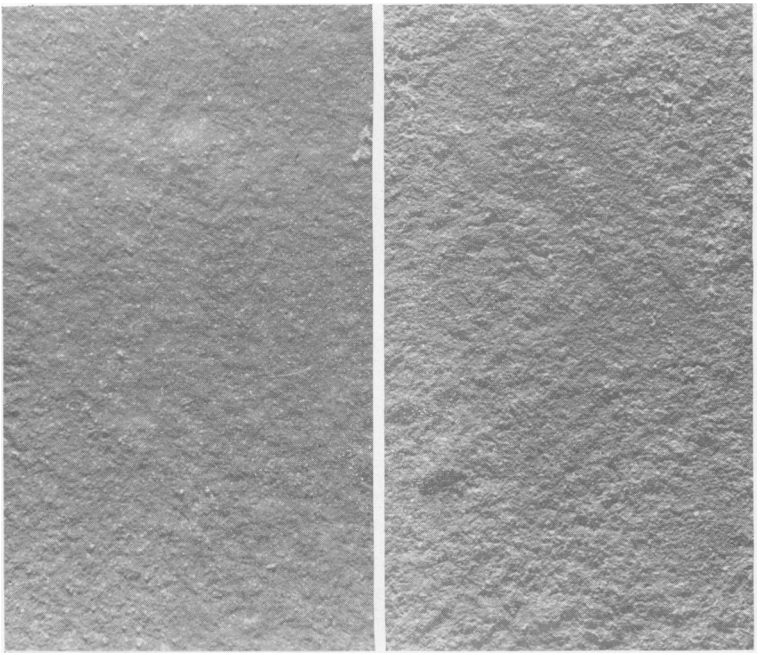

Figure 1. Left and right: minute roughness observed in most control specimens (7,200X).

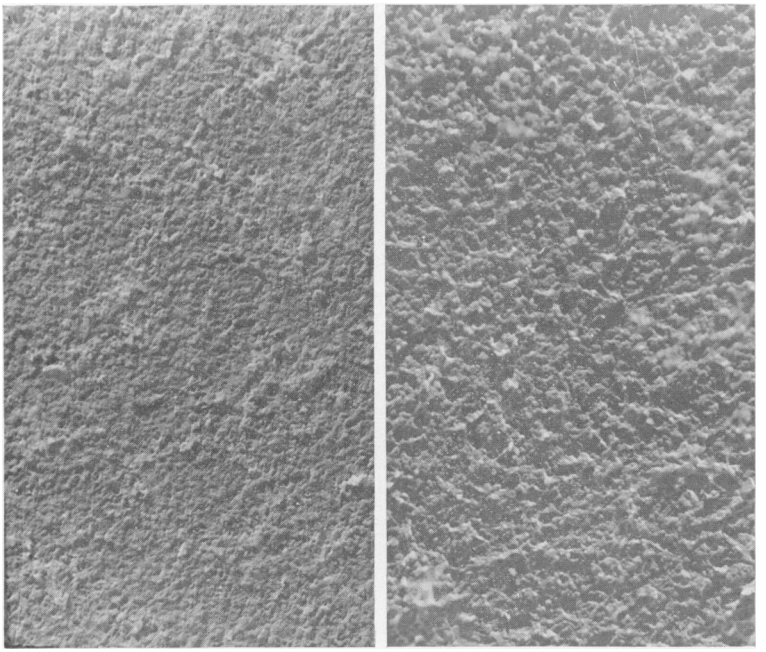

Figure 2. Left and right: more pronounced roughness observed in some control specimens (7,200X).

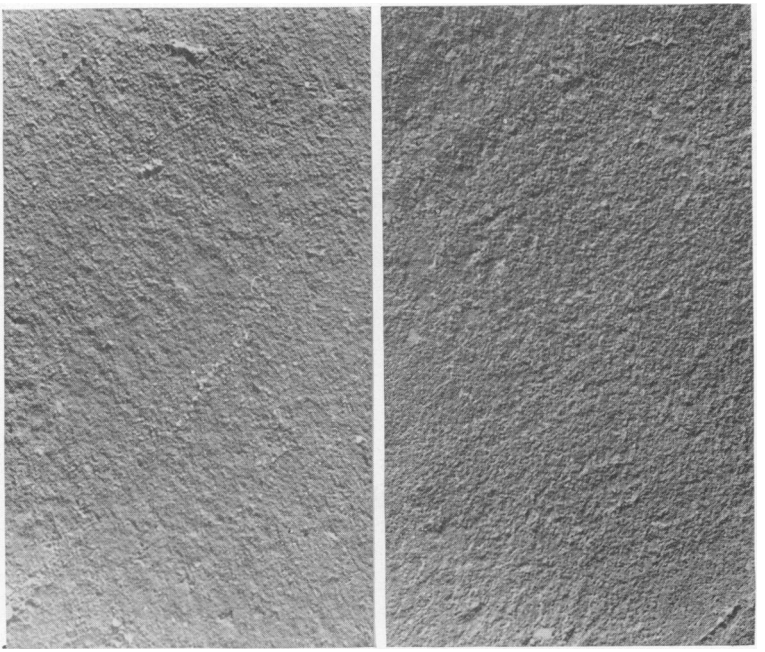

Figure 3. Typical appearance of enamel immersed in NaF for 5 minutes (left) and 24 hours (right) (7,200X).

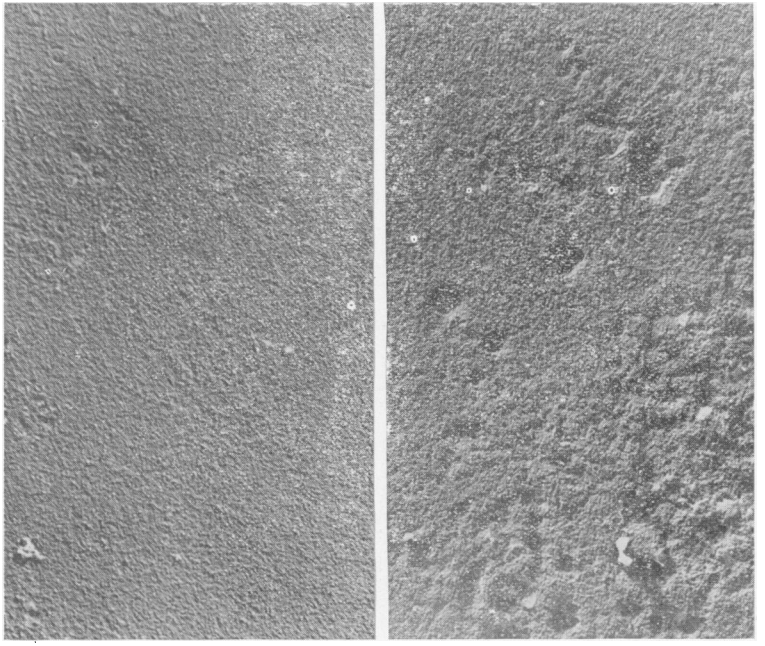

Figure 4. Normal (left) and slightly changed (right) areas on enamel after 2 and 7 days in NaF (7,200X).

