Section of Odontology.

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Circulation of Lymph in the Dentinal Tubules with some Observations on the Metabolism of the Dentine.

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PART I.

The Flow of Lymph along the Tubules and its Return to the Pulp.

In a preliminary report read before this Section last year,[1] it was described how substances injected either intravenously or subcutaneously into the body had been recognized subsequently in the dentine. Trypan blue, detected by its colour, and iron solution for which special tests were used, were found in the middle of the dentine within an hour or less of their injection into the blood-stream. When the dye was given in repeated weekly doses so that the tissues were kept saturated, the

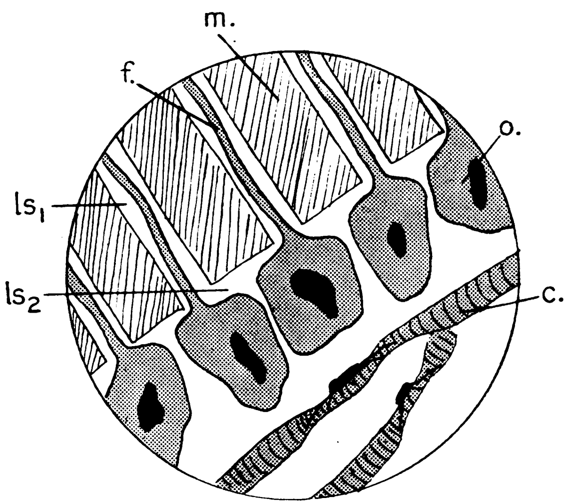

Fig. 1.—Schematic diagram to illustrate the observations that have been made on the circulation of lymph in the dentine. *o*, odontoblast; *f*, fibril; *m*, matrix substance of dentine; ls_1, lymph space in tubule; ls_2, subdentinal lymph space; *c*, capillary with Rouget cell.

dentine was quite deeply stained; but it could not be determined whether the colouring matter was carried into the dentine by an active circulation of body fluids along the tubules, or merely by diffusion along the cytoplasm of the dentinal fibril.

If it be assumed for the moment that an active circulation of lymph occurs, then one would expect to find, as in fig. 1, a lymph space between the odontoblast layer

[1] E. W. Fish, "Circulation of Lymph in the Dentine," *Proc. Roy. Soc. Med.*, 1925, vol. xviii (Sect. Odont.), pp. 35-37.

and the dentine, around the roots of the dentinal fibrils; and that this space would be continued up around each fibril in its tubule. If this supposition be correct, it should be possible to introduce a substance into the dental pulp, whence it would be carried into what we may call the subdentinal lymph space between the odontoblasts and the dentine, and from there into the tubules by the lymph stream.

In order to prove, however, that the foreign substance introduced is actually carried into the tubules by lymph circulation and not by simple diffusion, it is essential to use a non-diffusible substance such as indian ink, which consists of particles of insoluble pigment suspended in a fluid medium. This is necessary, because if the experiment be performed, as it has been, with methylene blue, neutral red, or similar soluble dye, the dye rapidly appears in the tubules; but it is difficult to say whether it is in the fibrils or outside them in the supposed lymph space. On the other hand, when indian ink is used, the finely divided particles of pigment cannot penetrate the cell membrane of the odontoblasts, and would not diffuse along the fibril even if they did penetrate the cells. When, therefore, these granules appear in the tubule, they must be lying in a space outside the fibril, and must have been carried there by a movement in the fluid contents of that space.

The following experiment was accordingly devised. A young dog of a large breed was selected in order to give as great a ratio as possible between the size of the pulp and that of the hypodermic needle used to introduce the ink, thereby minimizing the disturbance of the pulp elements by the needle. The animal was anæsthetized with chloroform and ether-chloralose sequence, and part of the crown of one of the

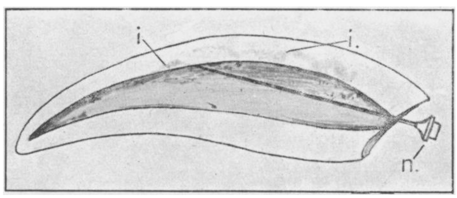

FIG. 2.—Drawing of canine of dog cut longitudinally. *i*, indian ink in substance of dentine; *n*, needle *in situ*. When the experiment is being made the ink is allowed to leak out as the needle is being withdrawn.

