IV. *The Relationship of Micro-Organisms to the Decay of Stone.*

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[Plates 16–20.]

Contents.

1. *Introduction and Historical Sketch.*

The first suggestion that bacteria might be agents in stone decay was probably that of Buchanan (1904) in a paper read before the Royal Philosophical Society of Glasgow in 1904. This paper contained evidence that bacteria, yeasts and moulds were associated

* The senior author (S. G. P.) is responsible for the preparation of the paper, the other authors assisted in the experimental work at various times during the course of the investigation.

with the decay of sandstones in the City of Glasgow, and included a brief sketch of the manner in which the autotrophic bacteria, sulphur bacteria, iron bacteria, and the nitrifying bacteria might play a part in sandstone decay. This paper was apparently unknown to later writers, ANDERSON (1910), MARSH (1923) and FOX and HARRISON (1925), none of whom makes reference to it. In 1928 BUCHANAN published the results afresh with no important addition, but confirming his earlier work. STUTZER and HARTLEB (1899) in an important paper, published five years before that of BUCHANAN, noted the solution of the lime of the cement on walls of reservoirs and dock sides. While the greater part of this solvent action was believed to be due to dissolved carbon dioxide in the water, part of it at least was attributed to the action of bacteria contained in a brown slime which covered the walls. The colour of this was shown to be due to the presence of oxide of iron, and the slime was found to contain bacteria capable of producing nitric acid from ammonia and simple nitrogenous materials like asparagine. These workers, however, did not apply their results to the problem of the decay of building stones. ANDERSON (1910) in a paper read at a Conference at York in 1910 did little more than suggest the possibility of bacterial or fungal action. His observation of the localization of decay and the spreading round a focus of apparent infection is in agreement with that of BUCHANAN in 1904, and is confirmed by the writer's own observation, for many instances can be found where infection appears to spread in this way, figs. 3 and 4, Plates 16 and 17. ANDERSON cites BOYD DAWKINS' suggestion that micro-organisms might secrete acids or other material capable of disintegrating the stone. In a footnote the author mentions the work of DELEPINE, who would appear to have had some considerable experience in the investigation of stone decay and to have attributed it definitely to bacteria.* MARSH (1923, 1926), chiefly on chemical considerations, held strongly the belief that nitrifying bacteria were active agents in stone decay, and was inclined to attribute a substantial part of the decay of the buildings of Oxford to this cause. He gives evidence of the presence of from 0·1 to 0·3 per cent. of nitrate in stones which, in virtue of their position, were protected from the leaching action of rain. Further, MARSH demonstrated the oxidation of ammonia when a "solution of ammonium sulphate was slowly forced through the stone together with a current of air." FOX and HARRISON (1925) provided confirmation of the occurrence of nitrate in decaying stone but expressed themselves as "doubtful whether the mere presence of small quantities of nitrate necessarily indicates bacterial action." They found that the nitrate content of sound stone from Rievaulx Abbey was considerably less than that of decayed stone from the same source. This support for the view of MARSH was, however, weakened by the presence of considerably more calcium sulphate in the decayed stone for which FOX and HARRISON were at a loss to account. These authors seem to have been, on the whole, in favour of a bacterial origin of decay in stone and they gave it as their opinion that "the whole subject of bacterial life in relation to

* Unfortunately no records of this work are available.

stone decay seems to require thorough investigation not only for the purpose of confirming or disproving its importance but also, in the event of positive evidence being forthcoming, to find a suitable sterilizing agent."

It is clear from the above that the biological theory of stone decay needs to be further investigated and that there is the possibility that biology in this field may elucidate phenomena which cannot otherwise be explained.

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At the outset of this investigation, which was begun in 1924, it was considered that the chief questions to be answered were the following.

1. Do bacteria exist in the interior of stone ?
2. If so, are they more numerous in stone which has been exposed to weather than in stone not so exposed ?
3. If bacteria are present in weathered or other stone at what depth are they to be found ?
4. Are the species of bacteria specially adapted to their peculiar habitat ?
5. Do the species isolated produce decay in stone when inoculated on or below the surface of sound stone and incubated under suitable conditions ?

2. *Enumeration of the Bacterial Population of Decaying and Sound Stone.*

In order to determine whether bacteria were present in weathered stone, surface organisms were destroyed, at first by flaming but in later experiments by means of hot wax. The lump of stone so treated was crushed in a vice with jaws protected by sterilized tin shields ; small pieces were then picked out aseptically and ground in a sterile glass mortar. One gram of the ground stone was shaken in 60 c.c. of sterile water and 1 c.c. of the water after sedimentation was further diluted in 49 c.c. of sterile water. 1 c.c. of each dilution was then plated in standard bouillon agar. The colonies were counted on the third and again on the seventh day.

Examination of the colonies on the plates of these experiments revealed an almost total absence of moulds. This does not agree with the findings of BUCHANAN (1928) ; it is accounted for by the sterilization of the outer surface of the stones. The lack of penetration of the stone by fungi has been taken to indicate their inability to cause decay of the stone, and consequently surface growths have been neglected throughout the subsequent investigations. Another fact worthy of note was a strong preponderance of non-sporing organisms. Considering the nature of the habitat, its varying conditions of moisture, and the probable periods of food shortage which almost certainly occur, the bacterial population might have been expected to consist mainly of sporing types.

The reliance to be placed upon the method adopted for enumerating bacteria in

TABLE I.—Numbers of Bacteria in Building Stone from various Localities.

Details of the Sample of Stone.	Bacteria per gram.
1. Stone removed at some time previously from King's Balcony, Buckingham Palace.	
Raspings to a depth of 3 mm.	72,000
,, ,, ,, 6–10 mm.	14,000
,, ,, ,, 16–22 mm.	520
,, ,, ,, 26–34 mm.	300
2. Bath stone crumbling rapidly on the coping of North Wing Balustrade of Buckingham Palace.	
Raspings to a depth of 2 mm.	2,480,000
,, from ,, 1 cm.	108,000
3. Rutland stone, sound stone being used to replace the above	7100
4. Portland stone. Sample I obtained from the Office of Works—source unknown.	
Sound stone, 0–1 cm. deep	180
,, 1–2 cm. deep	140
5. Portland stone. Sample II.	
Weathered on two faces—	
0–1 mm.	1300
1–3 mm.	700
The above five examples were obtained by rasping with a sterile wood rasp.	
1. Bath stone from King's Balcony repeat of above, broken in vice and lumps flamed and crushed in agate mortar.	
0·5–1·5 cm. deep	70,800
4. Portland stone, repeat of 4 above.	
Sound stone, 0–3 mm. deep	0
,, 0·5–1·5 cm. deep	0
6. Portland stone from Tower of London, thick grey corrosion of $CaSO_4$. This was unsterilized. Surface	29,000
0–1 cm. below grey layer	200
1–2 cm. ,, ,,	0
7. Portland stone (sound) cut from centre of large block	0
8. Bath stone from Jewel House, Tower of London, weathered in stonemason's yard since 1910.	
Outer centimetre	50,660
1–2 cm.	22,400
9. Caen stone from Officers' Quarters, Tower of London, weathered in yard since 1906.	
Outer centimetre	248,320
1–2 cm.	66,560
2–3 cm.	32,000
10. Grey corrosion on Portland stone, main entrance to the Tower of London; smells like wet soil when ground, consists largely of soot, dust and calcium sulphate	1,792,000
11. Gatton stone from Tower of London.	
Outer centimetre	334,720
1–2 cm.	250,880
12. Ketton stone from Tower of London.	
Outer centimetre	88,960
1–2 cm.	7600
13. Portland stone from Tower of London.	
Outer centimetre	210,560
14. Killas (slate)—Fowey Castle	0
15. Greissenized granite—Pendennis Castle	420
16. Hard stone—Farleigh Castle	320,000
17. Bath stone—Farleigh Castle	434,000

TABLE I—(continued).

Details of the Sample of Stone.	Bacteria per gram.
18. Sandstone—Rievaulx Abbey—	
(*a*) South Transept	278,000
(*b*) Chapter House (recently excavated) surface of this sample was covered with moulds	256,800
(*c*) Chancel and Transepts	8200
19. Yorkshire grit—Byland Abbey	1,183,200
20. Sandstone—Byland Abbey, Chapter House	46,400
21. Calcareous sandstone—Byland Abbey, south wall of Nave	12,100
22. Fine grain sandstone—Scarborough Castle	41,600
23. Oölitic limestone—Scarborough Castle	540,000
24. Sandstone—Whitby Abbey	118,400
25. Flaggy sandstone—Richmond Castle	3500
26. Sandstone—Richmond Castle	124 860
27. Calcareous sandstone—Helmsley Castle	267,200
28. Hard limestone—Helmsley Castle	60,000
29. Merton College, Oxford	1,535,000
30. Tintern Abbey, coping stone	1,278,000
,, ,, pier, 20 feet up	106,200
,, ,, window at west end	3100
31. Caerleon Castle, Entrance D, limestone	18,400
,, ,, ,, B, red sandstone	3500
,, ,, ,, H, brickwork	8000
32. Goodrich Castle, walls of the moat	192,000
33. Raglan Castle	103,900
34. Helmsley Castle, crumbling sandstone	736,000
,, ,, intact sandstone	23,000
,, ,, excavated limestone	0
35. Byland Abbey, crumbling sandstone	1,987,000
,, ,, intact sandstone	896,000
36. Rievaulx Abbey, sandstone	82,000
37. Hampton Court, Wren building	294,500
38. Pevensey Castle, Roman wall	9500
,, ,, Norman entrance	365,700
,, ,, Norman Tower	51,600
39. Victoria and Albert Museum, decayed terra-cotta	10,340
40. Limestone coping under a window-box periodically wetted by drainage from soil	18,440,000

stone has been critically examined in a series of experiments in which it has been shown :—

(1) that the shaking of finely powdered stone in water is not an effective way of separating the bacteria from the stone particles. The bacterial counts in the suspension after allowing the stone to settle give merely a rough quantitative data of the bacterial flora of the original stone, and must not be interpreted as in any way accurate.

(2) that the ratio of numbers of bacteria in the water after settling to numbers of bacteria in the shaken suspension shows wide variation from 1 : 2 to 1 : 21 ; the most usual finding being above 1 : 6 and below 1 : 9.

(3) that the numbers of bacteria found in a shaken suspension vary with the fineness of grinding of the stone, the optimum being that of a fine sand; finer grinding yields gradually diminishing numbers, bacteria being destroyed in the process.

(4) Samples from any individual block of stone, ground to what appears to the eye to be the same degree of fineness, give very different bacterial counts, indicating that the bacteria are not uniformly distributed in the matrix, but probably occur in pockets or fissures in the stone. See page 104.

From these considerations one is forced to the conclusions that the bacterial numbers given in Tables I, II and III, are to be taken only as rough indications of the bacterial content of the stone; that, since in the earlier experiments the sand was allowed to settle before plating, the first twenty-four numbers in Table I, with exception of the blanks, are under-estimates of the actual population; that, since in some cases figures as high as 2 million organisms per gram have been found in the water after settling of the stone, it may be assumed that the true state of the population in those cases is of the order of at least 10 million, a figure which is comparable with that of a cultivated soil.

3. *Enumeration of the Bacterial Population of Quarry Stone.*

Despite the shortcomings of the method of enumeration certain striking features have come to light in dealing with stones just removed from the quarry. The quarries selected were mostly limestone quarries in the west and south of England. Three sandstone quarries have been included so that together they form a fairly wide range of typical building stone. Samples were taken at the quarry face and from various depths below the face; they were surface-sterilized by dipping for a few seconds in a hot wax mixture (80 per cent. paraffin, 20 per cent. beeswax) raised to fuming point over a spirit stove, *i.e.* to a temperature of approximately 200° C. The wax-coated samples were conveyed to the laboratory as rapidly as possible in order to minimize the length of the anaërobic condition. When crushed only the material from the inside was taken, any pieces of stone with attached paraffin being rejected.

The number of blanks in Table II shows that the methods of collection and counting can at least be relied upon as regards freedom from contamination. With the exception of the Stancliffe stone the only samples showing a large bacterial population were those from closed mines, Beer, Corsham Down and Monk's Park. In these the stones were practically saturated with water from the roof, *i.e.* the leachings from the soil above the so-called "cap" of the quarry. Examination of the colonies on the plates showed the bacteria to be restricted to a comparatively few species. Further, comparing the flora of the Beer stone with that of the water percolating through the roof, the stone was found to have a selective or filtering action upon the organisms of the water which wets its surface. The water coming through the roof contained 16 million organisms per c.c. and included many species of bacteria which were not represented in the stone flora of this quarry. Moreover, the flora of the sample taken 24 inches

TABLE II.—Numbers of Bacteria in Quarry Stones.

Source of Stone.	Geological Description.	Number of Bacteria per gm. of stone.			Remarks.
		Surface.	12 inches depth.	24 inches depth.	
Beer	Cretaceous	2,900,000	1,500,000	9000	80 feet below cap; stone very wet, water dripping through roof.
Chilmark	Siliciferous limestone	350,000	0	0	Stone had been exposed to weather about 3 weeks and was very wet.
Cowcroft Portland	Upper oölite, Portlandian beds	0	0	0	Open quarry; 20 or 30 feet below cap, stone very dry.
Purbeck Portland	,, ,,	0	0 (15 inches)	—	Dry and hard stone.
Doulting	Inferior oölite . . .	3500	860	750	Quarry not working. Stone had been stacked in the yard some considerable time.
Ham Hill	Inferior, oölite, shelly limestone	30,400	0	0	Surface was weathered, green with algæ. Stone hard and dry.
Corsham Down . .	Great oölite, top bed	2,408,000	—	—	This stone was wetter than that from Monk's Park below, but not so wet as that from Beer.
	,, bottom bed	281,000	—	—	
Farleigh Down .	Great oölite	26,800	—	—	—
St. Aldhelm Box Ground	Great oölite, fine grain	0	—	—	—
Bradford-on-Avon	Great oölite, medium grain	0	—	—	Quarried some time, but left underground.

TABLE II—(continued).