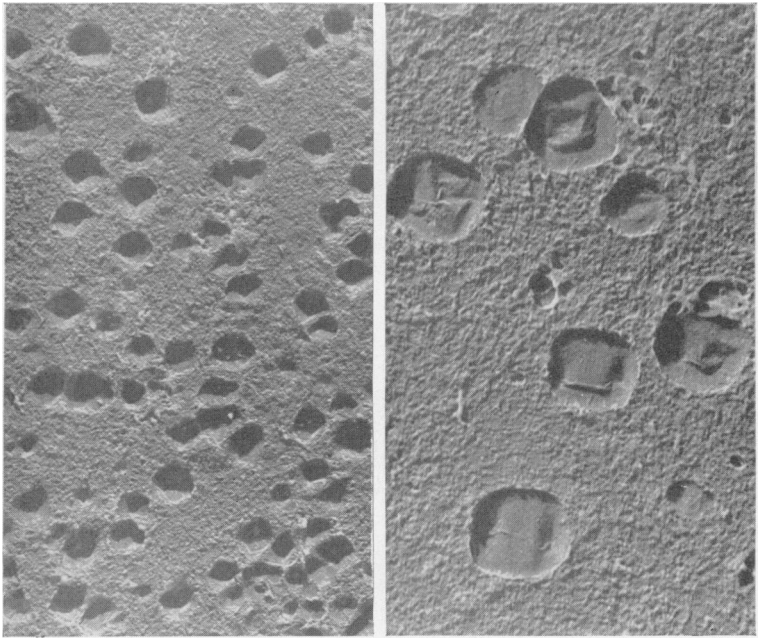

Figure 5. Deposit formed on enamel after 15 days in NaF. (Left, 7,200X; right, 11,000X).

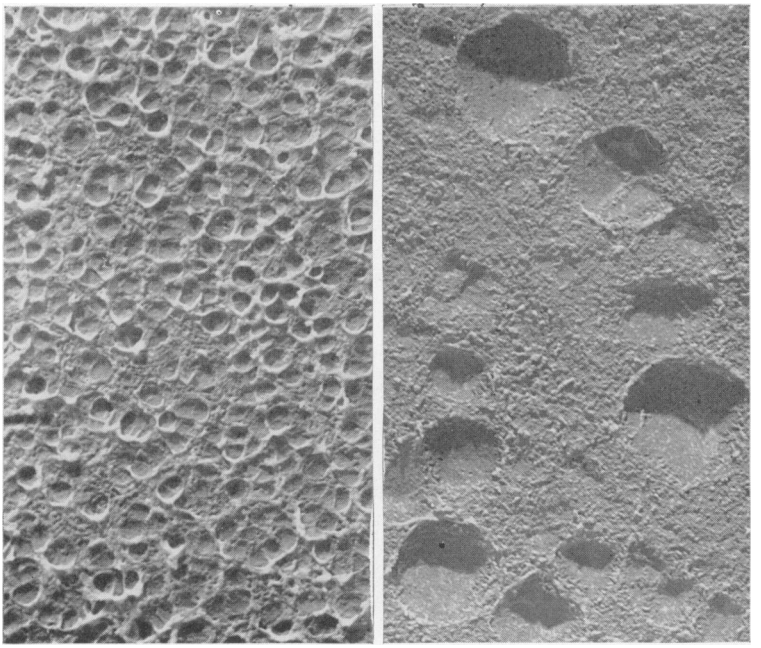

Figure 6. Deposit formed on enamel after 30 days in NaF. (Left, 7,200X; right, 11,000X).

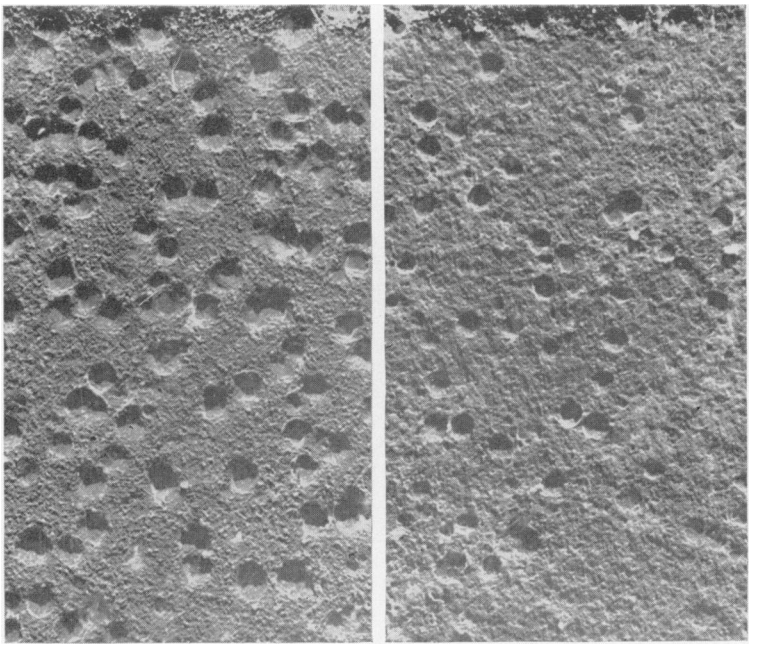

Figure 7. Appearance of 30-day NaF treated specimens after 1 day (left) and 3 days (right) washing (7,200X).

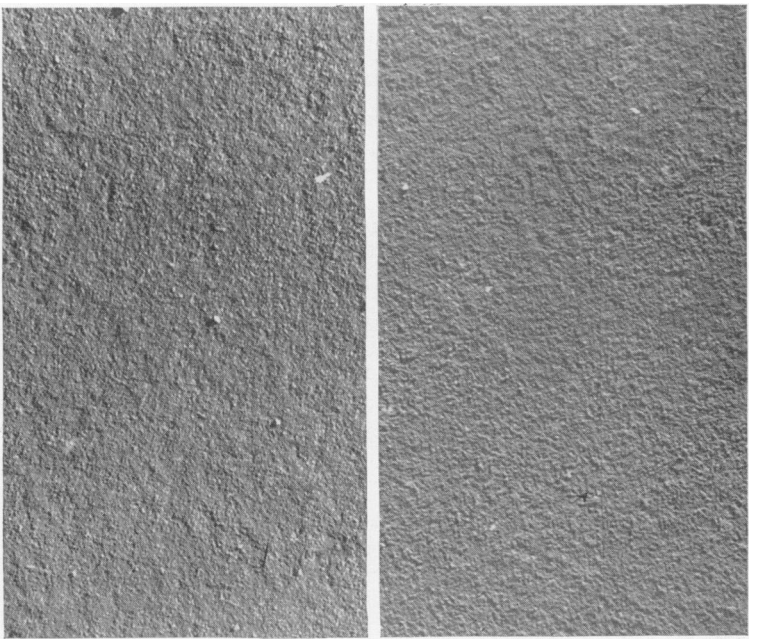

Figure 8. Appearance of 30-day NaF treated specimens (left) and controls (right) after 6 days washing (7,200X).

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with NaF are required to alter the appearance of enamel as seen at the high magnifications of the electron microscope. They show that immersion for about 7 days in 2 percent NaF solutions is required to establish the first obvious traces of a surface deposit, and that the effect is progressively more pronounced at 15 and 30 days.

The permanence of the deposit was investigated through washing experiments. The test objects were 36 slabs which had been immersed for 30 days in 4 percent NaF. To serve as controls, a similar number of slabs were immersed in water and the same number in 5 percent NaCl for 30 days. After immersion, each group of slabs was placed in a separate glass vessel containing two liters of distilled water agitated by a mechanical stirrer. The water was changed daily, and 12 specimens were removed from each group after 1 day of washing, 12 after 3 days, and 12 after 6 days. A pronounced deposit remained on all the NaF-treated specimens which had been washed for 1 day (fig. 7, left); traces of deposit were observed after 3 days of washing (fig. 7, right), but no deposit was seen after 6 days of washing (fig. 8, left). Both the water and NaCl controls appeared normal after each period of washing (fig. 8, right). It thus appears from these preliminary experiments that the surface deposit which results from long immersion in NaF solutions is slowly removed by immersion in water. Subsequent studies are planned to investigate further the permanence of such deposits.