canine teeth was sawn off to expose the pulp. A syringe was filled with indian ink and fitted with the finest ordinary hypodermic needle. The needle was introduced along the wall of the pulp chamber to about half-way down the root, and while it was being slowly withdrawn ink was allowed to leak out of it. It has been found important to use no pressure whatever, since if one does the pulp is torn from the dentine and circulation in that tissue immediately ceases. Various inks have been used, and ink diluted with saline, but the most constant results are obtained with Reeves' waterproof ink. After the injection it is not necessary to seal the pulp chamber, but the dog should be left for an hour or so before being killed. The tooth may then be cut in half, when the ink will be found to have penetrated into the dentine, as shown in fig. 2. Fig. 2 also shows the position of the needle before being withdrawn.

When one examines sections of such a tooth microscopically, it is seen that where the most massive deposit of ink occurs very little of it is carried into the dentine, since the normal circulation has been interfered with too seriously at this point. A little further along, however, where the odontoblasts are undisturbed, but where ink has infiltrated between the odontoblasts and the dentine, the particles of ink have also been carried into and along the tubules. In fig. 3 a collection of ink is seen in the subdentinal lymph space, and the granules have entered a number of tubules,

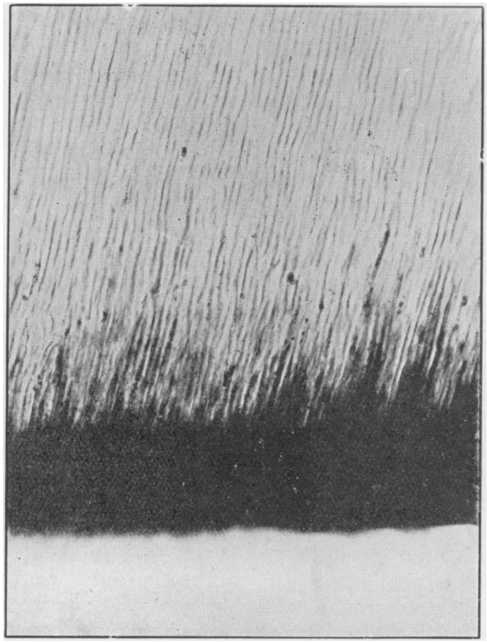

FIG. 3.—Photomicrograph. Indian ink in the subdentinal lymph space. The deposit of ink is so massive that while it has entered many of the tubules it has not penetrated very far. (1/6 in. objective.)

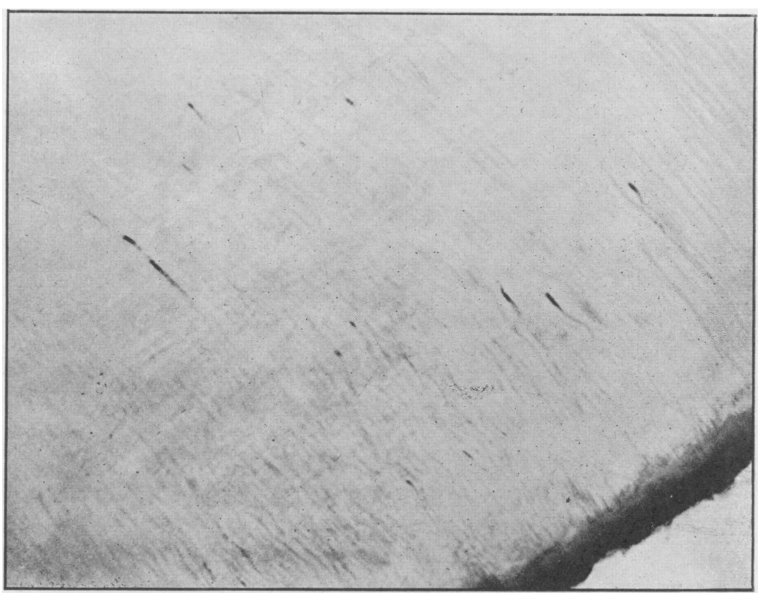

FIG. 4.—Photomicrograph. The indian ink has been carried further along the tubules in this section and can be seen in the middle third of the dentine. The ink was introduced into the pulp one hour before the animal was killed. (1/6 in. objective.)