Source of Stone.	Geological Description.	Number of Bacteria per gm. of stone.					Remarks.
		1 inch depth.	2 inches.	3 inches.	4 inches.	6 inches.	
Monk's Park . .	Great oölite sample .	484,100	0	0	0	0	Moist.
Ketton	Oölitic limestone . .	2000	0	0	0	0	—
Stancliffe . . .	Sandstone	1,273,000	—	0	0	—	—
Woodend Windhill	Sandstone	62,600	—	0	—	0	—

back from the cut surface (sawn three weeks before) was a simplified flora as compared with that of the surface sample. An interesting feature noticed in the quarries is a marked difference in the weathering qualities of stones from St. Aldhelm (Box Ground)

on the one hand, and Corsham Down and Monk's Park on the other; the former is described as a "weather stone," *i.e.* one which can be exposed to weather immediately after cutting, at any time of the year, without damage, while the latter stones quarried in the winter must be left below ground until March, otherwise exposure would cause crumbling. This difference may be associated with the relative wetness of the stones, it is, however, interesting to note that the difference is reflected in their bacterial content.

4. *Distribution of Bacteria in Stone.*

In the course of estimating the numbers of bacteria in various stones the content of two fragments of the same stone sometimes differed widely as shown in the first four examples given in Table III. These stones are all coarse grained, and showed the variation much more than the even textured samples from Corsham Down.

TABLE III.—Distribution of Bacteria in Stone.

Stone.	Sample.	No. Bacteria per gram.
Monk's Park	1 inch depth. Sample A	500
	,, ,, ,, B	52,000
	,, ,, ,, C	799,800
	,, ,, ,, D	1,084,000
Ketton	,, ,, ,, A	2,400
	,, ,, ,, B	1,500
Stancliffe	,, ,, ,, A	1,406,000
	,, ,, ,, B	1,140,000
Woodend Windhill	,, ,, ,, A	5,000
	,, ,, ,, B	120,000
Corsham Down	Top Bed. ,, A	2,429,000
	,, ,, B	2,377,000
Corsham Down	Bottom Bed. ,, A	299,300
	,, ,, B	272,000

These figures suggest that the bacteria probably occur unevenly in the stone, in pockets in the matrix, or in the interstices between the oölite grains in the limestones. A geological section of one of the oölite limestones was stained with fuchsin and examined under a microscope. The stain was mostly taken up by the accretion centres of the oölite grains and no definite sign of bacterial masses was observed. In an attempt to locate bacterial pockets by staining blocks of stone with dilute fuchsin for 24 hours or more, small areas staining darkly were observed. If these represent bacterial masses that would be consistent with the great difference in numbers found in separate parts of the sample of stone. Since, however, colloidal matter also takes up the stain, clay aggregates would give a similar appearance and the evidence cannot be said to be conclusive.

5. *Morphology and General Characteristics of the Heterotrophic Bacteria of Stone.*

All types of colony which occurred in the platings on ordinary nutrient media from decayed and quarry stones were examined, and the organisms grown in pure culture with the object of studying any physiological characteristics of importance in their possible destructive action on stone. Usually five to twelve different species were found on each plate but once a stone from Helmsley Castle gave a pure plate of a yellow rod-shaped organism, and a sample taken from 24 inches below the surface of stone in Beer quarry had an almost pure culture of a somewhat similar type. Fifty-eight organisms were studied in some detail and the results are tabulated below.

TABLE IV.—Characteristics of Heterotrophic Bacteria isolated from Stone.

Stone.	Index No.	Form.	Colour of culture.		Gram stain.	Motility.	Spores.*	Liquifaction of gelatine.	Action on Glucose, Lactose, Sucrose.†	Action on litmus milk.
			On agar.	On potato.						
Isolated from various stone in 1925	S 1	Rod	Pale yellow	Lemon yellow	Weak+	+	—	+	0·223	Alk., curd, peptonized.
	S 2	,,	Cream yellow	Pale yellow	+	—	—	+	0·222	No action.
	S 3	Long rod in chains	Orange	Dull orange	+	—	—	+	0·232	Alk., no curd or pepton.
	S 4	,, ,,	Orange yellow	Bright orange	+	—	—	+	0·232	,, ,, ,,
	S 5	Short rod	Transparent	Transparent	Weak+	+	—	+	0·332	No action.
	S 6	,,	Creamy yellow	Dirty cream	—	+	—	+	0·333	Acid, no curd or pepton.
	S 7	Sarcina type	Bright yellow	Lemon yellow	Weak+	—	—	+	0·233	No action.
	S 8	Coccus	,,	,,	Weak+	—	—	—	0·233	,,
	S 9	,,	White	Dirty white	Weak+	—	—	+	0·333	Alk., no curd or pepton.
	S 10	Short rod sporer	Cream	Buff	—	+	+	+	0·232	No action.
	S 11	,, ,,	,,	Deep cream	—	+	+	+	0·232	,,
	S 12	,, ,,	Cream-buff	,,	+	+	+	+	0·232	Alk., curd, peptonized.
1926–1930.										
Beer stone	BS 1	Small rod	Cream, transparent	Buff-cream	—	+		+	0·232	Neutral, curd, peptonized.
,,	BS 4 (i)	Rods-chains	White	Buff	Weak+	+		+	0·333	Acid, curd, peptonized.
,,	BS 4(ii)	Rods in chains	Creamy white	,,	Weak+	+		—	0·232	Neutral, curd, peptonized.
,,	BS 5	Small rods	Pale yellow	,,	+	—		+	0·333	Alk., no curd or pepton.
,,	BP 1	Coccus	Orange yellow	Orange	—	—		+	0·233	No action.
,,	BP 2	Rod	Transparent cream	Dirty buff	Weak+	+			0·233	Alk., no curd or pepton.
,,	BP 4	Coccus	White	No growth	+	—		+	No growth	No action.
,,	BP 5 (i)	Rods	Cream-white	Yellow cream	—	—		+	0·333	Acid, curd and peptonized.
,,	BP 5(ii)	Rod	,,	,,	—	—		+	0·111	Acid, curd, no pepton.
,,	BP 7	,,	,,	Buff	Weak+	—		+	0·333	Alk., curd, peptonized.
,,	BP 8	,,	,,	Dirty white	Weak+	—		+	0·233	Neutral, curd, peptonized.
,,	BP 11	,,	Lemon yellow	Buff	+	—		+	0·333	Alk., curd, peptonized.
Ham Hill	HH 1	Coccus	White	Dirty cream	—	—		+	0·333	,, ,, ,,
	HH 4	Rod	Transparent yellow	Deep yellow	+	—		+	0·230	,, ,, ,,
Corsham Down	CD 1	Small rod	Cream, transparent	Pink cream	—	—		+	0·233	—
,,	CD 4	Coccus	White	Dirty white	Weak+	—		+	0·330	Alk., curd, peptonized.
,,	CD 7	Rod	Opalescent (yellow)	,,	Weak+	+		+	0·333	,, ,, ,,
,,	CD 9	,,	Yellow cream	Deep yellow	—	—		+	0·333	No action.
Chilmark	Ch 1	,,	White	Dirty white	—	—		+	0·332	Neutral, curd, peptonized.
,,	Ch 3	Small rod	Creamy white	Buff	—	+		+	0·333	No action.
,,	Ch 5	Rod	,,	Cream	Weak+	—			0·032	Alk., curd, peptonized.
,,	Ch 7	Very small rod	,,	Buff	—	+		+	0·333	,, ,, ,,
Doulting	D 4	Rod	,,	Cream	Weak+	+		+	0·330	Alk., no curd or pepton.
,,	D 5	Sarcina type	Lemon yellow	Yellow	Weak+	—		+	0·330	No action.

* Tests for spores are recorded for the twelve organisms first isolated. Though not specially tested, most of the others were non-sporing types, —cocci, ovoid rods and small rods.

† The index-figures are those of the system adopted by the American Society of Bacteriologists. They relate successively to the sugars in the order given. 1 indicates acid and gas, 2 acid but no gas, 3 growth but no acid or gas.

TABLE IV—(continued).

Stone.	Index No.	Form.	Colour of culture. On agar.	Colour of culture. On potato.	Gram stain.	Motility.	Spores.*	Liquifaction of gelatine.	Action on Glucose, Lactose, Sucrose.†	Action on litmus milk.
Monk's Park	MP 1	Ovoid rod	Opalescent (yellow)	Yellow	−	+		+	0·303	Alk., no curd or pepton.
,,	MP 5	Coccus	Orange	Orange	+	+		+	—	No action.
,,	MP 7	Small rod	White	Dirty white	Weak+	−		+	0·203	,,
,,	MP 8	Ovoid rod	Greyish viscid	Deep cream	−	+		+	0·233	Acid, curd.
,,	MP 9	Small rod	Pale yellow	Yellow	−	+		+	0·303	Acid, no curd or pepton.
Bradford Church	BC 1	Rod	Dull white	Buff	+	−		+	0·302	Alk., no curd, peptonized.
Farleigh Down	F 2	Ovoid rod	Opalescent	Yellow	−	+		+	0·333	—
,,	F 3	Coccus	White	Dirty white	−	−		+	0·333	Acid, curd, peptonized.
,,	F 5	,,	Buff	Orange yellow	Weak+	−			0·333	—
,,	F 7	Rod	Creamy white	Buff	Weak+	−		−	0·330	No action.
,,	F 8	,,	Buff yellow	Deep yellow	−	−		−	0·333	,,
,,	F 9	Ovoid rod	Greyish viscid	Buff	Weak+	+		−	0·233	Acid, no curd or pepton.
Ketton	K 1	Small rod	White	Pinkish cream	Weak+	+		+	0·333	Alk., curd, peptonized.
Stancliffe	St 1	,,	,,	Creamy viscid	Weak+	+		−	0·333	No action.
,,	St 2	,,	,,	Dirty cream	Weak+	−		+	0·233	Alk., curd, no pepton.
Woodend Windhill	W 1	,,	,,	Cream viscid	−	−		+	0·333	Alk., curd, peptonized.
,,	W 2	Short rod	,,	Dirty cream	−	−		−	0·333	,, ,, ,,
,,	W 3	,,	,,	Creamy white	Weak+	−		+	0·333	Alk., no curd or pepton.
,,	W 4	Diplococcus	Yellow cream	Cream	+	−			0·333	No action.
,,	W 5	Small coccus	White	Dull white	+	−		+	0·222	Neutral, curd, no pepton.
,,	W 6	Ovoid rod	,,	Cream white	−	−		+	0·332	Alk., curd, peptonized.
,,	W 7	Small coccus	Pale yellow	Cream	−	−		−	0·333	No action.

* Tests for spores are recorded for the twelve organisms first isolated. Though not specially tested, most of the others were non-sporing types, —cocci, ovoid rods and small rods.

† The index-figures are those of the system adopted by the American Society of Bacteriologists. They relate successively to the sugars in the order given. 1 indicates acid and gas, 2 acid but no gas, 3 growth but no acid or gas.

TABLE V.—Analysis of the Characteristics of Stone Bacteria in Table IV.

1. Motility	Motile 27	Non-motile 31	—
2. Gram stain	Positive 12	Weak, positive 23	Negative 23
3. Liqn. gelatine	Liqd. 46	No. liqn. 8	Not recorded 4
4. Sugars—			
Glucose	Acid and gas 1	Acid only 21	No acid or gas 33
Lactose	,, ,, 1	,, 3	,, ,, 48
Sucrose	,, ,, 1	,, 14	,, ,, 36
5. Action on milk	Acid 8	Neutral 5	Alkaline 24
6. Coagulation of milk	Curd 25	Peptn. 22	No action 18

Of the fifty-eight strains isolated only six were found to occur in more than one sample of stone, one occurred in five of the fifteen or sixteen‡ samples, but this was the only one common to more than two stones so that it is evident that the heterotrophic flora of stone is not closely restricted to a few species. It must, like the soil flora, be considered as a complex population in which groups flourish locally and for a period when the food supply or other conditions favour them. No outstanding features characterize the stone bacteria as a group. They seem to be referable to known

‡ The uncertainty as to numbers is due to the fact that no record was kept of the number of samples from which cultures S_1 to S_{12} were obtained.

soil, water or air types; *e.g.* the organism from Beer stone (BS_1), found almost pure at a depth of 24 inches, is a slender motile rod agreeing in character with *Pseudomonas fluorescens*. Assuming the origin of the stone flora to have been the soil, a certain selective influence of the stone habitat is noticeable in the fewness of sporing organisms and the complete absence of large sporers like *Bacillus mycoides*, *B. megatherium*, and *B. mesentericus*. In order to determine whether this selection could be related to shortness of the food supply in the stone, a fairly exhaustive series of experiments was carried out with nine of the isolated stone organisms and nine typical soil organisms. The members of these two groups were compared as to their minimum food requirements, pairs of members of each group were grown together competitively in the same solution, and, finally, individual members of each group were cultivated together in a solution in which stone was immersed, so that by investigating their relative numbers at different depths below the surface of the stone, their respective power of penetrating the stone under these conditions was determined. The results of these experiments oscillated almost equally in favour of the two groups; on the whole the stone and soil organisms behaved similarly and no explanation of the apparent selectivity of the stone in the directions indicated above has been found.

6. *Carbon Dioxide Production from Stone and its Relationship with the Degree of Decay of the Stone and the Presence of Bacteria.*

It is of course inconceivable that the numbers of bacteria shown to be present in this investigation should be continually in a dormant condition, but without experimental evidence the activity of these organisms must remain in doubt. The activity of micro-organisms, be they bacteria, fungi or protozoa, with the exception of the small group of strict anaërobes, is accompanied by a corresponding interchange of oxygen and carbon dioxide. An experiment was therefore devised to determine the respiratory activity in a decaying stone. Use was made of a micro-respirometer of the Barcroft type, constructed rather on the principle of the cryophorus. A piece of decaying stone of about 3 grams weight was enclosed, together with a little potash solution, in one of two similar bulbs connected by a fine capillary glass tube in which a small drop of coloured alcohol occupied an intermediate position between the two bulbs. The second bulb contained a piece of stone which had previously been sterilized. The whole apparatus was immersed in a thermostat. The drop of alcohol moved slowly and steadily towards the vessel containing the unsterilized stone, thus indicating a measurable rate of respiration. A movement of 1 cm. per hour was obtained, corresponding to an absorption of ·006 c.c. of oxygen per hour. This rather successful beginning encouraged us to expect that carbon dioxide would be disengaged in an experiment performed on a larger scale.

Fig. 1, p. 108, represents the apparatus employed. In this experiment CO_2-free air was drawn, first through a vessel containing the stone broken into lumps of convenient

size, and then through an absorption vessel containing a standard solution of baryta. Experiments were set up in pairs, the one containing the untreated stone, the other, the control containing the same stone previously sterilized either by heat or by toluene. It at once became apparent in the relative cloudiness of the baryta solution that the untreated stone was yielding far more CO_2 than the control.