Electron Microscopy of Enamel Etched Before and After Immersion in Sodium Fluoride Solutions

It has previously been reported by Gerould (*6*) that a surface deposit is formed on enamel pre-etched with acid and immersed in NaF, and by Gerould (*6*), Holager and Syrrist (*7*), and Syrrist (*8*), that enamel becomes more resistant to acid after treatment with NaF. In the course of other studies in this laboratory on the fine structure of enamel, marked variations have been noted in its response to acid. The acid which has been most often used, and which has revealed much interesting fine detail, is 0.1 N HCl. Gerould (*6*), using silica replica techniques, and Syrrist (*8*), using a double replica method, etched enamel for 10 seconds in 0.1 N HCl, but this period seems to produce too great a depth of etch for satisfactory shadowed collodion replica studies. Although in many areas the resulting etch is sufficiently shallow to produce detail which can be visualized clearly, in most cases the elevations and depressions are so great, and their shadows so long, that much detail is obscured (fig. 9, left). We have found the most generally useful etch to be one obtained through 5 seconds contact with 0.1 N HCl. With this etch, it has been repeatedly observed that in different areas on a single specimen, and on different specimens, the enamel may be so etched as to produce a

pronounced enamel rod pattern (fig. 9, right), a faint rod pattern (fig. 10, left), or even no apparent change in structure at all (fig. 10, right). This makes it necessary to exercise considerable caution in the interpretation of structural differences seen in etched fluoride-treated specimens.

A deposit similar to that noted on unetched specimens is formed when pre-etched specimens are placed in NaF solutions. This was demonstrated in an experiment in which 28 enamel slabs were etched for 5 seconds in 0.1 N HCl, rinsed by agitation in distilled water for one minute, and placed in 4 percent NaF. Twelve specimens were removed after 15 days and the rest after 30 days. A deposit was observed under the electron microscope on most of the surfaces, and it was present in amounts roughly similar to those seen on the unetched slabs. In many instances an enamel rod pattern could no longer be seen (fig. 11, left); in some areas it was only faintly visible (fig. 11, right), and in a few cases it was as pronounced as that found on the controls, with no trace of deposit.

In a second experiment, 20 slabs were etched for 5 seconds, and washed for 24 hours in a vessel containing 2 liters of distilled water agitated by a mechanical stirrer prior to immersion in the NaF. This was done in order to be certain that all of the soluble salts and debris were thoroughly removed from the etched surfaces. The specimens were then immersed for 30 days in 4 percent NaF, washed, and replicated as usual. The deposit was noted on most of these preparations, but their study left the general impression that it was less plentiful than before.

It has proved equally difficult to interpret the results of etching specimens subsequent to their immersion in fluoride solutions because of their varied appearance after etch. In one trial 30 specimens, immersed in 2 percent NaF for periods of 5 minutes to 40 hours, rinsed for 1 minute, and etched for 5 seconds with 0.1 N HCl, showed no deposit and had surface structures similar to those in etched untreated enamel. There was, however, some suggestion of an increased acid resistance of the surfaces of 20 other specimens which had been immersed for 30 days, and similarly etched. After etching, some specimens still showed traces of deposit (fig. 12, left), but other specimens appeared to be free of deposit (fig. 12, right), and a few showed areas of apparently normal etched enamel. It is evident that much more work must be done with etched normal and treated enamel before definite evidence can be obtained concerning changes in acid solubility brought about by NaF.

Electron Diffraction of Enamel Treated with Sodium Fluoride

Although the electron microscope has revealed the formation of a visible deposit on enamel surfaces after prolonged immersion in NaF

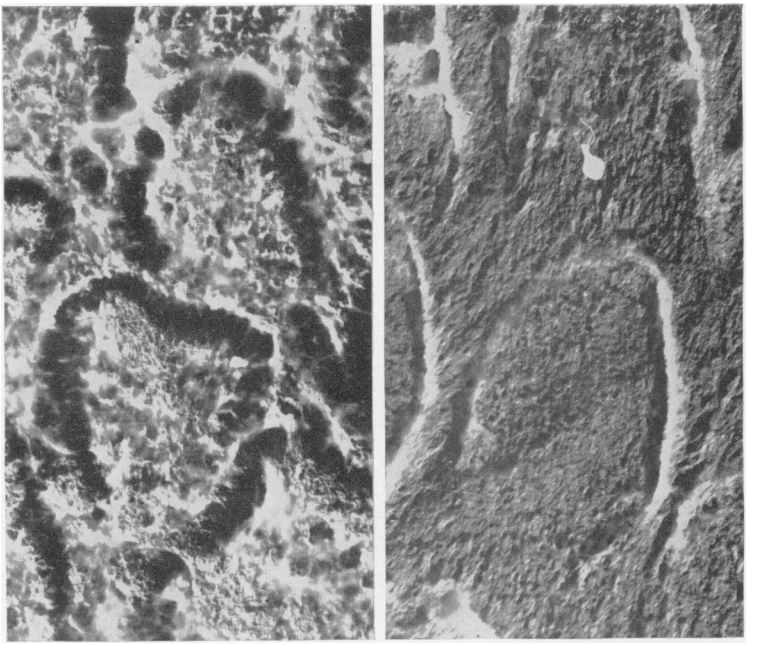

Figure 9. Left: untreated enamel etched 10 seconds with 0.1 N HCl. Right: untreated enamel etched 5 seconds with 0.1 N HCl (7,200X).

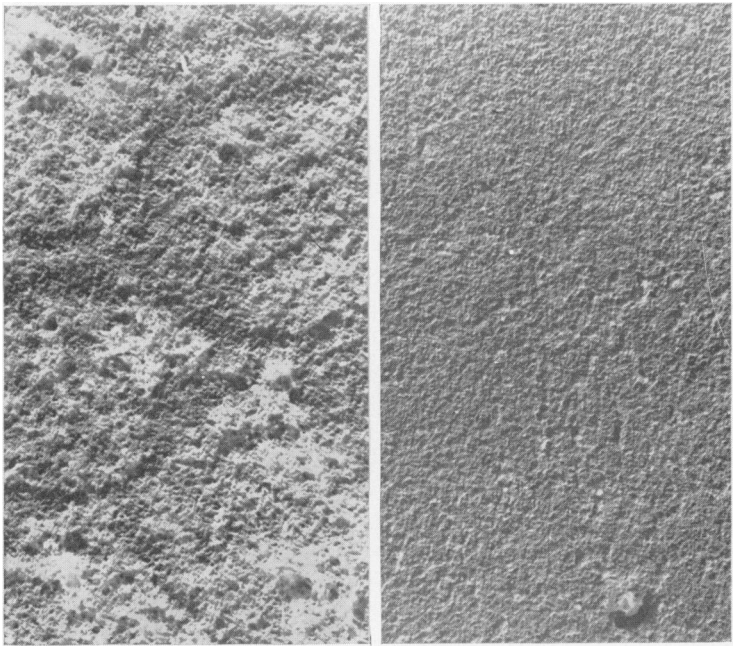

Figure 10. Untreated enamel etched 5 seconds with 0.1 N HCl. Left: faint rod pattern. Right: no change in structure. (7,200X).