but have not penetrated very far along them. The larger granules of ink have apparently blocked the way, and the lymph flow has ceased. This is due to the fact that the photograph displays a part of the dentine near the site where the ink was introduced. In other parts of the dentine, where there is less disturbance of the pulp elements, the ink is seen, as in fig. 4, to have been carried very much further along the tubules. In view of these findings, it became evident that there must be a lymph stream along the tubules in the space around the fibrils, and fortunately it was possible to demonstrate this perfectly clearly in transverse sections of the ink-bearing tubules where the particles of ink are invariably confined to the periphery of each tubule. Sometimes a large granule will displace the fibril to one side, and a crescent of ink presents. In other cases the granules are smaller and are disposed peripherally, showing as circles surrounding a clear centre, which is the fibril (fig. 5).

The next point was to observe the depth to which the ink was carried into the dentine, and to do this a large number of teeth had to be examined after ink had been introduced into their pulp chambers. It was possible to trace the ink two-thirds of the way across the dentine from the pulp, a distance which had been traversed in the course of an hour. This has been repeatedly observed, but it has not been possible to find it carried through to the cement or enamel junction, although a modification of technique, now to be described, was used in an attempt to demonstrate complete penetration of the dentine with ink. The modification consisted in leaving the ink in the pulp for twenty-four hours, and at the same time producing hyperæmia of the pulp with a trace of arsenic. A large young dog was put under chloroform and ether, part of the canine crown was sawn off, and ink infused as before. A fragment of arsenic fibre was then placed in the orifice of the pulp chamber, and the cavity was sealed up with cement. The dog was allowed to recover consciousness, and twenty-four hours later was killed.

In sections of such teeth (fig. 6) the ink is found to have penetrated two-thirds of the way across the dentine from the pulp to the surface, in several places. The granules also appear finer, or rather the coarser ones have not travelled down the pulp to that part of the dentine where this deep penetration is observed. The most interesting feature of these sections, however, is the amount of blood-pigment that appears in the tubules. The hæmorrhage which results from the operation, and perhaps from congestion due to the arsenic, is followed by hæmolysis, and the lymph is now tinged with hæmoglobin. It can, therefore, be traced to the most peripheral part of the dentine, but the colour becomes fainter and fainter as we pass outwards from the pulp. At first ink granules appear here and there in a red fluid in the tubules, then the ink ceases and the fluid is only faintly pink; then, just as we approach the cement or enamel, it is impossible to be sure that there is any pink colour left at all. If such a section be now stained with potassium ferrocyanide the hæmoglobin turns blue by virtue of its iron content, and can be seen in places to extend right up to the dentine-cement junction, where a marked accumulation is observed as a blue line just at the margin of the dentine.

This observation suggested a different method of investigation, as it seemed unlikely that anything further was to be learnt from the use of indian ink. The ink method had, however, served a useful purpose in that these experiments offer a marked contrast to those of other investigators who worked upon extracted teeth, introducing indian ink into the pulp chamber after removing the pulp, and subjecting the ink to a pressure of from five to ten atmospheres for twenty-four hours. By this means ink granules were forced into the tubules, but the experiments just described show that there exists a stream of lymph along the tubules during life of such a character and volume that ink granules are carried by it into remote parts of the dentine within so short a space of time as one hour. The tubules may, therefore, be looked upon as lymph channels, and by means of the circulation of the lymph in them, nutrient materials, oxygen and immune bodies, are transported to the living