Experiment 1.—The first stone used was a fragment from one of the pinnacles of the Houses of Parliament and gave a respiration equivalent of 0·18 mg. of CO_2 per hour per 350 gm. of stone.

Experiment 2. *The Effect of Surface Incrustation of the Stone.*—The stone selected was a surface-blackened decaying limestone which supported iron palings in a London

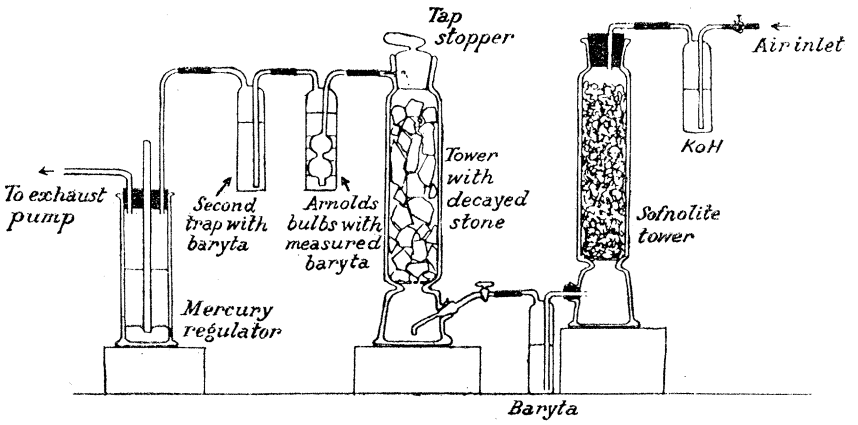

FIG. 1.—Unit of apparatus used for measuring carbon dioxide evolved by stone.

street. This coping was raised 6 inches or so from soil on one side and had received the attention of dogs on the other, the pavement, side. It had, therefore, every opportunity of acquiring a bacterial flora. The first results showed that the stone, as collected, gave far more CO_2 than the same stone sterilized, either by autoclaving or by toluene. The rate of action slowed down after the fifth day, but, although no nutriment was supplied, CO_2 continued to come off as long as the experiment lasted, in one case thirty-five days, so that presumably some of the organisms were living on the products of the dead bodies of others. In this experiment one of the containers was filled with stone from which the outer surface had been removed by rasping to a depth of 5 mm.

TABLE VI.—Milligrams of CO_2 produced during 5 days from 350 grams of Decaying Stone with (*a*) surface intact and (*b*) surface removed.

(*a*) Surface intact.		(*b*) Surface removed.	
Experimental.	Toluene Control.	Experimental.	Toluene Control.
62·9	4·1	5·8	2·5

Subtracting the mean of the controls from the experiments the relative respiration over these five days was (*a*) surface intact 59·1 mg. CO_2, (*b*) surface removed 2·0 mg. CO_2 —. Now 59 mg. of CO_2 is the equivalent of 134 mg. of $CaCO_3$. From the data available it is impossible to deduce how much of this carbon dioxide is the respiratory product of the bacteria and how much is liberated from the limestone in the neutralization of their metabolic acid products. Of the fifty-eight organisms which were isolated from stone and studied in detail, Table IV, only one produced any gas in the fermentation of carbohydrates though several of them produced acid ; there seems therefore some likelihood that the bulk of the CO_2 is derived from the stone. If it be assumed that the CO_2 has been derived wholly from the $CaCO_3$, the rate of destruction of the stone under the conditions of this experiment was 28 gm. per kilo of stone per annum. In a later experiment on the same stone only 2 mm. were removed from the surface with the following result :—mg. of CO_2 from 350 gm. of stone in 15 days, with surface intact 21·76, with surface removed 3·42. The rate is considerably slower than it was when the stone was freshly collected but is remarkably high considering that the stone had been standing for nearly two weeks under rather dry conditions and without any nutritive supply. During these experiments no moulds have been seen to develop on the stone, hence the bulk of the action is believed to be bacterial.

Experiment 3. *Comparison of the Respiratory Activity of Decayed and Sound Portions of the same Stone.*—The block of stone for this experiment was a large one removed from a balustrade on the roof of Lancaster House, London, about 80 feet from the ground and therefore free from contact with soil and apparently free from any organic matter such as bird droppings, growth of algæ, lichens or fungi ; there was a thin film of soot. The block was decayed at one end and comparatively sound at the other. Sound and decayed parts of the stone were placed in the apparatus and their respiratory action compared with toluene controls. As was to be expected the rate of respiration was lower than that of the stone used in experiment 2, but the difference between the two samples of the Lancaster House stone showed marked differences in the amount of CO_2 given up.

TABLE VII.—Milligrams of CO_2 produced during 26 days from 350 grams of Decayed and Sound Portions of the same Stone.

Decayed.		Sound.	
Experimental duplicates.	Toluene control.	Experimental duplicates.	Toluene control.
35·8 35·8	6·3 —	15·3 16·9	5·6 —

Subtracting the mean of the controls, the result from the decayed portion is 29·8 mg., and the relatively sound portion 10·1 mg.

Experiment 4.—A similar experiment was conducted on decayed and sound stones from other localities.

When, owing to the exhaustion of fermentable material, the rate of production of CO_2 in the above experiments, Table VIII, had fallen to a low level, the stoppers of the stone containers were removed and to each lot of stone 10 c.c. of a sterile 1 per cent. solution of cane sugar was added aseptic precautions being observed. The result of this addition was an immediate increase in the rate of output of CO_2. In one instance by periodically repeating such additions of nutriment the fermentation was continued for fourteen weeks, after which time the output of CO_2 fell off and increasingly weaker responses were made to the addition of fresh nutriment. This appeared to

TABLE VIII.—CO_2 Output, less that of Toluene Controls, from Decaying and Comparatively Sound Stone from the same Building.

Locality.	Nature of Stone.	Weight. gm.	Time. hrs.	mg. of CO_2.	
				Decayed.	Sound.
Carisbrook Castle .	Limestone	217	109	5·6	0·9
Pevensey Castle . .	Upper greensand calcareous sandstone	240	100	11·9	0·9
Dover Castle* . .	Calcareous sandstone	267	62·5	4·5	2·7

* Algæ were present on both stones and the so-called sound stone was considerably decayed, though noticeably not so much as the other.

be due to the accumulation of toxic metabolic products, for, after well washing with sterile distilled water and drying in a shady place, something like the original response to the supply of sugar was regained. It is perhaps significant that, as has already been shown, Table IV, of the species of bacteria isolated from stone only one has had the power to ferment sugars with the production of gas, although many have been shown to produce acids. It is therefore a fair assumption that part of the CO_2 evolved in these experiments came from the action of these acids on the carbonates of the stone. This statement is made, however, with reserve, since the relative importance of other living organisms, moulds, algæ, and protozoa, which may be present on the stone has not been determined. In any case some of the CO_2 would, under natural conditions, in a moist atmosphere, attack the limestone. The problem then, as regards the heterotrophic bacteria, resolves itself into whether these organisms can or cannot live a full active existence under the conditions of a stone used for building. It is of course unthinkable that they should be dormant all the time or even most of the time, the

more unlikely in view of the paucity of sporers. The extent of their periods of activity with accompanying acid production is mainly a question of food and water supply. At first thought one is inclined to assume that the organic food supply must fall far short of the requirements. In considering this, however, the minute size and body weight of the bacteria must be borne in mind. Assume a bacterial cell of dimensions $2\mu \times 1\mu$ (a fairly average size) and a bacterial number of 10 million organisms per gram of stone; the volume of a single cell $= 2 \times 10^{-9}$ cu. mm. so the volume of 10 million $= 2 \times 10^{-2}$ cu. mm., and assuming the specific gravity $= 1$, the weight of 10 million cells $= 2 \times 10^{-2}$ mg. In other words the weight of bacteria in 1 gm. of stone $= 0{\cdot}02$ of a milligram, of which mass approximately one-fifth is solid matter. The total solids then required for the population of 1 gram of stone is $0{\cdot}004$ of a milligram, which might conceivably be borne to them by rain-water.

Experiment 5. *Effect of adding Rain-water to Decaying Stone.*—In order to determine whether rain-water can support any considerable bacterial population, stone from Dover Castle, having a bacterial population of 750,000 organisms per gram, was used. A large quantity of fresh rain-water from the glass roof of a greenhouse was collected under toluene and evaporated to one-hundredth of its volume, hydrochloric acid being added to fix any free ammonia. The concentrated solution was shaken with $CaCO_3$ to neutralize the free acid, and the filtered liquid was shaken with 20 gm. of dust from the roof. The extract was filtered and sterilized. Two towers filled with decaying stone were set up, one being treated with toluene to act as control. After determining the initial CO_2 output, 10 c.c. of the concentrated rain-water-dust-extract was added to each tower twice a week, the CO_2 output being determined as before. After three and a half weeks of this treatment the addition of food was interrupted for nineteen days, though the measurement of CO_2 output was continued. The result of this experiment was an increase in the weekly output of CO_2 from $6{\cdot}2$ mg. to $21{\cdot}6$, $24{\cdot}9$, $41{\cdot}6$ mg. in successive weeks during the addition of the concentrated rain-water, followed by a fall to 24, 20, 14 mg. during the subsequent three weeks without food. After this an attempt was made to determine the effect of rain-water in its natural concentration 10 c.c. were first added and that proved sufficient only to check the falling output of CO_2. In order to give a measurable effect the amount was then increased to 40 c.c.* with resulting increase to $17{\cdot}4$ mg. which was maintained practically constant for four weeks. Then came a week without rain when only 5 c.c. of dew drained from the roof: the addition of this, however, produced an output of CO_2 of 21 mg. It was then decided to contrast the effect of rain-water and distilled water; on the addition of distilled water the rate fell to $13{\cdot}6$ mg., it rose again with rain-water to $17{\cdot}5$, and fell with distilled water to $11{\cdot}5$ mg. in successive weeks. The result of this experiment is shown graphically in fig. 2.

* The increase of the amount of water to 40 c.c. necessitated the provision for drainage of the sump before each subsequent addition. This was arranged for by a three-way tap at the inlet to the tower, so that the liquid collected at the bottom could be removed by suction.

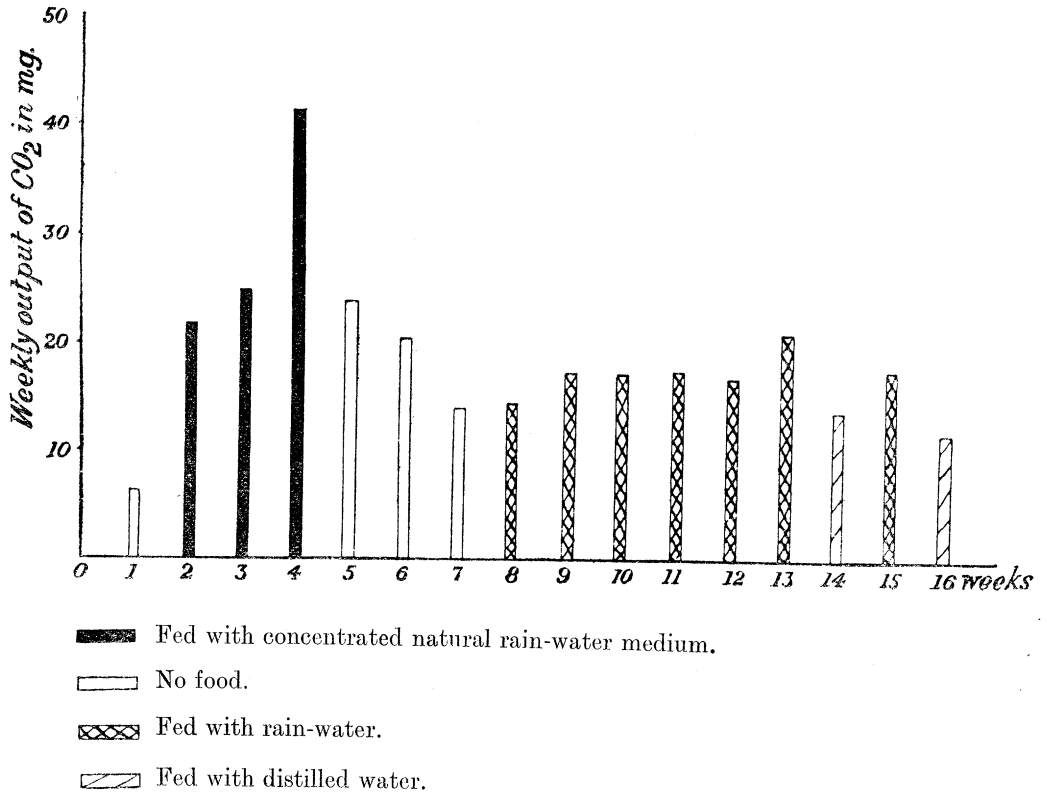

FIG. 2.—Feeding experiments with decaying stone and varying amounts of food for the bacteria.

7. *Investigation of Nitric Acid Production in Decaying Stone.*

The conditions found to enhance the process of nitrification in soil, as given by WAKSMAN (1927) (p. 527 *et seq.*), are mostly to be found in the stone of buildings; ammonia passes into the air by the combustion of coal and is found in appreciable amount in the accumulated soot on the walls; other sources of ammonia, horse dung and sewage, unimportant nowadays except locally, were more considerable while the decay now in question was developing; carbonates in the stone serve to produce an optimum p_H; the porosity of the stone provides, at least at or near the surface, ideal conditions for the life of aërobic bacteria; and periodical drought and freezing, both of which have been found to increase nitrification (WAKSMAN *loc. cit.*), are even more potent factors in stone than in soil. In view of these considerations, and of the suggestions of MARSH (1923), BUCHANAN (1928) and FOX and HARRISON (1925) that nitrification occurs in stone, a survey of the amount of nitrate in decaying stones was undertaken.

Method of Estimation of Nitrate.

The method was based on that given by HARPER (1924).

Extraction.—Stones were dried at about 100° C. to constant weight and 250 grams (or available quantity) of powdered stone weighed out. About 50–100 c.c. of hot distilled water was poured on and allowed to stand overnight; further extraction was made on a Büchner funnel, the stone being washed with hot water and the extract made up to 500 c.c. (or appropriate concentration). Spotting tests with diphenylamine in sulphuric acid were used to determine when extraction was complete. (This is a smaller proportion of water than HARPER uses since the nitrate content of stones is usually low.)