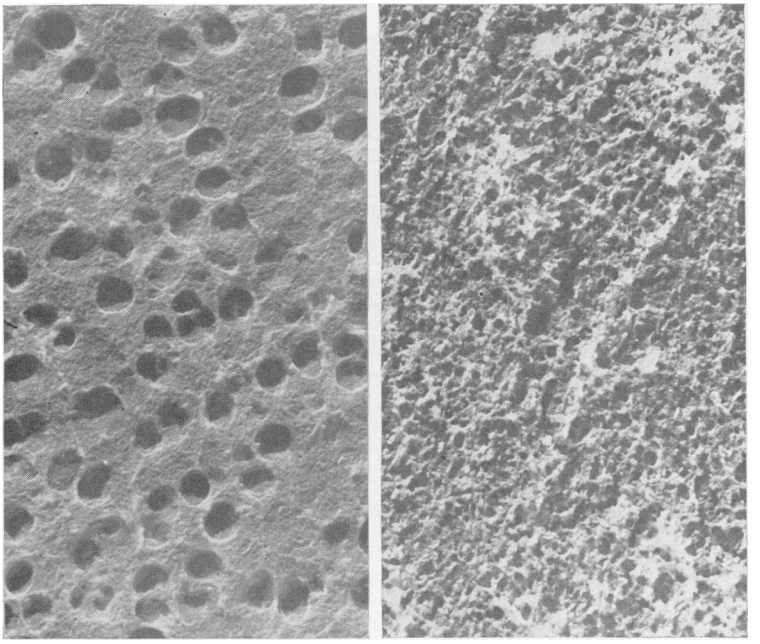

Figure 11. Pre-etched enamel immersed in NaF for 30 days. (7,200X).

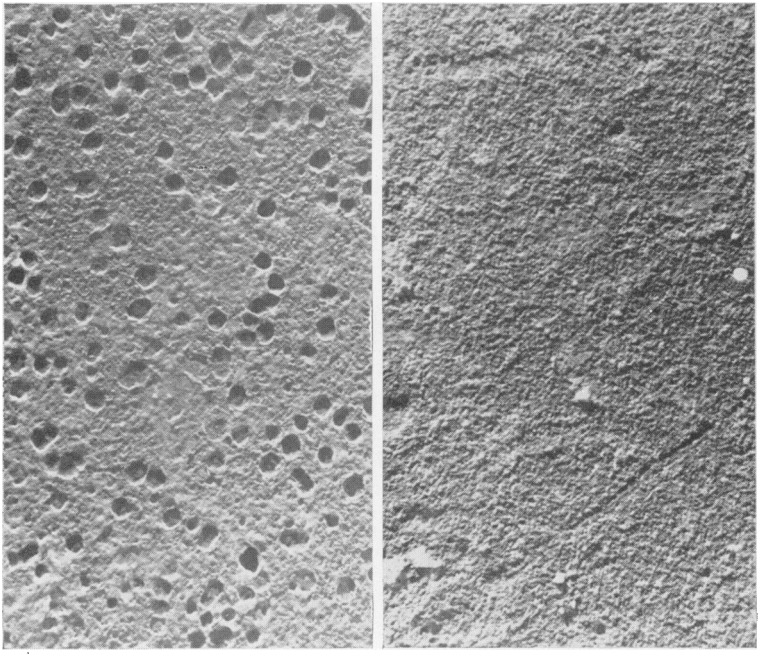

Figure 12. Enamel immersed 30 days in NaF and etched 5 seconds with 0.1 N HCl. (7,200X).

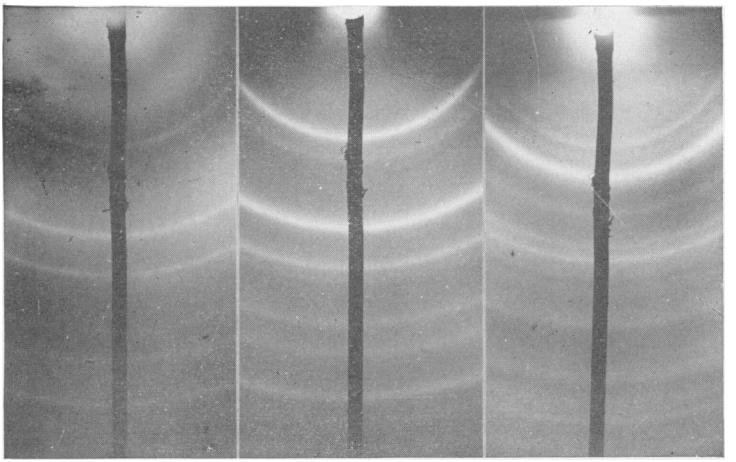

Figure 13. Diffraction patterns from untreated enamel (left); from enamel treated with NaF (center); and from CaF_2 (right).

solutions, it has failed to show changes after the shorter treatments that are of clinical significance. Changes resulting from these short treatments can, however, be demonstrated through electron diffraction.

Taking advantage of the ease and precision with which the new RCA type EMD electron diffraction unit can provide electron diffraction patterns from the surfaces of solid objects, we have used it to follow chemical changes in the surfaces of enamel slabs which have been treated by NaF in a fashion identical to those studied by electron microscopy. Such diffraction studies of enamel surfaces have a two-fold advantage over diffraction studies using powdered enamel. They permit a direct comparison with the results of electron microscopy since patterns can be obtained from a surface before and after treatment, and from the exact surface that is to be replicated for microscopy. They also allow determination of chemical changes that may be brought about in enamel by the relatively short exposures to NaF that are clinically feasible.

In a first diffraction experiment, 30 enamel slabs were immersed in 2 percent NaF and 10 in distilled water, both for 30 days. At the end of this time, the specimens were washed for 1 minute in water, dried, and electron diffraction patterns made from their surfaces. The pattern obtained from untreated enamel was that of apatite (fig. 13, left), while a completely different pattern was obtained from the specimens treated with NaF (fig. 13, center). This pattern has been identified as that of CaF_2, both by measurement of the observed spacings and by comparison with patterns from CaF_2 itself (fig. 13, right). The CaF_2 pattern was given by all the treated specimens, although, when replicas of the surfaces of the slabs were examined

under the electron microscope, the usual variety in appearance and quantity of visible deposit was noted.

After experimenting with progressively shorter immersion periods in NaF, it was found that the apatite pattern is replaced by that of CaF_2 after as short an exposure as 3 minutes. This was demonstrated through an experiment in which patterns were taken from the surfaces of 28 enamel slabs before and after 3 minutes' immersion in 2 percent NaF; in all cases the CaF_2 pattern was present after the treatment. Replicas of 12 of these surfaces were then examined under the electron microscope and no deposit could be detected.

Preliminary washing experiments were performed similar to those in the electron microscopic studies. Patterns were taken from the surfaces of 12 specimens before and after a 3-minute immersion in 2 percent NaF. They indicated that the surface of every specimen had become microcrystalline CaF_2. These slabs were then washed in circulating water and new patterns made after 15 and 90 minutes. The CaF_2 pattern was invariably present after the shorter period, but after 90 minutes every pattern had reverted to the apatite pattern obtained before treatment with NaF.

It is apparently more difficult to bring about the reversion of the CaF_2 pattern to the apatite pattern in specimens which have been immersed for long periods in NaF. Thus 12 specimens, which gave CaF_2 patterns after 30 days' immersion in 2 percent NaF, were washed for 6 days in water and, at the end of this time, 9 of the slabs still yielded the CaF_2 pattern while the other 3 gave that of apatite.

These observations demonstrate that there is a definite modification of the surface of enamel treated with NaF for periods as short as 3 minutes. Taken in connection with the microscopic studies, they show that this alteration is superficial and that prolonged exposures are necessary before definite visible changes in surface structure can be recognized, even under the high magnifications of the electron microscope. These facts, together with the relatively short time required to wash it away, clearly show the thinness of the CaF_2 layer that is formed after short treatments with NaF.