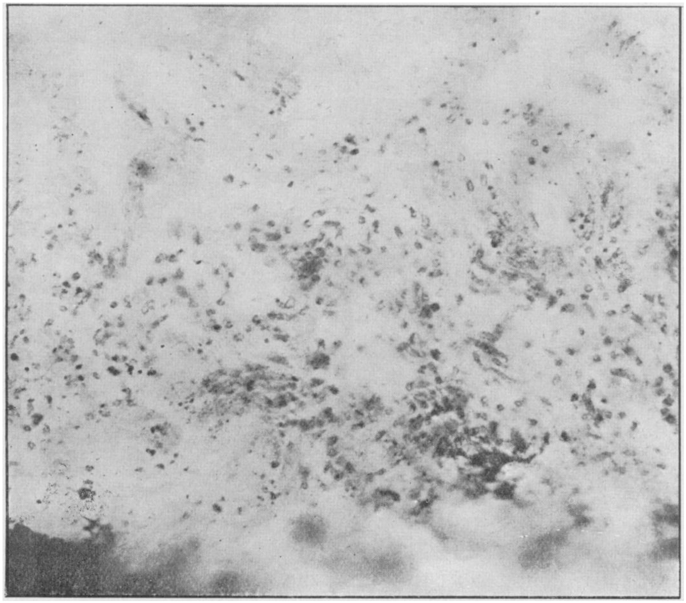

FIG. 5.—Photomicrograph. Transverse section of tubules containing indian ink. The granules are seen placed peripherally round the fibril in the tubule. The fibril appears as a clear centre surrounded by a ring of ink. In some tubules the fibril is displaced eccentrically and the ink shows as a crescent. (1/6 in. objective.)

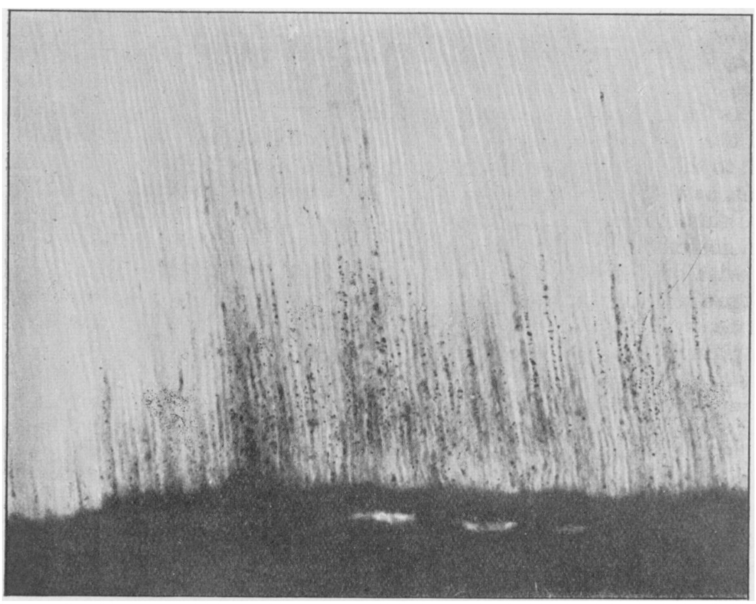

FIG. 6.—Photomicrograph. Section of dentine from canine of dog. Indian ink and As_2O_3 placed in pulp twenty-four hours before animal was killed. The hæmoglobin-stained lymph shows grey with black granules of ink at intervals along the tubules. (1/6 in. objective.)

dentine, and the products of its katabolism are carried away. It appears that the fibrils of the odontoblasts which lie in these lymph channels exert a controlling influence upon the metabolic processes in the dentine.

It was therefore decided, at this point, to discard indian ink as an indicator, and to attempt to trace further the course of the circulation by means of a reagent which did not contain suspended particles, and which, therefore, would not interrupt the lymph flow by blocking the tubules. For this purpose a solution of ferrous ammonium citrate 0·5 per cent. was introduced into the pulp and the animal was killed an hour later. The tooth was placed in formalin solution to fix the dentine and then decalcified. The iron solution became mixed with the lymph in the pulp and was carried along the tubules as the lymph circulated, during the time which elapsed between its introduction and the animal being killed. It was, therefore, only necessary to immerse a section of the tooth in potassium ferrocyanide solution, thereby producing the Prussian-blue reaction to render the lymph visible as a dark, blue fluid throughout the whole of its course.

It now became evident that the lymph penetrates all the tubules of the dentine right up to the cement margin, and where a tubule is cut in transverse section the lymph shows as a blue ring surrounding the fibril precisely as the ink had done.

The most striking feature of these sections, and one that appears in all of them, is a marked accumulation of lymph in a zone just at the periphery of the dentine, both in the crown and in the root. This shows, under a low power, as a narrow blue band immediately under the granular layer of Tomes (fig. 7). Under a higher power (fig. 8) it is evident that this blue zone corresponds to the area in which the terminal branches of the tubules occur. These branches can be seen very clearly, charged with lymph, while between them and the cement in most places is the granular layer of Tomes free from lymph. The branches form a plexus into which the tubules pour their contents, thereby producing the blue zone which is such a striking and constant feature of these sections. From this plexus the greater part of the lymph returns to the pulp along neighbouring tubules. There can be no doubt, however, that some of the tubules reach the cement margin, and it may be that the spaces of the granular layer of Tomes are lymph spaces.