Decolouration, when necessary, was effected as follows. To 100 c.c. extract 150 c.c. distilled water, containing 5 c.c. N. $CuSO_4$ solution, was added and shaken for 10 mins. 0·4 gm. $Ca(OH)_2$ and 1·0 gm. $MgCO_3$ were added and shaken for 5 mins. The solution was filtered on a dry filter, the first 20 c.c. filtrate being discarded. If too dilute, extracts had to be concentrated, or a large proportion used for estimation.*

Estimation.—25 c.c. of the extracts (or larger volume if necessary) were evaporated to dryness on a water bath in $2\frac{1}{2}$ inches diam. evaporating dishes. 25 c.c. of standard KNO_3 (0·7221 gm. per litre) solution were treated likewise. The dishes were cooled and 2 c.c. of phenol disulphonic acid were added to each. The reagent was brought in contact with the residue, and allowed to act for 10 minutes. It was then diluted with 15 c.c. distilled water and well stirred so that all the residue dissolved. When cool, dilute ammonia (1 vol. 0·880 sp. gr. in 2 vols. water) was added till slightly alkaline, usually about 17–20 c.c. This was transferred to a measuring flask and made up to 100 c.c. The yellow colour was compared with the standard in a Klett colorimeter, diluting the standard or solution as necessary.

Method of Estimation of Nitrites.

The estimation of nitrites was made by a modification of the Greiss method (LIPMAN and BROWNE (1911)). It was found (i) that a standard sodium nitrite solution did not long remain constant, and (ii) that heating was necessary to develop a more or less stable colour from the reagent within a suitable time. Therefore slides made by Tintometer, Ltd., were used as colour standards, and calibrated with freshly prepared sodium nitrite standard solutions under standard conditions adopted throughout the experiments. The 10 c.c. (or more) of the test treated with 16 c.c. Greiss reagent and diluted to 100 c.c. in a measuring flask, were heated at 70°–80° in a water bath for 10 minutes, cooled rapidly, and comparisons made at once with the colour standard.

* Some specimens gave strongly yellow extracts which could not be cleared by this or any known method. The estimation of nitrate by the phenol disulphonic acid method had to be abandoned.

(The Tintometer slides used were red 4·0, and in combination with the test solution, yellow 0·25.)

TABLE IX.—Amount of Nitrate in Decaying Stones and Relation to their Bacterial Content.

Source of Stone.	Condition or Situation.	Parts NO_3 per 1,000.	Population on Bouillon Agar Plate.	Presence of Nitrifiers.
Goodrich Castle Keep .	(i) Fragments	6·5	—	—
	(ii) Whole	2·4	—	—
	4 feet above ground.			
Helmsley, 3 samples . .	Blocks, fairly soft	3·4	(i) 22,950	—
			(ii) 736,000	—
Hampton Court, Fish Court	Loose surface, archway sheltered from rain	3·0	—	No
Tewkesbury Abbey . .	Surface fragment	1·3	0	No
Hampton Court, Wren Building	Decayed surface exposed to rain . .	0·3	294,450	Not in subculture.
Hampton Court, Fountain Court	Decayed surface sheltered from rain	0·2	—	No
Goodrich Castle	Decayed outer ½ inch	0·1	—	—
	Sound inner block	0·02	192,000	—
Tintern Abbey	Fragments	0·1	—	—
	Coping stone	0·02	1,279,000	—
Byland Abbey	Fragments	0·04	2,000,000	—
	Block	0·03	896,000	—
Merton College, Oxford	Fragments	0·04	1,535,000	—
Raglan Castle	Fragment	nil.	103,880	—
Rievaulx Abbey . . .	Soft stones	0·01	80,000	No
Caerleon, 3 samples . .	Various	nil.	3,000–18,000	—
Goodrich, Portcullis room	Fragments	nil.	—	No

These results on the whole confirm the finding of nitrate by other observers, the highest amounts were in stones from sheltered positions where the surface of the stone had crumbled or flaked off. The material was not aseptically collected so it was not possible to obtain the bacterial population of crumbling samples, but where counts were made they showed no relation to the amount of nitrate, as might have been expected, since many heterotrophic bacteria can use nitrates in their metabolism. Where indicated tests for the presence of nitrifying bacteria were made by inoculation of small pieces of stone into a medium containing ammonium sulphate (GIBBS (1919), LIPMAN and BROWNE (1911)), but with negative results; a trace of nitrate found in a few cases was not produced in subcultures so may have been that present in the stone used for inoculation. In the search for the evidence of nitrifiers later experiments gave a few positive results, and it is possible that lack of success at this stage was owing to dryness of the samples, collection having been made during the summer months.

In several experiments, continuing over two years, blocks of stone and chalk, provided with cups chiselled in their upper surface, have been allowed to imbibe from the cups, solutions of ammonium sulphate and urea, replenished weekly. A small addition of soil provided the inoculum for a culture of nitrifying bacteria. Periodically the stone lining the cups was chiselled out and the contained nitrate was determined. The monthly rise and fall in the amount of nitrate agreed with the seasonal rise and fall characteristic of the nitrification process as it occurs in soil, thus affording evidence that the nitrifying bacteria had established themselves in the stone habitat and were there functioning in their normal manner.

8. *Isolation of Nitrifying Bacteria from Decaying Stone.*

Attempts to isolate nitrifying bacteria from the stones in the above experiments were unsuccessful though fluid cultures made by inoculating small pieces of the stone into solutions containing ammonium sulphate gave evidence of their presence in the formation of nitrite from the ammonia, both in the original culture and in sub-cultures from it. Quite recently nitrifying bacteria have been isolated from three different sources, the decaying limestone from near ground level in a London street (p. 108), the decaying sandstone from Dover Castle (p. 110), and a crumbling limestone from the Fountain Court at Hampton Court (p. 114). At the same time failures to obtain cultures of nitrifying bacteria were recorded in twelve other cases, so, including the earlier failures shown in Table IX, the ratio of successes to failures is 3 : 21.

Method of Isolation.—A small quantity of the sample was introduced into the solution containing ammonium sulphate commonly employed for this purpose. In the three successful cases oxidation of the ammonia to nitrite and finally to nitrate took place. Repeated sub-culturing gave similar results from which it was concluded that nitrifying organisms were present. Enrichment cultures in ammonium sulphate medium were carried forward until a really active culture was obtained before an attempt was made to isolate the organism on plates of silica jelly. The technique employed was that described by Fred and Waksman (1928). After six weeks' incubation at 30° C. small brownish-yellow and white colonies appeared while none were visible on control plates uninoculated. Six of these colonies were transferred by means of sterile, drawn-out glass rods to 10 c.c. of liquid medium in small conical flasks, the thin ends of the glass bearing the colonies being broken off and left in the medium. After two weeks' incubation three of the six colonies were found to have "taken," but unfortunately, on transferring $\frac{1}{2}$ c.c. of each into bouillon, growth took place, showing the presence of heterotrophic organisms. That these were not nitrifying heterotrophic strains similar to those of Ward Cutler (1930) was shown by plating on nutrient agar from the bouillon when the resulting colonies proved to possess no nitrifying power. On microscopic examination of a stained preparation from one of the yellow colonies on silica jelly the field of organisms, though obviously mixed, contained a large preponderance in zoogloeal formation of Gram-positive short oval rods of the characteristic size

and shape of WINOGRADSKY'S *Nitrosomonas europæa*, fig. 19, Plate 20. Microscopic examination of the organisms in bouillon showed them to be morphologically distinct from these.

9. *Inoculation of Stone with Cultures of Nitrifying Bacteria.*

The effect of nitrifying bacteria upon stone has been tested in the following experiment. Weighed small blocks of Corsham Down limestone were placed at the bottom of Erlenmeyer flasks, partially covered with a culture solution containing ammonium sulphate and inoculated with a strong enrichment culture of *Nitrosomonas*. The disappearance of ammonia was investigated and, when found to be complete, further additions of sterile 1 per cent. ammonium sulphate were made. The experiment was carried on for about ten weeks with monthly additions of ammonium sulphate. At the end of this period the surface of the stones in the inoculated flasks showed a brown stain, not present in the controls, and a precipitate, believed to consist of calcium and magnesium phosphates, had formed in the inoculated fluid. The stones were extracted first with cold, then with hot water and estimations were made of the Ca, SO_4, NH_4, and NO_3 from which the amounts of $CaCO_3$ and $MgCO_3$ dissolved from the stone could be determined.

TABLE X.—Effect of Nitrifying Bacteria on Stone in Culture Flasks.

No. of Block.	Description.	Weight of Stone.		Loss (Total).	Loss due to Solution.
		Before Incubation.	After Incubation.		
		gm.	gm.	gm.	gm.
4	Control	11·199	11·093	0·106	—
6	,, ...	11·941	11·829	0·112	—
12	Inoculated	10·601	10·232	0·369	0·284
10	,, ...	9·300	8·868	0·432	0·327
9	,, ...	9·010	8·545	0·465	0·267
		Period of incubation $2\frac{1}{2}$ months.			

The effect of the solvent action is clearly seen in the accompanying photographs the edges of the stone A from inoculated solution being markedly rounded as compared with those of stone B from the uninoculated control, fig. 18, Plate 20.

Experiments with Stone Columns inoculated with the Nitrifying Organism.

It was considered advisable to determine whether ammonia supplied to small columns of stone inoculated with the nitrifying organism would be oxidized to nitrous acid. The columns used were of dimensions $4\frac{1}{2}$ inches by 1 inch by 1 inch and were cut from one block of Corsham Down stone.

Method of Inoculation.

The blocks were placed with 50 c.c. of ammonium sulphate medium in tall, narrow boiling tubes, which were then plugged and sterilized. One was inoculated with the organism (by adding a few drops of a filtered enrichment culture) and the other served as control. The inoculated tube was placed at an angle of about 35°–40° to the vertical, and incubated at 30° C., in this sloping position and, in order that the whole surface of the stone might become inoculated with the organism, the tube was turned through 90° each day. In order to obtain a strong culture of the organism on the stone several fresh additions of ammonium sulphate were made as the previous ones became used up. The stones were then removed from the tubes, and placed on glass tripods standing in 1-litre beakers, each being covered by a bell jar. On each column 100 c.c./ per day of the following solution were allowed to drop slowly: $(NH_4)_2SO_4 = 0{\cdot}3$ gm., $K_2HPO_4 = 0{\cdot}5$ gm., NaCl = 1 gm., Distilled Water = 1000 c.c. The top of each column had been previously fitted with a cap of Winceyette (a wool and cotton cloth) which served to ensure that all sides of the column were equally wet. Each column was placed in a warm room at 28°–30° C. Nitrification was prevented in the liquid after passing over the columns by addition of 10 c.c. of N·NaOH to the beaker before the commencement of the experiment, and the washings were not allowed to exceed 500 c.c. This procedure caused the p_H of the drainings to rise to 13–14, effectively preventing any biological oxidation of the ammonium sulphate, for which the upper limit, according to MEYERHOF (1917), is p_H 9·5. After periods of 10–12 days' incubation, the amount of nitrite in the washings from each column was estimated by means of Griess' reagent, the washings for the first two periods being discarded, as these would contain some of the nitrite formed during the period of inoculation. The results are shown in Table XI.

TABLE XI.—Milligrams of Nitrite in Washings from Stone Columns fed with Ammonium Sulphate.

Period of Incubation.	Milligrams of Nitrite in Washings from	
	Control Block.	Inoculated Block.
10 days	0·345	73
11 days	0·305	243
10 days	0·312	210

These results show clearly that nitrification can take place on a column of stone fed with ammonium sulphate solution. This is not surprising for, after all, the type of experiment is little different from the original classical experiment of SCHLOËSING and MUNTZ, in which they demonstrated the biological character of the nitrification

of ammonia in soil. In view of the numerous occasions when it has been impossible to demonstrate the presence of nitrifying bacteria in decaying stone the presence of small quantities of nitrate cannot be regarded as proof of this process as a common feature in stone decay ; the few instances in which the presence of nitrifying organisms were found were not beyond the possibility of chance infection from soil on stone already partially decayed from some other cause.

10. *Occurrence of Sulphur-oxidizing Bacteria in Decaying Stone.*

For some time attention has been focussed upon that particular form of decay which is marked by the formation of considerable masses of white deposit 1–2 mm. below the surface, causing the stone to blister or flake away often in large patches, leaving the white deposit exposed to the air. It has been observed that in some instances the white incrustation is accompanied by brown discolouration of the decayed stone, sometimes faintly and often clearly marked. A good example of this kind of decay is found on the inner walls of Westminster Hall. This has been under observation since 1926, when the accompanying photograph, fig. 10, Plate 19, was taken. Subsequent photographs at yearly intervals reveal very little change although the surface is continually breaking away and falling to the ground. From this it is quite clear that the agent of decay is still active. Tests for the ordinary bacteria of the air and for the evidence of nitrification have yielded no positive results and the stone was considered almost sterile. It has been discovered, however, during the past year that, although free from the more ordinary forms of bacteria, the stone harbours an organism, or group of organisms, capable of oxidizing sulphides and thiosulphates with the formation of sulphuric acid. The presence of such organisms in stone had been suggested by BUCHANAN (1904), but no evidence in support of the view has hitherto been produced. The importance of this discovery is at once obvious, and it has been followed up by a search for these bacteria in other cases where decay of the stone is accompanied by a crystallization of calcium or sodium sulphates. The method of investigation is as follows. About a gram of the decaying stone is inoculated into 50 c.c. of a culture fluid contained in a 300–c.c. Erlenmeyer flask. The culture solution (WAKSMAN) (1927) is made up of sodium thiosulphate 5 gm., magnesium chloride 0·1 gm., ammonium chloride 0·1 gm., potassium phosphate 0·2 gm., sodium bicarbonate 1 gm., excess of calcium carbonate, 1000 c.c. of tap water. After incubation for fourteen days at 30° C. the amount of thiosulphate titratable by iodine is found to be almost exhausted in the experimental flask, but not much reduced from the original in the control. Transferring 1 c.c. of the fluid to fresh medium carries over the oxidizing power. The presence of sulphur-oxidizing bacteria is accepted for any sample of stone only after oxidation of the thiosulphate has been proved to occur in sub-cultures from the original. Working in this way positive results were obtained for sandstones from Kelso Abbey, fig. 6, Plate 18, Jedburgh Abbey, Stirling Castle, Linlithgow Palace, Holyrood Palace, Crichton

Castle, Dryburgh Abbey, Melrose Abbey, and sand which had fallen down a chimney; for limestones from Westminster Hall, fig. 10, Plate 19, the Terrace and the Star Chamber Court, and other places at the Palace of Westminster, fig. 5, Plate 17, fig. 7, Plate 18; for a decaying Portland stone on the new Science Museum at South Kensington, fig. 4, Plate 17; for brickwork at the Royal College of Music; and for terra-cotta at the Victoria and Albert Museum.