Summary

Electron micrographs have been made of replicas of the surfaces of enamel slabs treated with NaF solutions for lengths of time between 5 minutes and 30 days. Few visible surface changes were recognized when the treatment was less than a week. Small amounts of deposit were noted after 7 days. Greater quantities were seen after 15- and 30-day treatments. It was not visible after the prolonged washing of 30-day treated specimens. Preliminary but inconclusive tests were made of the relative acid solubilities of untreated enamel and enamel after immersion in NaF.

Electron diffraction patterns have been made from the surfaces of enamel slabs before and after immersion in NaF for periods from 3 minutes to 30 days. In all instances the original apatite pattern was converted to that of CaF_2. The CaF_2 pattern reverted to that of apatite when specimens treated for 3 minutes with NaF were washed for 90 minutes in water, but when specimens treated for 30 days were washed for 6 days the reversion did not occur in all instances. Electron micrographs of surfaces after diffraction showed amounts of deposit to be expected from the treatments they had undergone.

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Serological Survey for Murine Typhus Infection in Southwest Georgia Animals

By Harvey B. Morlan, B. S., Elmer L. Hill, M. D., and Joseph H. Schubert, Ph. D.*

Detailed studies of domestic rats were conducted during the evaluation of county-wide DDT dusting operations in murine typhus control (*1*). In addition to information regarding the prevalence of typhus in the principal rat reservoir, data were obtained to contribute toward a determination of any supplemental reservoir of typhus among other animals. From October 1945 through January 1949, sera for the typhus complement fixation test were collected from a wide variety of animals. Most of the animals were collected in Decatur, Grady, Thomas, and Brooks Counties, with a few from the nearby and similar Cook and Berrien Counties.

Recent observations of murine typhus in Georgia have been reported by Bowdoin (*2*) and Boston (*3*). References to literature prior to 1938 are given by Brigham and Dyer (*4*) who list animals found by various workers to be susceptible to infection with either epidemic or endemic typhus as follows: monkey, guinea pig, rabbit, gray rat, white rat, white mouse, gerbil, ground squirrel (*Citellus citellus*), squirrel (Xerus [*atlantoxerus*] *getulus*), dog, cat, meadow mouse (*Arvicola arvalis*), dwarf mouse (*Mus minutus*), garden mouse, wood mouse, hedgehog, and pigeon.

On the basis of re-isolation of the laboratory inoculated Wilmington strain, Dyer (*5*) and Brigham (*6*, *7*, *8*) reported the following species of animals to be susceptible to infection with murine typhus rickettsiae:

1. Opossum, *Diedelphis virginiana.*
2. Old-field mouse, *Peromyscus polionotus polionotus.*
3. Cotton mouse, *Peromyscus gossypinus gossypinus.*
4. Cotton rat, *Sigmodon hispidus hispidus* (=*S. hispidus komareki*).
5. Rice rat, *Oryzomys palustris palustris.*
6. House mouse, *Mus musculus musculus.*
7. Cottontail rabbit, *Sylvilagus floridanus mallurus.*
8. Skunk, *Mephitis elongata.*
9. Fox squirrel, *Sciurus niger niger.*
10. Golden mouse, *Peromyscus nuttalli aureolus.*
11. Flying squirrel, *Glaucomys volans saturatus.*

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* Sanitarian (R), Surgeon, and Scientist, respectively, Communicable Disease Center, Atlanta, Ga. This study from the Typhus Investigations Project at Thomasville, Ga., was made cooperatively with the Georgia Department of Public Health, C. D. Bowdoin, M. D., Director, Division of Preventable Diseases, and Roy J. Boston, Director, Typhus Control Service.

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12. Gray squirrel, *Sciurus carolinensis carolinensis.*
13. Cat, *Felis domestica.* [1]
14. Wood rat, *Neotoma floridana rubida.*
15. Swamp rabbit, *Sylvilagus aquaticus aquaticus.*
16. Woodchuck, *Marmota monax monax.*
17. Meadow mouse, *Microtus pennsylvanicus pennsylvanicus.*
18. Whitefooted mouse, *Peromyscus leucopus noveboracensis.*
19. Chipmunk, *Tamias striatus striatus.*

One or more sera collected in southwest Georgia from animals of the first nine species listed were found positive to the complement fixation test for murine typhus fever. All sera from species 10 through 14 were negative to the typhus complement fixation test. The remaining animals listed are not known to occur in the Typhus Investigations study area.

Brigham (*7*, *8*) reported the raccoon, *Procyon lotor lotor*, and the gray fox, *Urocyon cinereoargenteus cinereoargenteus*, to be insusceptible to murine typhus. This finding was strengthened by serological evidence obtained in the Georgia survey where different subspecies of the raccoon and fox were found to be negative to the typhus complement fixation test.

After inoculation of wild rodents with passage strains of endemic typhus, Lillie, Dyer, and Topping (*10*) observed characteristic reactions in brains of the following animals: *Peromyscus polionotus*, *P. leucopus*, *P. eremicus*, *P. maniculatus*, *Reithrodontomys* sp., *Mus musculus*, and *Rattus norvegicus.*

Brigham (*11*) reported the recovery of endemic typhus virus from an old-field mouse, *Peromyscus polionotus polionotus*, which was trapped on rural premises in the southeastern part of Alabama.

Irons et al. (*12*, *13*) report the recovery of endemic typhus rickettsiae from individual pools of the common cat flea, *Ctenocephalides felis*, taken from kittens, opossums, and puppies; and from a pool of the oriental rat flea, *Xenopsylla cheopis*, from a kitten. Mazzotti and Varela (*14*) tested 24 Mexico City dogs for the Weil-Felix reaction with *Proteus* OX 19 and reported that 10 sera reacted at a dilution of 1 : 20, 10 at 1 : 40, and 4 at 1 : 80.

In working with experimental typhus infection in the eastern cotton rat, *Sigmodon hispidus hispidus*, Anderson (*15*) found sera from recovered rats had neutralizing, complement fixing, and antitoxic antibodies, but no agglutinins for *Proteus* OX 19.

Using the complement fixation test for endemic typhus and tissue inoculations into guinea pigs, Beck and van Allen (*16*) found naturally infected California specimens of *Rattus norvegicus*, *R. rattus*, *R. alexandrinus*, and *Mus musculus.* Other California animals tested with negative results included *Citellus fisheri*, *C. beecheyi*, *Sigmodon hispidus*

[1] This finding was in confirmation of previous work by Lépine and Lorando (*9*).

eremicus, Citellus tereticaudus tereticaudus, Neotoma albigula, Neotoma fuscipes macrotis, Ammospermophilus leucurus, Dipodomys deserti, Peromyscus eremicus eremicus, cat, ground owl, and gopher. Their experimental inoculation of *Citellus beecheyi* demonstrated this ground squirrel may be infected with a murine strain of typhus fever.

In addition to those animals previously shown to be susceptible, the Georgia survey found sera from the weasel and blue jay positive to the murine typhus complement fixation test.

Although 3,202 specimens were examined serologically, the survey was definitely limited through difficulty of collection in the less common species. Small numbers of several species were obtained by chance collection in traps set for other animals. While negative results on small numbers of specimens have no significance, they are reported as a contribution toward the eventual objective of determining the extent of any supplemental reservoir of murine typhus. The serological evaluation is limited by the lack of any strain isolation studies in conjunction with the survey.