In the coronal part of the tooth, dentinal tubules have been described by many workers as entering the enamel, and the present writer has, in the last few days observed the penetration of lymph into the cervical part of the enamel; but it is not proposed to discuss the movements of the lymph outside the confines of the dentine at present, as they have not been completely worked out.

The ultimate fate of the lymph which returns to the pulp from the tubules is clearly indicated in fig. 9. Here are seen numerous small lymphatic vessels heavily charged with indian ink which had been introduced into the tissue of the pulp an hour before the animal was killed. These vessels are seen to enter channels through the dentine in its apical portion, and in this way to make their escape from the pulp chamber. There also appear to be perivascular lymph spaces around the blood-vessels, where they lie in the apical canals of the dentine.

It now remains to discuss the mechanism by which this movement of lymph in the tubules is maintained, and it will be seen at once that this circulation differs materially from lymph movement in some other tissues in that it is so isolated that the action of the neighbouring muscles can exert no influence over it. The lymph must, therefore, gain its motive power entirely from the blood-pressure. A. W. Wellings[1] has described the occurrence of contractile Rouget cells on the capillary walls, in the network of vessels which appears at the surface of the pulp, immediately below the odontoblast layer. These cells, by alternately contracting and expanding the calibre of the capillaries, would be capable of causing a pumping action on the

[1] A. W. Wellings, "Some Points in the Anatomy of the Capillary of the Tooth Pulp," *Proc. Roy. Soc. Med.*, 1926, xix (Sect. of Odont.), p. 27.

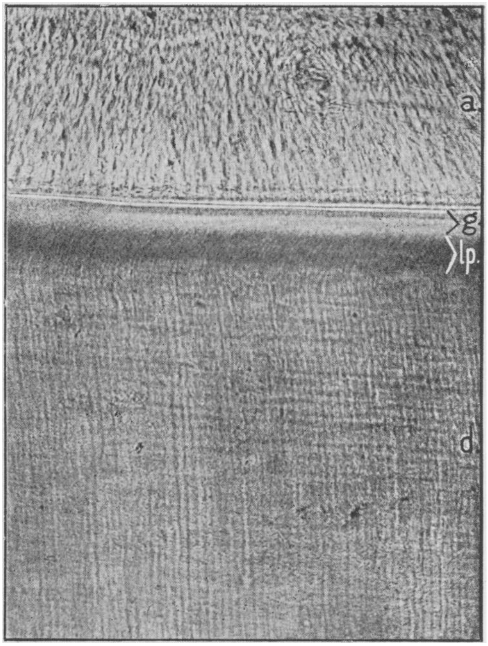

FIG. 7.—Photomicrograph. L.S. canine of dog. Ferri et ammon. cit. solution introduced into pulp one hour before killing animal. Section immersed in potassium ferrocyanide and HCl. Photographed through a red screen, hence the shading represents the depth of the blue colour which in turn indicates the presence of lymph carrying the iron solution. The lymph plexus is seen as a dark band immediately under the granular layer of Tomes, which is relatively free from lymph. *a.*, alveolus ; *g.*, granular layer of Tomes ; *lp.*, lymph plexus ; *d.*, dentine. (2/3 in. objective.)

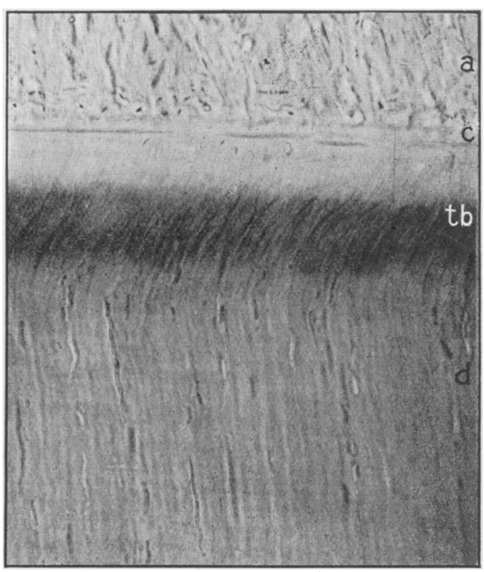

FIG. 8.—Photomicrograph. L.S. canine of dog. Prepared and photographed as in fig. 7. This section shows the accumulation of lymph in the lymph plexus, which is seen to be formed of the terminal branches of the tubules. *a.*, alveolus; *c.*, cement ; *tb.*, terminal branches of the tubules; *d.*, dentine. (1/6 in. objective.)