It is seen then that these bacteria have been found in a surprisingly large number of cases (in fact it is unusual to fail to demonstrate their presence), it must however be clearly stated that the bacteria are not held to be responsible for the formation of the sulphates in all these cases. This can readily and adequately be explained on chemical and physical grounds (see SCHAFFER (1931)), and it is not necessary to invoke the aid of bacteria to explain the oxidation of atmospheric sulphur dioxide. The existence of organisms possessed of the power to effect the oxidation of lower sulphur compounds may however assist to explain certain difficult cases where a straightforward explanation on other grounds is not easily forthcoming. Such an explanation on biological grounds postulates the existence in stone of some form of oxidizable sulphur. The brown colouration which frequently accompanies the deposition of sulphates suggests that one source of sulphur may be iron pyrites. The occurrence of iron oxide in the neighbourhood of the decay, at many of the castles in Scotland given in the above list, was a notable feature, and it is perhaps significant that the stone for these Castles was quarried in a coal-mining area.

11. *Morphology and General Characteristics of the Sulphur-oxidizing Bacteria from Stone.*

The organism grows well on plates of washed agar containing mineral salts, including sodium thiosulphate and precipitated chalk. The colonies appear in about two weeks at 28° C., surface colonies being round, depth colonies spindle-shaped. As the colonies develop they become surrounded by a clear halo in which the chalk has been dissolved by the sulphuric acid produced, figs. 11 and 12, Plate 20. At the same time they present a peculiar dual structure, fig. 12, Plate 20, a dense cyst-like centre, coloured yellowish-brown, surrounded by a clear wide envelope of mucilage. On examination of the colony under higher magnification the yellow colour of the central zone is found to be due to the presence of sulphur granules, and, under the $\frac{1}{12}$-inch objective, both the outer and inner regions are seen to contain masses of minute bacteria, figs. 13 and 14, Plate 20. Attempts to stain these bacteria with carbol fuchsin result in failure owing to the strong staining capacity of the mucilage and the impossibility of separating the organisms from it. By careful staining with Leishmann's stain the organisms are revealed as minute cocci. When photographed, unstained, by ultra-violet light ($\lambda = 0{\cdot}275$), the size of the organism is found to be only $0{\cdot}28$ μ diameter, fig. 14, Plate 20. The minute size of this organism is sufficient to distinguish it from the

organism first discovered by NATHANSOHN (1902) in the mud of the seashore and named by BEIJERINCK (1904) *Thiobacillus thioparus*. Moreover our species differs from this in forming granules of sulphur within the colony, and not in the surrounding medium as described by NATHANSOHN (1902). ELLIS (1932) in a brief account of thionic-acid bacteria describes *Thiobacillus thioparus* as a small slender rod of $0{\cdot}3\mu$ to $0{\cdot}5\mu$ in length. Such an organism would be comparable in size with the stone organism. The dimensions are, however, incorrectly stated by ELLIS; BEIJERINCK's organism was a rod of $3{\cdot}0\mu \times 0{\cdot}5\mu$. ELLIS possibly obtained his figures from a paper by DÜGGELI (1919), also cited by WAKSMAN (1927). Reference to DÜGGELI's paper leaves doubt as to whether he made an actual isolation; it seems more probable that he misquoted BEIJERINCK and that the dimensions of $0{\cdot}3\mu$ to $0{\cdot}5\mu$ in length should have read $3{\cdot}0\mu \times 0{\cdot}5\mu$. The accompanying illustration in DÜGGELI's paper makes it quite clear that an organism of the latter proportions was there described. BEIJERINCK's measurements have recently been confirmed by WUDTKE (1932). The stone organism differs in size and properties from the acid-loving *Thiobacillus thio-oxidans*, of WAKSMAN and JOFFE (1922), whose growth limit, the notably low p_H value of $0{\cdot}6$–$1{\cdot}0$, is far below the extreme limit of acid tolerated by the stone organism. GICKLHORN (1920) described under the name *Bakterium crystalliferum* an organism of this group which, like our organism, produced a double-zoned colony with crystals of sulphur deposited in the central zone. *Bakt. crystalliferum* differed morphologically from our organism in having a length of 1–2μ and diameter of $0{\cdot}3$–$0{\cdot}5\mu$. ISSATSCHENKO and SALIMOWSKA (1929) name three organisms from the mud of salt lakes, *Bakterium Beijerinckii*, *Bakt. Nathansohnii* and *Bakt. Beijerinckii f. Jacobsenii*. These were all rod forms similar to *Thiobacillus thioparus*, but differing from it and from each other in the size of the cells.

The question of the identity and nomenclature of the stone organism remains undecided until more of its characteristics have been discovered. The writer views it as a common dust organism and thinks it highly probable that some of the previously named sulphur-oxidizing organisms may also be found in connection with stone decay.

12. *Result of Inoculation of Sterile Stone with Sulphur-oxidizing Bacteria.*

To each of two cultures of the organism in TRAUTWEIN's (1921) medium, a mineral salt solution containing thiosulphate, a block of sound stone was added, a similar block being added to the control flask. The fate of the thiosulphate was watched by titrating at weekly intervals with standard iodine. When the thiosulphate was found to have disappeared more was added in sterile solution, the control flask being similarly treated. This became necessary about once every two weeks. After nine weeks' incubation at 28° C., a white deposit had formed on the blocks of stone in the culture flasks but none was visible on the controls. As more and more $Na_2S_2O_3$ was

added this deposit grew in size and a brown stain was noticed. At the end of two months the piece of stone from one of the culture flasks was carefully removed so as not to disturb the deposit, placed in a weighing bottle, dried and weighed, the stone from a control flask being used for comparison. After being weighed both were well washed with cold water (the washings being collected), when it was found that the white deposit dissolved. Each block was transferred to a Gooch crucible, dried and weighed, with the following result:—Loss of weight of stone from culture flask approximately 0·06 gm.; loss of weight of stone from control flask approximately 0·02 gm. (the rather large loss of the control block is due probably to the solution of absorbed $Na_2S_2O_3$, the concentration of which had become rather high in the flask from which this stone was taken. The filtrate was tested for calcium and sulphate which were present. Estimations of SO_4 showed that, while no SO_4 was present in the filtrate from the control block, 88 per cent. of the loss in weight of the other block was due to the solution of $CaSO_4$. It was also noticed that the block of stone from the culture flask was considerably eroded, the control not showing this at all, fig. 15, Plate 20.

This experiment was repeated with weighed blocks of stone. Some free sulphur formed in the inoculated flasks, and as before, a brown stain appeared on the exposed surface of the inoculated stones. During the course of the experiment samples were withdrawn with a sterile pipette in order to discover the fate of the added thiosulphate —1 c.c. was withdrawn at each sampling so that some of the products of the reaction were removed. The complete balance sheet, which might otherwise have been obtained at the close of the experiment after three months' incubation, was therefore deficient by these amounts, moreover some difficulty was experienced in removing the free sulphur from the blocks of stones and from the loosened oölites. In view of these deficiencies the results obtained can be regarded as satisfactory.

TABLE XII.—Analysis of Extracts of Limestone Blocks after the Action of Sulphur-oxidizing Bacteria.

Block No.	Nature.	Ca in Extract.	$CaCO_3$ Dissolved.	$Na_2S_2O_3$.
		gm.	gm.	gm.
1	Control	Nil	Nil	1·65
2	,, ...	Nil	Nil	1·78
3	Inoculated	0·065	0·163	Nil
4	,, ...	0·070	0·175	Nil
5	,, ...	0·059	0·148	Nil

In each case the sulphate also was determined but this was always considerably in excess of the equivalent of the Ca found, part of the thiosulphate is probably oxidized according to the reaction $Na_2S_2O_3 \longrightarrow Na_2SO_4 + S$.

TABLE XIII.—Loss of Weight in Limestone Blocks resulting from the Action of Sulphur-oxidizing Bacteria.

Block No.	Description.	Weight before Incubation.	Weight after Incubation.*	Loss.	Weight of Blocks alone after Experiment.	Loss.
		gm.	gm.	gm.	gm.	gm.
1	Control	10·538	10·528	0·010	10·498	0·040
2	,, ...	9·473	9·459	0·014	9·431	0·042
3	Inoculated	11·294	11·058	0·236	10·878	0·416
4	,, ...	10·768	10·458	0·310	10·337	0·431
5	,, ...	9·277	9·068	0·209	8·809	0·468

* Loosened oölites and small flakes of stone were included.

Balance Sheet.

Block No.	Description.	$CaCO_3$ lost by Stone.	$CaCO_3$ gained by Solution.
1	Control	0·10	Nil
2	,, ...	0·14	Nil
3	Inoculated	0·236	0·163
4	,, ...	0·310	0·175
5	,, ...	0·209	0·148

In another experiment blocks of Bath stone about 3 inches by 2 inches by 1 inch were drilled with an augur of 0·7 cm. diameter to a depth of 4 cm. at a distance of approximately 2 mm. from one edge of the stone. They were sterilized and treated as under.

A Mineral culture media + thiosulphate, inoculated with sulphur bacteria.
B Mineral culture media + thiosulphate, uninoculated.
C Mineral culture media + thiosulphate, inoculated.
D Mineral culture media + thiosulphate, uninoculated.
E Mineral culture media — thiosulphate, inoculated.
F Mineral culture media — thiosulphate, uninoculated.

These were maintained at a temperature of 28° C. in a moist atmosphere and supplied with fresh additions of sterile culture fluids to the point of saturation. After six months they were removed from the moist air and allowed to evaporate. Calcium sulphate crystallized out on the surface of A and C, but none appeared on B, D, E and F. Moreover a marked brown colouration appeared on A and a less apparent though quite distinct tinge on C. The brown stain when dissolved in HCl was shown to give the test for iron with KCNS.

In another experiment a column of Bath stone 4 inches long by 1 inch square, in section, was inoculated with the sulphur-oxidizing organism, and fed with a solution containing 2·5 gm. of $Na_2S_2O_3$ per litre. The uninoculated controls received the same treatment. The solution was allowed to drip on to a muslin cap designed to equalize the flow down all sides. After passing over the surface of the column the fluid was collected and the sulphate determined. During the first four weeks no determinations were made.

TABLE XIV.—Oxidation of Thiosulphate on the Surface of Limestone Columns inoculated with Sulphur-oxidizing Bacteria.

Time.	Temperature.	Gm. of Sulphate in Fluid after passing over the stone.	
		Inoculated Column.	Control Column.
5th week	30° C.	0·672	0·462
6th week	30° C.	0·635	0·457
7th and 8th weeks	Outside winter temperature . .	0·445	0·389
9th, 10th and 11th weeks . . .	,, ,, ,, . .	0·825	0·700

The muslin cap failed to serve its purpose and the flow occurred down one face of each column only. The path of flow was marked by a brown stain much more pronounced on the experimental than on the control-stone. At the end of the experiment the columns were allowed to dry, when a blister raised itself along the line of flow on the inoculated stone. This consisted of a thin brown skin which in drying tore away at one of its edges, fig. 16, Plate 20. The skin was carefully removed with forceps and laid over on its back, fig. 17, Plate 20, when it was observed that some of the oölites at the surface had been carried away with the skin and some had been actually split in the process. The skin proved to be composed of the mucilage of the bacterial zoöglœa which had developed on the surface. The tearing away of the surface of the stone is in keeping with the finding of MELLOR (1922) that lichens growing on glass cause the removal of small flakes from the surface, and of FRY (1924) that the tearing away of rock surfaces by lichens results from the powerful forces which their gelatinous tissues exert on desiccation. The tearing of the surface in this experiment indicates a possible way in which the sulphur-oxidizing bacteria may disintegrate stone quite apart from the solvent action of the sulphuric acid they excrete. Even the smallest colony buried in stone would, under the influence of alternations of wetness and drought, exert locally an enormous strain.

In conclusion the authors desire gratefully to acknowledge the grant from the Department of Scientific and Industrial Research and to express their thanks to

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13. *Summary.*

1. Bacteria have been enumerated in decaying stones from various localities and from quarry stone, and have been found to be present in considerable numbers.

2. The general characteristics of the heterotrophic bacteria of stone have been studied and while they can be related to soil, air, and water types, certain features distinguish them as a group from soil bacteria.

3. Many of the heterotrophic bacteria of stone have been found to produce acids by the fermentation of organic compounds.

4. Evolution of carbon dioxide is found to occur when these acid-forming bacteria are inoculated on the surface of sound stone.

5. Evolution of carbon dioxide is found to occur when decaying stone is fed with rain-water.

6. Evidence is given of the presence of small quantities of nitrate to a maximum concentration of 0·3 per cent.

7. Nitrifying bacteria agreeing with the description of *Nitrosomonas europœa* have been isolated from stone, and it has been shown that cultures of this organism in contact with sound stone effect a partial solution of the material of the stone.

8. The view is expressed and supported by evidence that the nitrifying bacteria in decaying stone are to be regarded as chance infections from soil rather than as an essential factor in stone decay.

9. Sulphur-oxidizing bacteria (thionic acid bacteria) have been isolated from decaying stone.

10. These sulphur organisms have been shown to be widely distributed on types of decaying stones exhibiting efflorescence of crystalline sulphates of calcium and sodium.

11. Since the sulphuric acid of the atmosphere can cause the production of sulphates in decaying stone bacteria are not necessary for the formation of these salts. It is, however, suggested that this biological factor may help to elucidate certain phenomena of stone decay which are difficult to explain on other grounds.

12. Colonies of the sulphur organism growing on culture media are found to be embedded in masses of mucilage. If colonies formed in stone are similarly embedded an easy explanation of the disintegration of the stone is provided by the tearing strain resulting from the alternating expansion and contraction brought about by imbibition and desiccation of the mucilage.

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15. DESCRIPTION OF THE PLATES.

PLATE 16.

†FIG. 3.—Church Anston dolomite, Commons' Outer Court, Houses of Parliament, Palace of Westminster. Decay appears to have spread like a disease from an initial point of origin. This doorway was blocked up in 1913 and at that time no decay was noticeable.