Methods

From October 1945 to January 1947 small numbers of miscellaneous animals were caught by chance in number 0 steel traps that had been set in buildings for collection of domestic rats. This chance collection continued, and, in addition, during 1947 and 1948, two men were assigned to the collection of small mammals in rural areas of the four-county Typhus Investigations study area. Native rats and mice were caught in several types of live traps, including a box trap with gravity drop hardware cloth doors at each end, the modified rabbit box trap described by Richter and Emlen (*17*), and a box trap described by Hubbard (*18*). Opossums and many of the carnivorous mammals were caught in number 0 to number 2 steel traps. Many of the squirrels and rabbits were collected with a shotgun. [2]

While a few specimens of wildlife were collected from farm buildings, collections were predominately from the fields and woods. Since house mice are often found in field habitats and domestic rats are only infrequently found away from man-made harborage, it is apparent that there is some but not extensive overlapping of ecological areas. Collection records indicate a limited exchange of ectoparasite species among domestic rats and several native mammals. The opossum, cotton rat, wood rat, and the spotted skunk were occasionally trapped in rural barns. Cotton rats were easily collected in large numbers in broom sedge fields.

Many of the small mammals were brought alive to the Thomasville laboratory where they were anesthetized and bled directly from

[2] The authors are indebted to E. V. Komarek (*19*) for valuable aid and advice in the collection and determination of mammals.

the heart with a sterile glass pipette. Animals killed in the field with a gun were bled with a sterile syringe. The blood was centrifuged twice and serum sent to the Communicable Disease Center Serological Laboratory for complement fixation test. Chicken blood was obtained at poultry houses supplied from rural sections of Decatur, Grady, and Thomas Counties. Dog blood was obtained for the most part from Thomas County through the cooperation of local veterinarians.

The complement fixation technique used throughout the survey was developed by Bengtson (*20*). Serial twofold dilutions from 1:4 to 1:1024 of inactivated serum in 0.2 cc. amounts were employed, to which were added 4 units of antigen in 0.2 cc., and 2 full units of complement in 0.2 cc. Fixation was allowed to proceed for 1 hour in the water bath at 37° C. Sensitized sheep erythrocytes consisting of 2 hemolytic units of hemolysin in 0.2 cc. mixed with an equal volume of 2 percent suspension of washed sheep erythrocytes were then added. The tubes were further incubated for 1 hour in the water bath at 37° C. Appropriate serum and antigen controls, as well as positive and negative sera, were included with the test. Positive results were reported from the highest serum dilution which gave 3 or 4 plus fixation. The antigen was a purified soluble product prepared from murine typhus-infected yolk sacs by the ether extraction process.

Results

Results of the complement fixation tests are shown in table 1. Of the 12 species that yielded positive sera, samples of over 100 specimens were obtained from only seven species (opossum, cottontail, house mouse, cotton mouse, old-field mouse, cotton rat, and Florida skunk). Among these seven species the percentage of positive sera varied from 0.5 for the cottontail to 2.7 for the cotton rat. In comparison, domestic rats from untreated Grady County during a similar period averaged about 38 percent positive to the complement fixation test. Thus, these limited results tend to show a very low incidence of typhus in animals other than domestic rats.

Available data are inconclusive as to whether or not typhus in wildlife has been affected by the DDT dusting of domestic rat harborages conducted during 1946 and 1947 in Thomas and Brooks Counties (*1*, *21*). For example, of the cotton rats collected in 1947 from Decatur and Grady Counties where there was no dusting program, over 4 percent were positive while only 2 percent of those collected in dusted Thomas and Brooks Counties were positive. In 1948, about 0.6 percent of the cotton rats from Decatur and Grady Counties were positive while 1.6 percent of these animals from Thomas and Brooks Counties were positive.

The negative results that were obtained from small numbers of

Table 1. *Results of complement fixation tests for murine typhus fever*

Animal	Number examined	Number positive	Percent positive
Class Mammalia			
Florida opossum, *Didelphis virginiana pigra* Bangs	345	3	0.9
Little brown bat, *Myotis lucifugus lucifugus* (LeConte)	2	0	0
LeConte free-tailed bat, *Tadarida cynocephala* (LeConte)	3	0	0
Seminole red bat, *Lasiurus borealis seminola* (Rhoads)	1	0	0
LeConte lump-nosed bat, *Corynorhinus macrotis* (LeConte)	1	0	0
Eastern cottontail, *Sylvilagus floridanus mallurus* (Thomas)	199	1	0.5
Marsh rabbit, *Sylvilagus palustris palustris* (Bachman)	21	0	0
Southeastern flying squirrel, *Glaucomys volans saturatus* Howell	1	0	0
Southern gray squirrel, *Sciurus carolinensis carolinensis* Gmelin	86	0	0
Southern fox squirrel, *Sciurus niger niger* Linnaeus	39	1	2.6
House mouse, *Mus musculus* Linnaeus	294	6	2.0
Eastern harvest mouse, *Reithrodontomys humulis humulis* (Audubon and Bachman)	2	0	0
Swamp rice rat, *Oryzomys palustris palustris* (Harlan)	25	1	4.0
Cotton mouse, *Peromyscus gossypinus gossypinus* (LeConte)	235	5	2.1
Old-field mouse, *Peromyscus polionotus polionotus* (Wagner)	217	1	0.5
Southern golden mouse, *Peromyscus nuttalli aureolus* (Audubon and Bachman)	4	0	0
Eastern cotton rat, *Sigmodon hispidus komareki* (Say and Ord)	841	23	2.7
Florida wood rat, *Neotoma floridana floridana* (Ord)	36	0	0
Pine mouse, *Pitymys pinetorum pinetorum* (LeConte)	4	0	0
Florida raccoon, *Procyon lotor elucus* Bangs	102	0	0
Florida bobcat, *Lynx rufus floridanus* (Rafinesque)	2	0	0
Cat, *Felis domestica*	220	0	0
Dog, *Canis familiaris*	56	3	5.4
Florida gray fox, *Urocyon cinereoargenteus floridanus* Rhoads	14	0	0
Spotted skunk, *Spilogale putorius* (Linnaeus)	101	0	0
Florida skunk, *Mephitis elongata* (Bangs)	109	1	0.9
Southern weasel, *Mustela noveboracensis notia* (Bangs)	3	1	33.3
Class Aves			
Domestic duck, *Anas boschas*	2	0	0
Bob-white quail, *Colinus virginianus*	1	0	0
Common hen, *Gallus gallus*	209	0	0
Domestic turkey, *Mellagris gallapava*	1	0	0
Domestic pigeon, *Columbia livia*	4	0	0
Mourning dove, *Zenaidura macroura*	1	0	0
Screech owl, *Outs asio*	4	0	0
Great horned owl, *Bubo virginianus*	2	0	0
Blue jay, *Cyanocitta cristata*	12	1	8.3
Brown thrasher, *Toxostoma rufum*	3	0	0

various species collected does not necessarily indicate that infection does not occur in these species. A sufficient number of sera were obtained from the gray squirrel, raccoon, domestic cat, and common hen to indicate that if these animals are naturally infected, the prevalence of demonstrable antibodies is not very high.

The number of positive animals was too small to make valid comparison of prevalence in the various counties. Of the 47 sera that were positive to the complement fixation test for murine typhus, 15 were collected in Decatur County (9 cotton rats, 1 opossum, 2 cotton mice, 1 house mouse, 1 cottontail, and 1 Florida skunk), 5 were from Grady County (4 cotton rats and 1 blue jay), 18 were from Thomas County (8 cotton rats, 2 opossums, 3 cotton mice, 1 house mouse, 1 fox squirrel, and 3 dogs), 5 were from Brooks County (3 house mice, 1 old-field mouse, and 1 rice rat), 2 were from Cook County (cotton rats), and 2 were from Berrien County (1 house mouse and 1 weasel).