tubules. It is difficult to see what other function these cells could have, while on the other hand they appear to be perfectly adapted to serve this special purpose, particularly as their action is controlled and regulated by the nervous system. The circulation would, therefore, be in the nature of an "ebb and flow," and would not always be in the same direction in a given tubule.

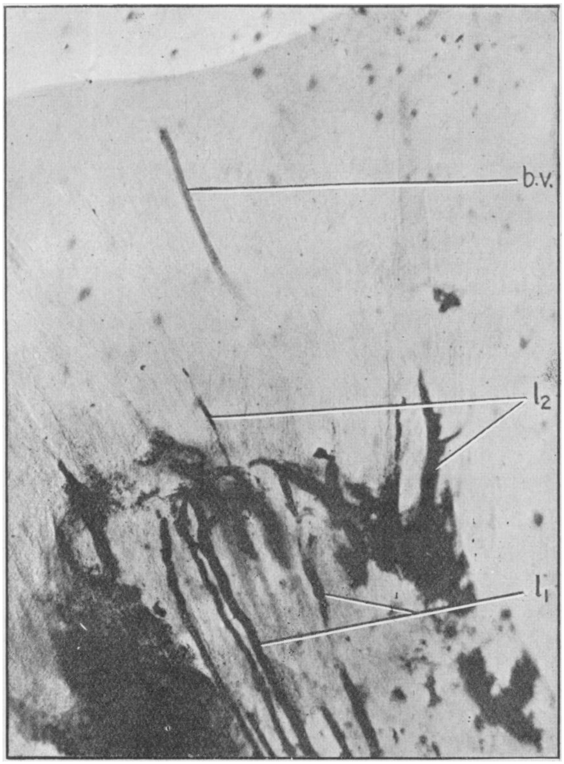

FIG. 9.—Photomicrograph. Longitudinal section of canine of dog, apical portion. Indian ink introduced one hour before animal was killed. l_1, lymphatic vessels in pulp; l_2, lymphatic vessels in canals in dentine; *b.v.*, blood-vessel in canal in dentine. (1 in. objective.)

PART II.

THE CALCIUM CONTENT OF DENTINE: PHYSIOLOGICAL AND PATHOLOGICAL VARIATIONS.

In the light of the observations recorded in Part I, it seemed natural to inquire into the purposes which the lymphatic circulation serves in the metabolic processes of the dentine, As a working basis we may perhaps suppose that the fibril exerts a controlling action on the metabolism of this tissue, while the lymph supplies the necessary materials and carries away the waste products. For the purpose of a preliminary investigation calcium was selected as the particular constituent of the dentine upon which to make determinations, and a fairly large series of analyses have now been made.

Estimation of Calcium: Method.—Specimens of dentine were prepared on a uniform plan from a number of human teeth extracted in dental practice. A transverse section of the tooth was taken, about 2 mm. thick, from the cervical region. This was mechanically freed from cementum and enamel and the pulp canal was cleaned out, leaving a ring of dentine comprising the outer and therefore oldest part of the dentine in each case. The specimen was then dried at 105 ° C. for eighteen hours and weighed into a silica crucible. This was taken as the dry weight of the tooth, upon which percentage calculations were based, and does not vary appreciably however long the tooth is dried. To determine this a tooth was dried and weighed at intervals, thus :—

Weight after 18 hours drying	0·2217 grm.
,, ,, additional 6 hours drying ...	0·2211 ,,
,, ,, ,, 16 ,, ,, ...	0·2214 ,,
,, ,, ,, 3 days ,, ...	0·2213 ,,