PLATE 17.

†FIG. 4.—Portland limestone. New Science Museum, South Kensington. A faulty stone from which apparent disease has spread to its neighbours. The front of this building was faced with stone in 1927 and this stone is the only faulty one in the whole of the façade.

†FIG. 5.—Church Anston dolomite. Terrace, the Palace of Westminster. A strictly localized constellation of six blisters.

PLATE 18.

FIG. 6.—Ferruginous sandstone. Left side of entrance, Kelso Abbey. An isolated stone deeply excavated as though by the sea—the cavernous depressions are from 2 to 3 inches deep and the white felt of crystals consists of calcium sulphate.

†FIG. 7.—Anstone stone ? Mullion of window on the Terrace, Palace of Westminster. The mass of crystals of sulphates seen at the top of the decayed area was $\frac{3}{16}$ of an inch thick, and was lying under the surface at a depth of 3 mm.

FIG. 8.—A piece of the stone removed from this mullion, no mark of decay is obvious on the outside, but just under the soot is a surface skin of sulphate (not visible in the figure) as described by SCHAFFER (1931). The fragment was split off by a fracture running parallel with the outer surface at a distance of 3 mm. from it.

FIG. 9.—The underside of this fragment exhibiting masses of crystalline sulphates.

PLATE 19.

†FIG. 10.—Ketton stone, limestone. North side of Westminster Hall, Palace of Westminster. One of three large decaying areas developed on the length of this wall—the stone is stained brown with iron oxide, and flakes from the surface as large as 5 inches square separate through the development of a fissure parallel to the surface at a depth of 2 to 3 mm. The decay seems slow as judged by the change seen in photographs at three yearly intervals, yet oölites and flakes are continually falling and may be swept up daily. The stone flakes are free from heterotrophic and nitrifying bacteria but an organism of the thionic-acid group capable of producing sulphuric acid from lower sulphur compounds is associated with sulphate crystals.

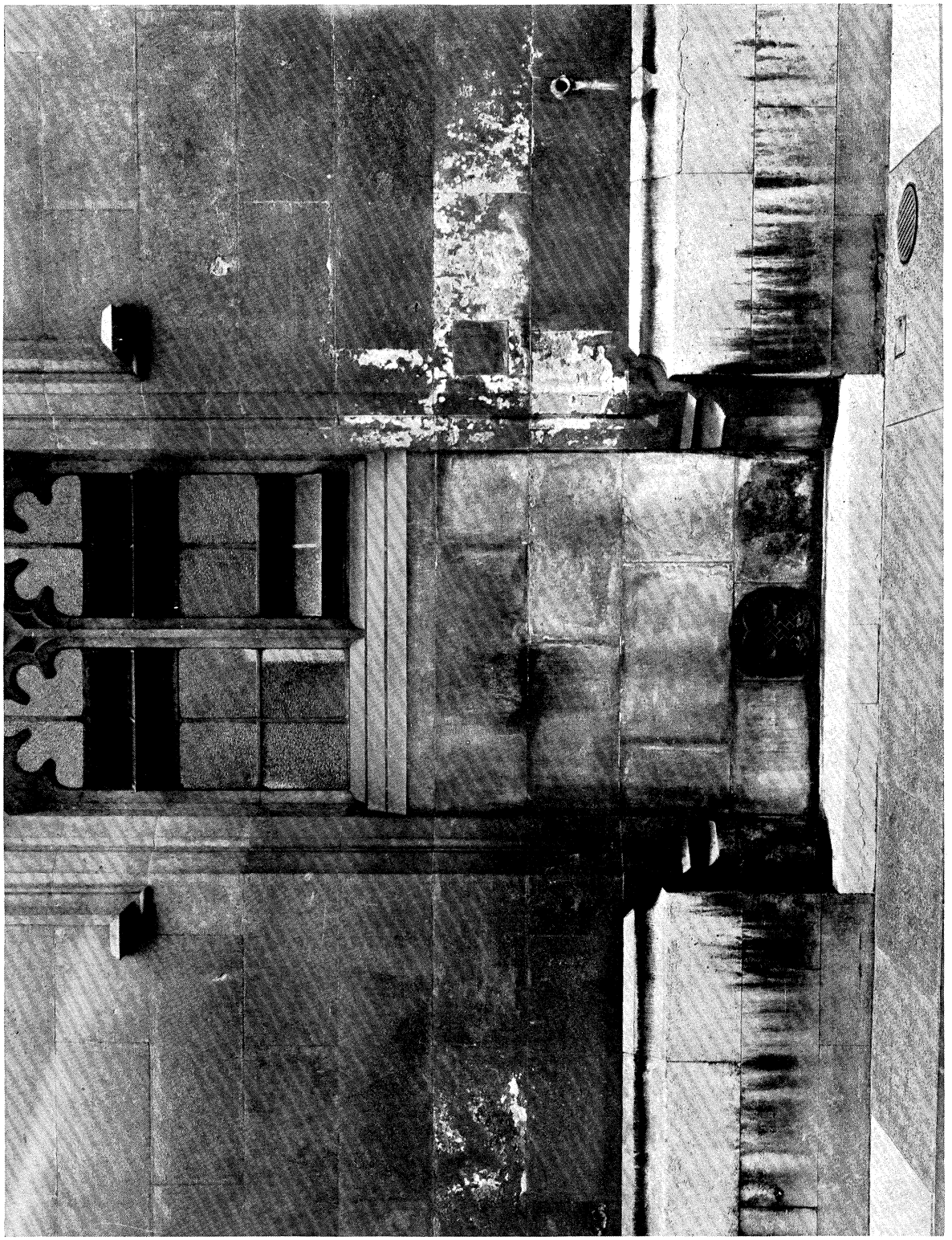


Fig. 3

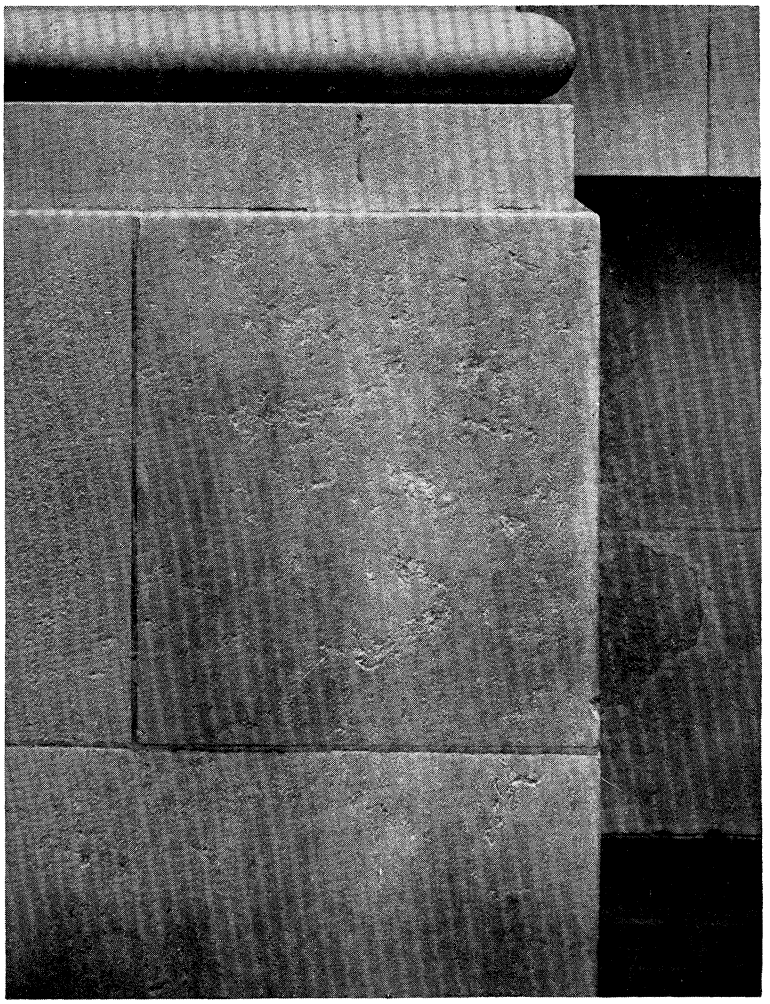


Fig. 4

Fig. 5

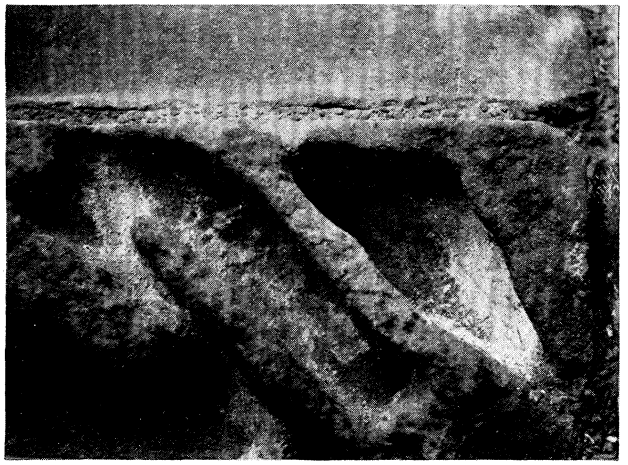


Fig. 6

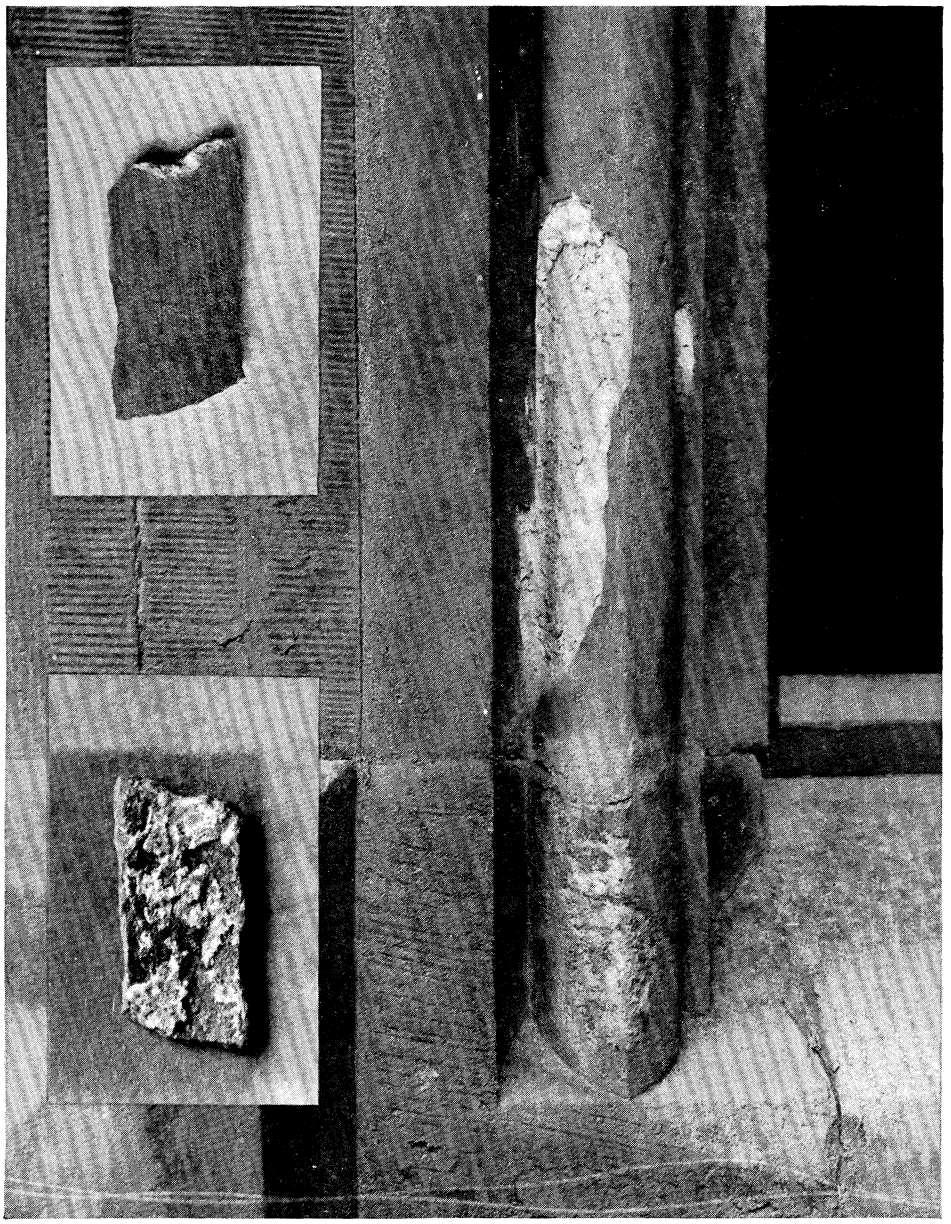


Fig. 8 Inset above.
Fig. 9 Inset below.