Although the weasel and blue jay are not known to be susceptible

to murine typhus, the titers of positive sera, shown in table 2, are certainly suggestive of a previous natural infection in these animals. The indication of natural infection among the other animals found positive is strengthened by the knowledge of their susceptibility to typhus.

Table 2. *Titers of animal sera positive to the complement fixation test for murine typhus*

Source of sera	Titer						
	1:8	1:16	1:32	1:64	1:96	1:128	1:1024
Florida opossum	1		2				
Eastern cottontail	1						
Southern fox squirrel			1				
House mouse	3	1	2				
Swamp rice rat				1			
Cotton mouse	3	2					
Old-field mouse	1						
Eastern cotton rat	10	5	4	1	1	1	1
Dog	2	1					
Florida skunk	1						
Southern weasel	1						
Blue jay			1				

Summary

In a limited survey of southwest Georgia animals, exclusive of domestic rats, 3,202 sera from 37 species were examined by the complement fixation test for murine typhus. A low level of natural infection was indicated by the finding of 47 positive sera from 12 species of animals including the opossum, cottontail, fox squirrel, house mouse, rice rat, cotton mouse, old-field mouse, cotton rat, dog, Florida skunk, weasel, and blue jay.

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INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED DECEMBER 24, 1949

For the eighteenth consecutive week the total reported incidence of poliomyelitis in the Nation decreased from the preceding week. The total number of cases reported is 154 as compared with 243 last week and 90 for the 5-year (1944–48) median. Five States reported an aggregate increase from the preceding week of 13 cases ranging from 1 in 2 States to 5 (from 18 to 23) in Michigan. Twenty-five States reported an aggregate decrease of 102 cases, ranging from 1 in 7 States to 16 (from 28 to 12)in New York.

Compared with last week, increases occurred in infectious encephalitis (from 7 to 12), measles (from 1,774 to 2,008) and typhoid fever (including paratyphoid fever) (from 33 to 52). In addition, the cumulative totals for these diseases are slightly above those for the corresponding period last year. Other than the above mentioned, no other notifiable disease increased over the preceding week. Diphtheria, influenza, scarlet fever and whooping cough in addition to decreases for the week, showed decreases in the cumulative totals for the year as compared with the corresponding period last year.

One case of anthrax was reported in Pennsylvania. No cases of smallpox were reported in the United States. Hawaii reported 551 cases of influenza.

Of 41 States and the District of Columbia reporting on rabies in animals, 23 States and the District of Columbia reported no cases, while the remaining 18 reported a total of 103 cases. The States reporting the largest numbers were Texas (23), Georgia (11), West Virginia (11) and New York (10). The total number of rabies in animals reported to date is 5,553.

A total of 9,321 deaths was recorded during the week in 92 large cities in the United States, as compared with 9,315 last week; 8,833 and 8,737, respectively, for the corresponding weeks of 1947 and 1948; and 8,833 for the 3-year (1946–48) median. For the year to date the total is 463,177, as compared with 463,113 for the same period last year. Infant deaths for the current week totaled 627; for the last week, 591; for the corresponding week last year, 592; and the 3-year median, 642. The cumulative figure is 32,952, as compared with 33,640 for the corresponding period last year.

Telegraphic case reports from State health officers for week ended Dec. 24, 1949

[Leaders indicate that no cases were reported]

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Poliomyelitis	Rocky Mt. spotted fever	Scarlet fever	Smallpox	Tularemia	Typhoid and paratyphoid fever [1]	Whooping cough	Rabies in animals
NEW ENGLAND														
Maine				46		13			13				10	
New Hampshire						5			1					
Vermont		1		1					8				9	
Massachusetts	5			15	1		2		59				84	
Rhode Island			1			5			4				4	
Connecticut			1	20	4	52	4		15			1	82	
MIDDLE ATLANTIC														
New York	9		[2]	154	2	259	12		[3] 64			5	154	10
New Jersey	2	1	1	97		60	2		18				112	
Pennsylvania	1	1		66	7	72	2		76			1	156	1
EAST NORTH CENTRAL														
Ohio	6			34	5	40			124		2		102	3
Indiana	2			7		9			20			1	8	4
Illinois		1	1	28	3	62	7		32		5	2	55	3
Michigan [4]	3	4	1	449	4	38	23		87		1	2	172	5
Wisconsin			15	44		9	16		49			2	100	
WEST NORTH CENTRAL														
Minnesota	2	1		53		6	2		18			1	13	1
Iowa	1	2		172		1	5		11				2	5
Missouri					1	2	3		12		2		6	
North Dakota				3					12				3	
South Dakota				4		1	1						1	
Nebraska			10	1		4	1		17			1	4	
Kansas			20	1		13	4		22				12	
SOUTH ATLANTIC														
Delaware				1	2		1		2			1	6	
Maryland [4]	2		1	10	1	38	1		18		1		43	
District of Columbia	2			24		10			3				1	
Virginia	2		224	8	1	74	1		18		4		11	2
West Virginia	3		12	15	3	7			9		2	1	7	11
North Carolina	8			42	3		2		54		1		7	
South Carolina			22	19		16			4					6
Georgia	3		170	1		17			13		2		6	11
Florida	3		9	11		17	2		7			1		
EAST SOUTH CENTRAL														
Kentucky	4			2	2		5		16					
Tennessee	6		17	22	4	69	3		34		1	4	11	8
Alabama	6		28	6	2	46	2		19			1	2	4
Mississippi [4]	1			9	1	4	2		1		1		1	

See footnote at end of table.

Telegraphic case reports from State health officers for week ended Dec. 24, 1949—Continued

[Leaders indicate that no cases were reported]

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Poliomyelitis	Rocky Mt. spotted fever	Scarlet fever	Smallpox	Tularemia	Typhoid and paratyphoid fever [1]	Whooping cough	Rabies in animals
WEST SOUTH CENTRAL														
Arkansas	8		78	2	1	49			2		4	3	16	1
Louisiana	3		5	4		21	1		4			3		
Oklahoma	3		50	9		19	2		5				4	2
Texas	16		1,469	26	4	283	6		43			4	62	23
MOUNTAIN														
Montana	1			29			2		4		3			
Idaho	2		7	2		18	3		3					
Wyoming				4					1					
Colorado	3		19	106		13	3		9				19	
New Mexico	1		1	83		12	1		4		1	1	8	
Arizona	3		119	34	1	15	1		9		1	3	7	
Utah [4]	1			85		1	3		2		1		12	
Nevada	1													
PACIFIC														
Washington	1			144	1		1		52				15	
Oregon			4	37	1	9	4		26				16	
California	4	1	4	78	3	26	24		59			14	51	3
Total	118	12	2,289	2,008	57	1,415	154		1,083		32	52	1,394	
Median, 1944–48	319	5	3,338	2,696	55		90	1	1,956	4	38	41	1,541	
Year to date, 51 weeks	7,868	750	103,777	604,870	3,360	75,987	[5] 42,184	560	[6] 72,891	48	1,106	3,592	66,668	
Median, 1944–48	13,795	615	334,374	596,757	5,584		19,196	522	111,108	332	1,003	3,966	98,565	
Seasonal low week ends	(27th) July 9		(30th) July 30	(35th) Sept. 3	(37th) Sept. 17		(11th) Mar. 19		(32nd) Aug. 13	(35th) Sept. 3		(11th) Mar. 19	(39th) Oct. 1	
Since seasonal low week	4,100		27,910	16,352	844		[5] 41,269		[6] 14,631	7		3,132	20,066	
Median, 1944–45 to 1948–49 [7]	7,265		32,861	23,401	918		18,933		24,813	53		3,491	22,690	

[1] Including paratyphoid fever currently reported separately as follows: Connecticut 1; Louisiana 2; Texas 1; California 10. Cases reported as salmonella infection not included in the table were as follows: Massachusetts 1; New York 5; Pennsylvania 1.