After being weighed the tooth was incinerated to a clean white ash. To the ash was added 10 c.c. phosphoric acid solution (1 vol. syrupy H_3PO_4 to 3 vols. H_2O), and on warming the ash completely dissolved. The solution was washed out into a beaker with 50-100 c.c. of water, and 50 c.c. of a solution of oxalic acid containing 37·8 grm. oxalic acid per litre was added. To effect complete precipitation an equivalent quantity of ammonia was added (50 c.c. of solution containing 10·2 grm. NH_3 per litre). The precipitated solution was allowed to stand for a few hours and was then filtered through a sugar tube prepared with asbestos. The precipitate was washed seven times with hot water to remove all ammonium oxalate and the precipitate and the asbestos transferred to a beaker. Ten c.c. H_2SO_4 solution (1 vol. H_2SO_4 to 1 vol. H_2O) were added and the solution heated nearly to boiling. It was then titrated with N/10 potassium permanganate solution. A titration was performed on an equal quantity of asbestos and H_2SO_4 as a small quantity of $KMnO_4$ is absorbed.

Calculation.—One c.c. N/10 $KMnO_4$ is equivalent to 0·0028 grm. CaO. The titration was therefore multiplied by 0·0028, the result being the amount of CaO in the tooth taken. The percentage was obtained by multiplying by 100 and dividing by the dry weight of the tooth. To calculate the percentage of calcium salts contained in the dentine it may be assumed that they occur as nine parts of calcium hydroxyapatite to one part of calcium carbonate, hence 1 per cent. of calcium oxide is equivalent to 1·82 per cent. of the mixed salts.

It was found that the specimens lost from 8 to 10 per cent. of water during the drying process. It was noticed that the percentage of ash obtained from the dentine was no criterion of the amount of calcium contained in it. This would account for the somewhat conflicting results obtained by certain earlier investigators.

The results of these analyses are tabulated below in four groups: premolars, molars, canines and incisors. The specimens in each group are arranged according to age.

PREMOLARS.

No.	Sex	Age	CaO per cent.		Notes
1	F	9	35·5	|4	
2	F	9	37·15	|4	
3	F	10	36·9	4|	
4	M	11	35·76	4|	Open apex
5	M	11	36·63	4|	
6	F	12	37·05	4|	
7	F	13	36·78	|5	
8	F	13	37·52	4|	
9	F	13	36·37	|4	
10	F	13	37·35	4|	
11	F	14	37·19	5|	
12	M	15	37·99	5|	
13	M	18	38·08	|4	Caries
14	F	19	36·36	5|	,,
15	F	20	37·47	|4	,,
16	F	22	38·51	|5	,,
17	M	23	36·68	4|	,,

No.	Sex	Age	CaO per cent.		Notes
			PREMOLARS.		
18	F	30	39·02	5|	
19	F	30	38·92	|5	Same patient
20	F	30	39·09	|5	
21	F	30	38·20	5|	
22	F	35	36 73	|5	
23	M	35	37·97	4|	
24	F	35	38·09	5|	Same patient
25	F	35	38·77	5|	
26	F	38	38·19	|5	
27	F	40	37·32	5|	
28	F	40	38·19	5|	Same patient
29	F	40	38·20	|5	
30	F	45	36·96	|5	
31	F	45	38·9	|4	Pyorrhœa
32	F	46	38·18	|5	Very translucent
33	F	60	38·64	|5	Pulp calcified, but cut away before analysis
34	F	62	38·87	5|	,, ,, ,,
35	F	70	38·54	|4	
			CANINES.		
36	F	25	38·49	|3	
37	M	27	37·23	3|	
38	F	50	41·05	|3	
39	M	59	38·7	3|	Very firm tooth
40	M	59	39·4	3|	
41	F	60	36·5	3|	Pyorrhœa, loose
42	F	70	34·68	|3	
43	F	70	38·68	|3	
44	M	71	37·6	|3	
			MOLARS.		
45	F	11	37·18	|6	Caries
46	M	16	37·77	6|	,,
47	M	20	38·5	|6	,,
48	F	21	40·09	|6	,,
49	M	23	36·40	6|	,,
50	M	25	38·04	|8	,,
51	M	25	37·49	|7	Marked absorption of roots. Caries
52	F	25	38·66	|6	
53	F	26	37·37	6|	Patient neurotic. Endocrine disturbance (?) Caries
54	F	27	37·20	|6	Exostosis. Caries
55	M	29	38·9	|7	Caries
56	F	35	38·70	7|	Eight months pregnant. Caries
57	M	38	38·4	|6	
58	F	55	36·50	|6	Exostosis
59	F	56	37·93	|8	Pyorrhœa. Tooth loose
60	M	60	38·10	6|	Marked attrition
			INCISORS.		
61	F	50	38·3	|2	Root had been crowned for twenty-three years. Good condition
62	M	62	34·30	1|	Loose. Tooth fell out
63	F	65	37·78	|2	Very translucent
64	F	70	37·80	|2	Pulp calcified