Fig. 7

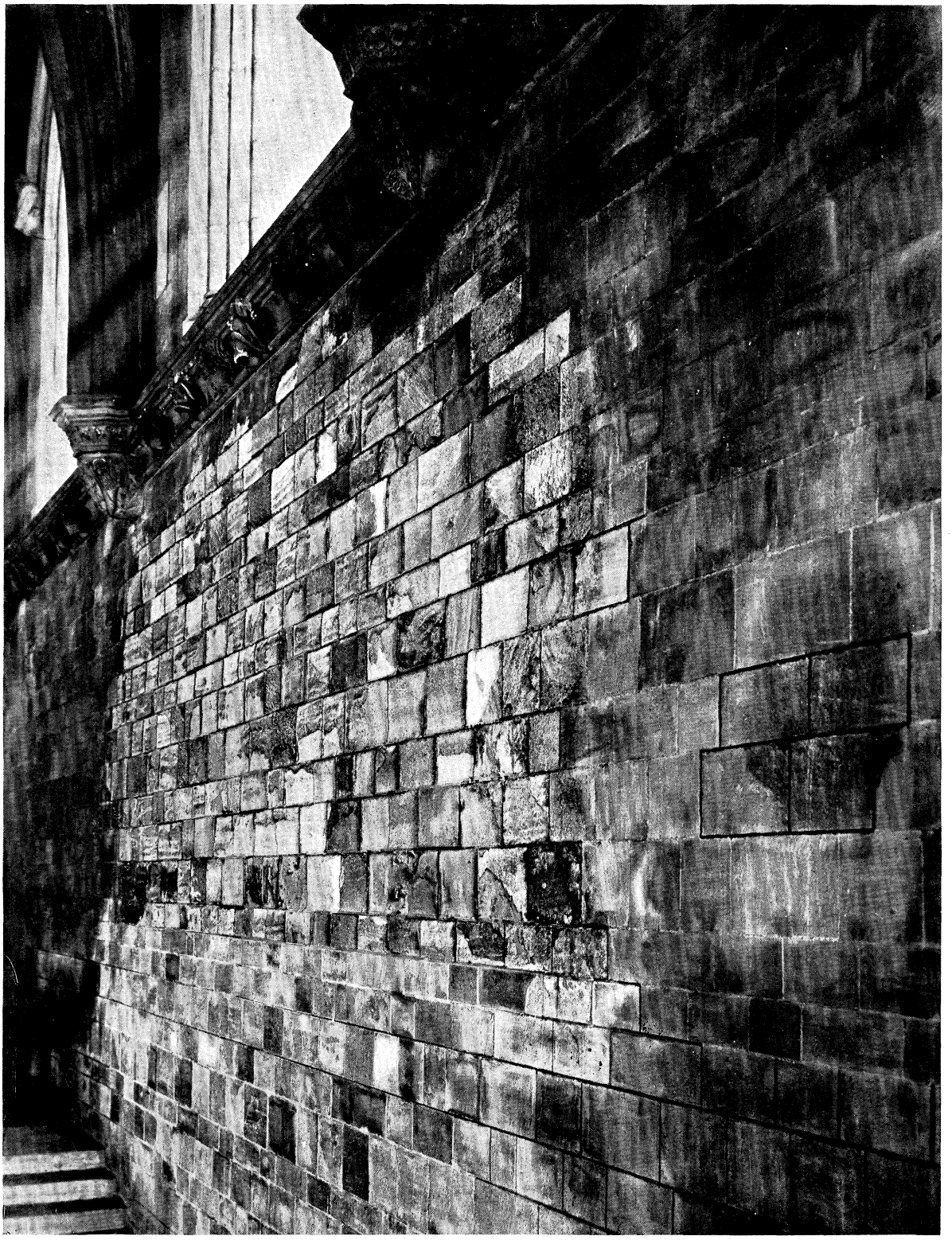
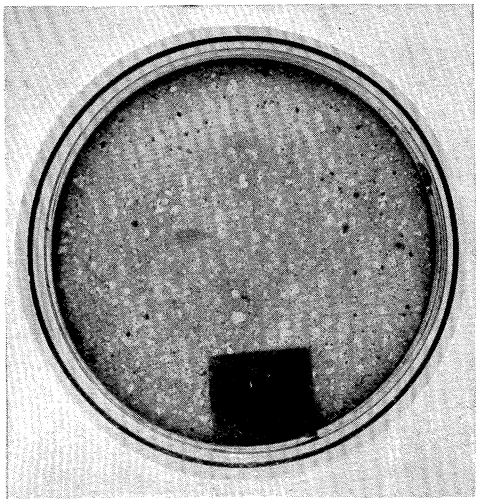


Fig. 10



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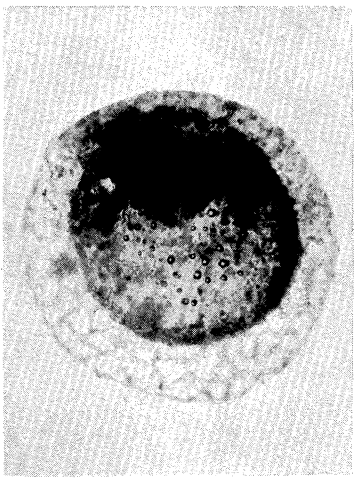


Fig. 13

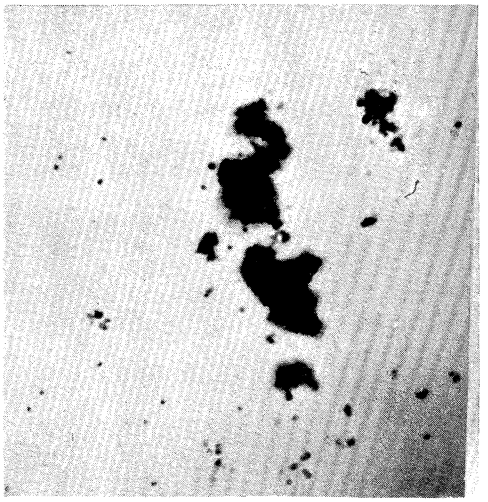


Fig. 14

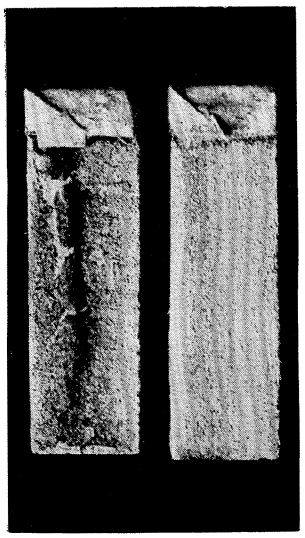


Fig. 16

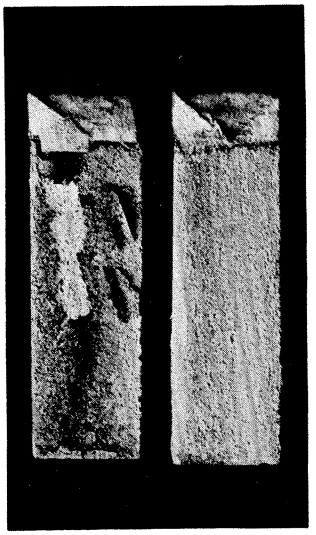


Fig. 17

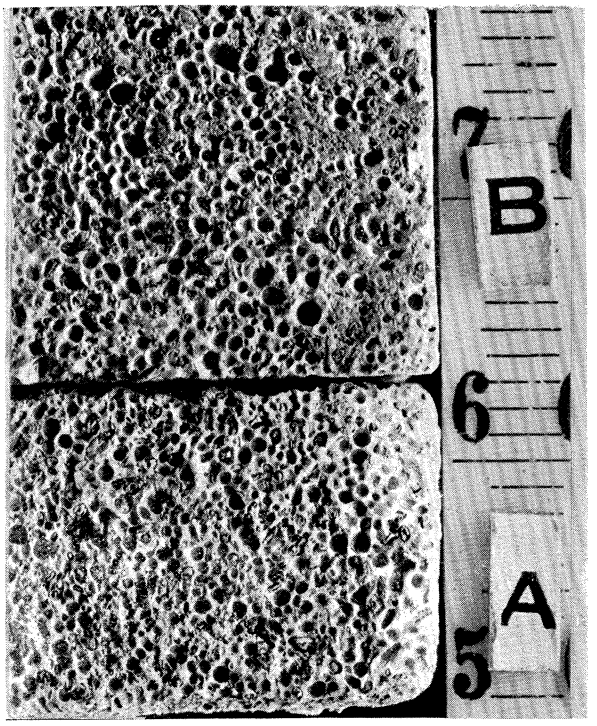
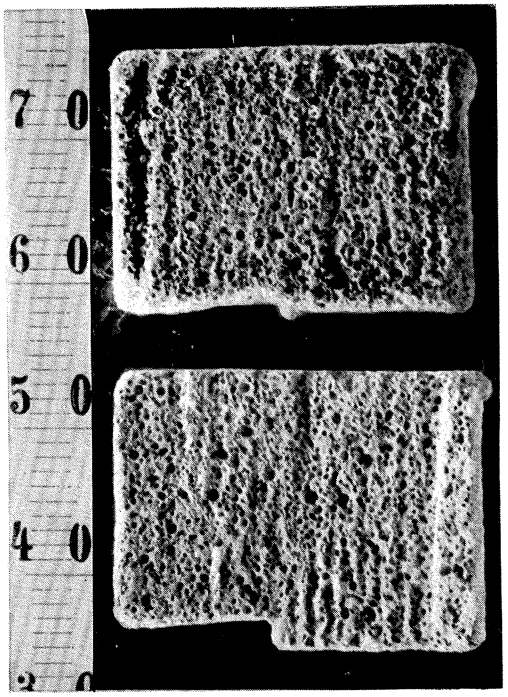


Fig. 18



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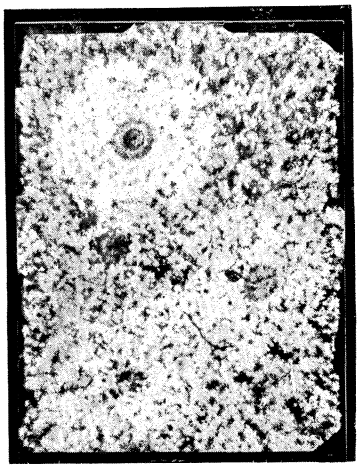

Fig. 12

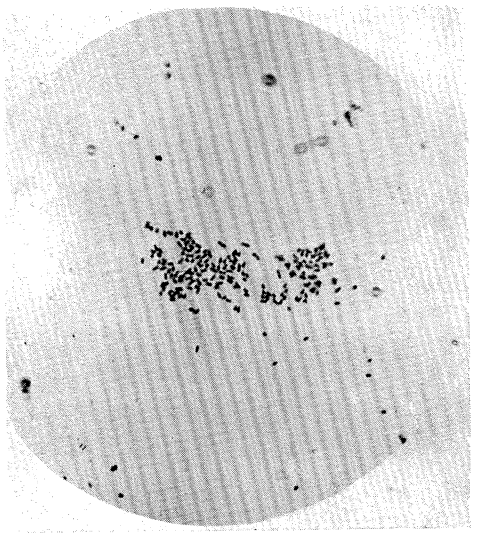

Fig. 19

PLATE 20.

FIG. 11.—A petri dish culture of the sulphur-oxidizing organism on silica jelly containing thiosulphate and precipitated chalk. Each colony of the organism is surrounded by a space in which the calcium carbonate has been dissolved by sulphuric acid produced by the organism.

FIG. 12.—Part of the same plate magnified. The colony possesses a dual structure, an outer transparent mucilaginous mass enclosing a yellowish-brown cyst-like core.

FIG. 13.—The central mass more strongly magnified showing the yellow colour to be due to granules of sulphur.

FIG. 14.—The sulphur-oxidizing organism magnified 1750 diameters photographed unstained by ultra-violet light of wave-length $\lambda = 0{\cdot}275\mu$.

FIG. 15.—The solvent action on a block. A. of Corsham Down limestone brought about by immersion of the stone in a liquid culture of the sulphur-oxidizing organism in sodium thiosulphate solution. B. the control uninoculated block for comparison of its edges. The scale divisions are millimetres.

FIG. 16.—Result of inoculation of the surface of limestone with the sulphur-oxidizing organism fed by a slow stream of thiosulphate culture fluid. A mucilaginous film formed a blister on drying and in doing so tore oölites from the surface of the stone.

FIG. 17.—The blister removed and turned over to show oölites on the underside.

FIG. 18.—Result of immersion of Corsham Down limestone in a culture solution containing ammonium sulphate inoculated with nitrifying bacteria isolated from stone. A, experimental. B, control. The scale divisions are millimetres.

FIG. 19.—The nitrifying bacteria, believed to be *Nitrosomonas europœa*, isolated from decaying stone and used in the above experiment. Fuchsin stained preparation, photographed with a Wratten B, green light filter using a $\frac{1}{15}$ objective and No. 8 ocular.

The photographs marked † are published with the kind permission of the Controller of H.M. Stationery Office.

Fig. 3

PLATE 16.

†FIG. 3.—Church Anston dolomite, Commons' Outer Court, Houses of Parliament, Palace of Westminster. Decay appears to have spread like a disease from an initial point of origin. This doorway was blocked up in 1913 and at that time no decay was noticeable.

Fig. 4

Fig. 5

PLATE 17.

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†FIG. 5.—Church Anston dolomite. Terrace, the Palace of Westminster. A strictly localized constellation of six blisters.

Fig. 6

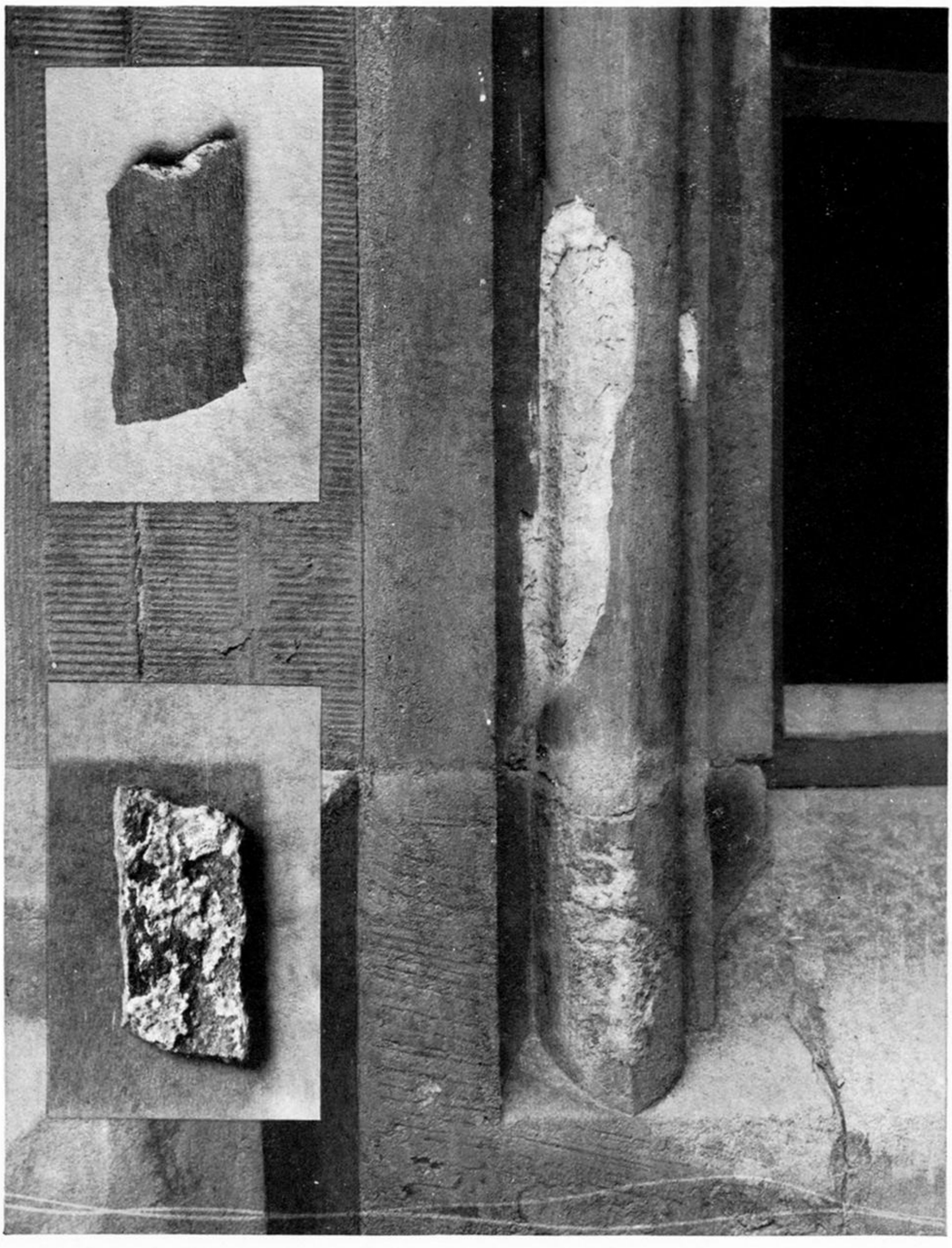


Fig. 8 Inset above.
Fig. 9 Inset below.