[2] New York City only. [3] Including cases reported as streptococcal sore throat. [4] Period ended earlier than Saturday.

[5] Deductions—Michigan: 1 case, week ended Sept. 10; 2 cases, week ended Sept. 24. Mississippi: 2 cases, week ended Nov. 19. Additions—Kentucky: 10 cases not assignable to specific weeks, July, 4 cases; October, 4 cases; and November, 2 cases.

[6] Figures have been adjusted to exclude streptococcal sore throat, excepting those of New York.

[7] The median of the 5 preceding corresponding periods (1944–45 to 1948–49).

Anthrax: Pennsylvania 1 case. Alaska: Measles 20. Hawaii: Influenza 551, measles 1.

PLAGUE INFECTION IN KITTITAS COUNTY, WASH.

Under date of December 22, plague infection was reported in 123 fleas from 21 *Lagurus curtatus* (sagebrush voles) trapped December 13, 1949, on U. S. Highway 10, 18 miles east of Ellensburg, Kittitas County, Washington.

DEATHS DURING WEEK ENDED DEC. 24, 1949

	Week ended Dec. 24, 1949	Corresponding week, 1948
Data for 92 large cities of the United States:		
Total deaths	9,321	8,737
Median for 3 prior years	8,833	
Total deaths, first 51 weeks of year	463,177	463,113
Deaths under 1 year of age	627	592
Median for 3 prior years	642	
Deaths under 1 year of age, first 51 weeks of year	32,952	33,640
Data from industrial insurance companies:		
Policies in force	69,928,911	70,740,929
Number of death claims	12,277	12,153
Death claims per 1,000 policies in force, annual rate	9.2	9.0
Death claims per 1,000 policies, first 51 weeks of year, annual rate	9.1	9.2

FOREIGN REPORTS

CANADA

Provinces—Notifiable diseases—Week ended December 3, 1949.—Cases of certain notifiable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Newfoundland	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox			54	1	193	395	109	72	90	125	1,039
Diphtheria					15		1	1			17
Dysentery:											
Amebic									1		1
Bacillary					1						1
Encephalitis, infectious							1				1
German measles			1		8	40	1		70	55	175
Influenza			16			6	1	1			24
Measles			15	1	165	123	114	163	65	269	915
Meningitis, meningococcal									1		1
Mumps			79		36	225	8	32	29	90	499
Poliomyelitis						2	1		2	2	7
Scarlet fever	3		5	7	76	30	36	6	45	10	218
Tuberculosis (all forms)	64		3	9	209	27	18	15		34	379
Typhoid and paratyphoid fever					3	1		1		1	6
Undulant fever					2	1	1			1	5
Venereal diseases:											
Gonorrhea	7		9	6	89	69	32	9	44	68	333
Syphilis	2		4	6	58	43	5	5	5	11	139
Other forms										1	1
Whooping cough			7		135	59	2	9		7	219

MADAGASCAR

Notifiable diseases—October 1949.—Notifiable diseases were reported in Madagascar and Comoro Islands during October 1949, as follows:

Disease	October 1949			
	Aliens		Natives	
	Cases	Deaths	Cases	Deaths
Beriberi			6	
Bilharziasis			76	1
Cerebrospinal meningitis			10	3
Diphtheria			7	2
Dysentery:				
Amebic	12		376	1
Bacillary	1			
Erysipelas			13	1
Influenza	64		3,984	32
Leprosy	1		61	1
Malaria	332	1	30,155	125
Measles			179	4
Mumps			139	
Plague			20	20
Pneumonia:				
Broncho	2	1	334	58
Pneumococcic			454	61
Poliomyelitis			2	1
Puerperal infection			5	1
Relapsing fever	1			
Trachoma	1			
Tuberculosis, pulmonary	1		118	21
Typhoid fever			4	3
Whooping cough	1		384	6

NORWAY

Notifiable diseases—September 1949.—Cases of certain notifiable diseases were reported in Norway as follows:

Disease	Cases	Disease	Cases
Cerebrospinal meningitis	13	Mumps	143
Diphtheria	21	Paratyphoid fever	1
Dysentery, unspecified	2	Pneumonia (all forms)	1,409
Encephalitis, epidemic	3	Poliomyelitis	17
Erysipelas	384	Rheumatic fever	97
Gastroenteritis	3,846	Scabies	1,543
Gonorrhea	292	Scarlet fever	439
Hepatitis, epidemic	106	Syphilis	92
Impetigo contagiosa	2,829	Tuberculosis (all forms)	283
Influenza	1,795	Typhoid fever	4
Laryngitis	7,883	Weil's disease	1
Malaria	3	Whooping cough	5,050
Measles	780		

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

Note.—The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

Burma—Moulmein.—During the week ended November 12, 1949,

1 imported case of cholera was reported in Moulmein, Burma.

Ceylon.—Correction: According to information from Colombo, Ceylon, dated December 13, 1949, the 10 suspected cases of cholera reported in Eastern Province, week ended November 19, 1949 (see PUBLIC HEALTH REPORTS 64: 1629, December 16, 1949), have been determined not to be cholera cases, but suspected food poisoning. Eastern Province was stated to have been declared free from cholera as of December 6, 1949.

Plague

Belgian Congo—Costermansville Province.—On December 9, 1949, 1 fatal case of plague was reported in Malihi, northeast of Lubero, Costermansville Province, Belgian Congo.

Madagascar.—During the period November 21–30, 1949, 11 cases of plague, with 10 deaths, were reported in Madagascar.

Netherlands Indies—Java—Jogjakarta.—For the week ended December 3, 1949, 49 fatal cases of plague were reported in Jogjakarta Residency, Java. Twelve of these cases were reported in Jogjakarta City. For the week ended December 10, 1949, 15 cases, all fatal, were reported in Jogjakarta City.

Union of South Africa—Cape Province.—During the week ended December 3, 1949, 1 fatal suspected case of pneumonic plague was reported in Hay District, Cape Province, Union of South Africa.

Smallpox

Arabia—Jedda and Mecca.—During the week ended November 26, 1949, 15 cases of smallpox, with 3 deaths, were reported in Jedda, and 7 cases, with 2 deaths, in Mecca, Arabia.

India—New Delhi.—During the week ended December 10, 1949, 54 cases of smallpox, with 32 deaths, were reported in New Delhi, India.

Indochina (French)—Tonkin.—For the week ended December 10, 1949, 97 cases of smallpox were reported in Tonkin, French Indochina.

Pakistan—Chittagong.—During the week ended December 3, 1949, 10 cases of smallpox were reported in Chittagong, Pakistan, and 11 cases were reported during the week ended December 10.

Peru—Pacasmayo.—According to information dated December 29, 1949, an outbreak of smallpox has been reported in the port of Pacasmayo, Peru. No figures were given in the report as received.

Yellow Fever

Sierra Leone—Freetown.—On December 4, 1949, 1 fatal confirmed case of yellow fever was reported in the port of Freetown, Sierra Leone. The patient was stated to have come from Musaia, about 247 miles from Freetown. Death occurred in Freetown.