Fig. 7

PLATE 18.

FIG. 6.—Ferruginous sandstone. Left side of entrance, Kelso Abbey. An isolated stone deeply excavated as though by the sea—the cavernous depressions are from 2 to 3 inches deep and the white felt of crystals consists of calcium sulphate.

†FIG. 7.—Anstone stone ? Mullion of window on the Terrace, Palace of Westminster. The mass of crystals of sulphates seen at the top of the decayed area was $\frac{3}{16}$ of an inch thick, and was lying under the surface at a depth of 3 mm.

FIG. 8.—A piece of the stone removed from this mullion, no mark of decay is obvious on the outside, but just under the soot is a surface skin of sulphate (not visible in the figure) as described by SCHAFFER (1931). The fragment was split off by a fracture running parallel with the outer surface at a distance of 3 mm. from it.

FIG. 9.—The underside of this fragment exhibiting masses of crystalline sulphates.

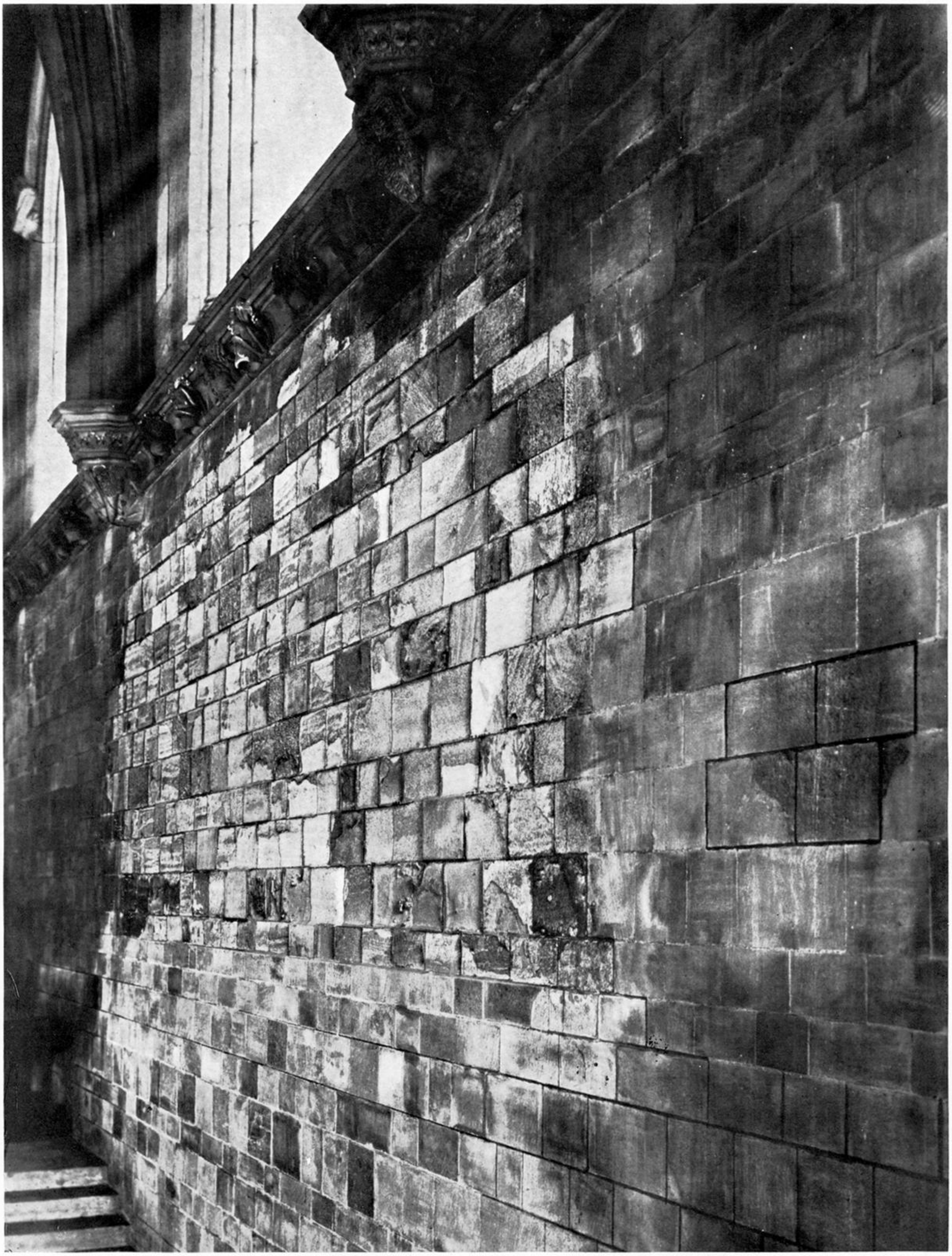


Fig. 10

PLATE 19.

†FIG. 10.—Ketton stone, limestone. North side of Westminster Hall, Palace of Westminster. One of three large decaying areas developed on the length of this wall—the stone is stained brown with iron oxide, and flakes from the surface as large as 5 inches square separate through the development of a fissure parallel to the surface at a depth of 2 to 3 mm. The decay seems slow as judged by the change seen in photographs at three yearly intervals, yet oölites and flakes are continually falling and may be swept up daily. The stone flakes are free from heterotrophic and nitrifying bacteria but an organism of the thionic-acid group capable of producing sulphuric acid from lower sulphur compounds is associated with sulphate crystals.

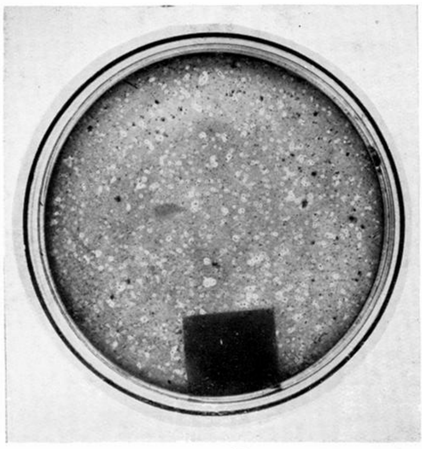


Fig. 11

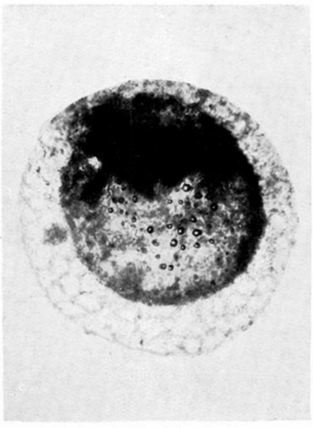


Fig. 13

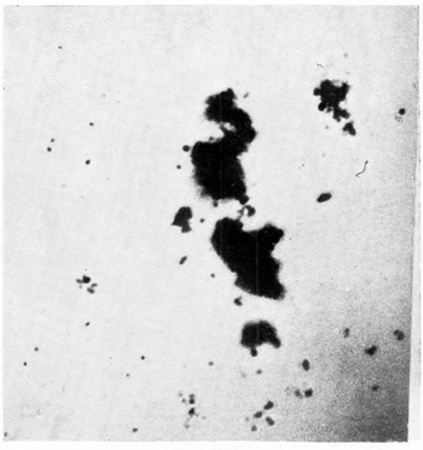


Fig. 14

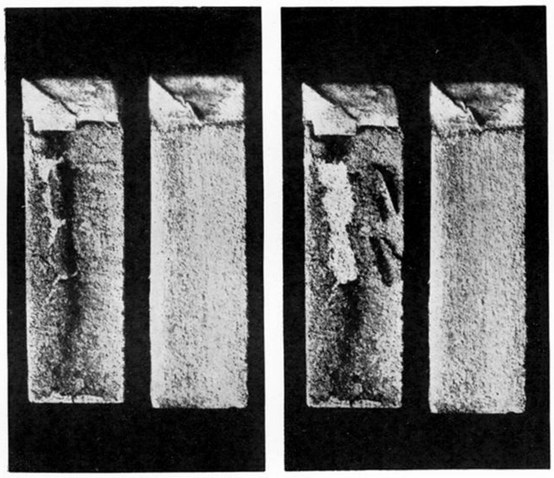


Fig. 16

Fig. 17

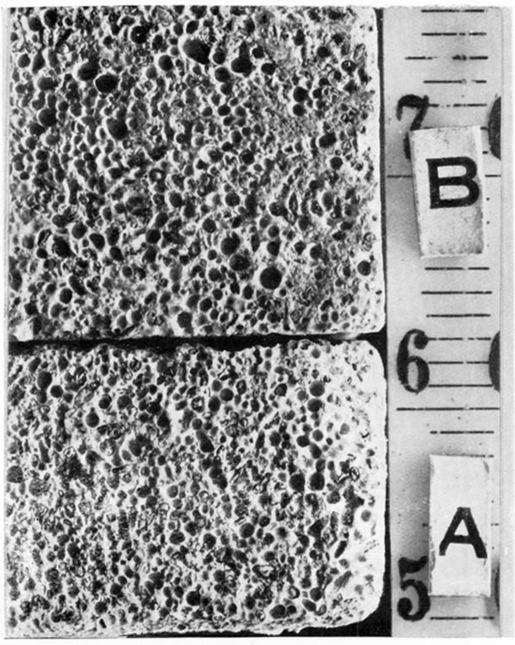
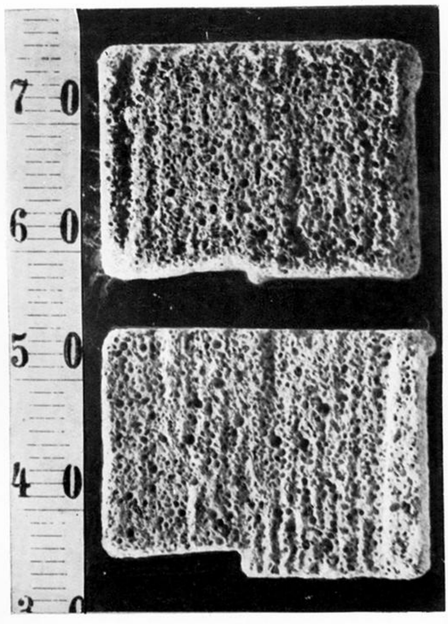
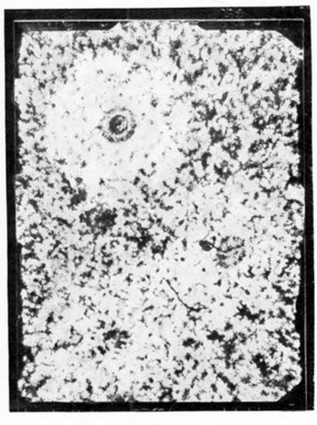


Fig. 18





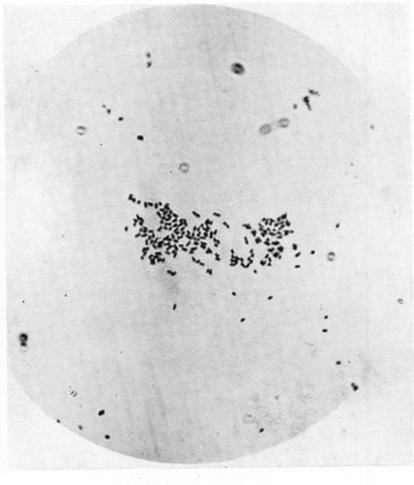


Fig. 15

Fig. 12

Fig. 19

Plate 20.

Fig. 11.—A petri dish culture of the sulphur-oxidizing organism on silica jelly containing thiosulphate and precipitated chalk. Each colony of the organism is surrounded by a space in which the calcium carbonate has been dissolved by sulphuric acid produced by the organism.

Fig. 12.—Part of the same plate magnified. The colony possesses a dual structure, an outer transparent mucilaginous mass enclosing a yellowish-brown cyst-like core.

Fig. 13.—The central mass more strongly magnified showing the yellow colour to be due to granules of sulphur.

Fig. 14.—The sulphur-oxidizing organism magnified 1750 diameters photographed unstained by ultra-violet light of wave-length $\lambda = 0{\cdot}275\mu$.

Fig. 15.—The solvent action on a block. A. of Corsham Down limestone brought about by immersion of the stone in a liquid culture of the sulphur-oxidizing organism in sodium thiosulphate solution. B. the control uninoculated block for comparison of its edges. The scale divisions are millimetres.

Fig. 16.—Result of inoculation of the surface of limestone with the sulphur-oxidizing organism fed by a slow stream of thiosulphate culture fluid. A mucilaginous film formed a blister on drying and in doing so tore oölites from the surface of the stone.

Fig. 17.—The blister removed and turned over to show oölites on the underside.

Fig. 18.—Result of immersion of Corsham Down limestone in a culture solution containing ammonium sulphate inoculated with nitrifying bacteria isolated from stone. A, experimental. B, control. The scale divisions are millimetres.

Fig. 19.—The nitrifying bacteria, believed to be *Nitrosomonas europœa*, isolated from decaying stone and used in the above experiment. Fuchsin stained preparation, photographed with a Wratten B, green light filter using a $\frac{1}{15}$ objective and No. 8 ocular.

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