An examination of the calcium content of these specimens indicates that the composition of the dentine is not constant. The maximum difference is between Nos. 38 and 62, where the latter contains 6·75 per cent. CaO less than the former. The difference is thus 20 per cent. of the total amount of calcium in the less highly calcified tooth. This finding was not unexpected in view of the extensive irrigation with lymph that the dentine has just been shown to undergo.

The most important positive conclusion that may be drawn is that in a newly-erupted tooth the dentine is not fully calcified, and that for a few years, at any rate, after its complete formation calcium is constantly being carried into the dentine. The average percentage of calcium oxide, and therefore of calcium salts in the premolars, is appreciably lower during the ages 9 to 14 than is the average in premolars from patients of 30 to 45 years of age.

Age of patient	Average per cent. CaO	Average per cent. Ca salts
9-14	36·75	66·86
30-45	38·33	69·76

There is, therefore, in the first few years after the eruption of a tooth an increase of calcium salts in the dentine of the order of 5 per cent. of the amount originally laid down at the time of calcification.

Very little indication of a normal physiological reduction in the calcium content of dentine in old age is available, although No. 42 shows a very low reading. Similarly, teeth with pyorrhœa which show great translucency have a normal calcium content, though here again No. 62 is a marked exception. We may perhaps say that there appears to be no insurmountable obstacle in the way of calcium being removed from the dentine, though it does not seem to occur in a high percentage of cases.

The average CaO percentage in badly carious teeth does not appear to be specially low. The carious premolars have an average of 37·42 per cent. CaO, but they are all in the 18-23 age-group, which is intermediate in age between the two groups already summarized. The carious molars also have a normal content of calcium. It may be mentioned in this connexion that the calcium content of the dentine in dogs is appreciably lower than that in human beings, although the dog is naturally immune from dental caries. The table also shows that although young human teeth, which are known to be particularly susceptible to caries, have a low calcium content, yet Nos. 42 and 62, which were taken from the teeth of older people, free from any indication of caries, have the lowest percentage in the whole series. There would therefore appear to be no direct relationship between dental caries and the calcium content of the dentine.

A few general observations may be made: The analyses establish a reliable estimate of the calcium content of dentine, especially as specimens have been analysed in duplicate and have agreed to within ± 0·02 per cent. CaO. Nos. 18 and 19 and Nos. 28 and 29 suggest that symmetrical teeth have an almost identical calcium content, though Nos. 20 and 21 do not bear this out. There does not appear to be any great difference between the calcium content of the different groups of teeth. Adult premolars, molars, canines and incisors all show much the same percentage of lime salts. The only specimen of dentine from a patient in the later months of pregnancy exhibited no deviation from the normal in its calcium content.

PART III.

Preliminary Note on Experimental Procedures leading to Changes in the Calcium Content of Dentine.

When it became apparent that changes occurred in the calcium content of dentine, it was thought to be of interest to attempt to induce modifications experimentally. In recent years much has been said of the influence of the parathyroid on calcium metabolism. Though the interpretations placed by MacCallum on his experimental findings have been freely criticized, yet it has been shown by Erdheim, and later by

Fleischmann, that the parathyroids exerted a definite influence on the calcification of *forming* dentine. It was considered of importance therefore to observe the influence of parathyroidectomy upon dentine which was *already formed*, since it seemed possible that the operation might cause a reduction in its calcium content. A number of cats were accordingly subjected to partial parathyroidectomy. In the experiments tabulated below, the cats used were as follows:—

Cat 1 was from the same litter as Cat 2. The cats were identical in appearance except for a patch of white in the coat. A third cat of the same litter had to be destroyed because it developed tetany four days after the operation—apparently the whole of the parathyroid tissue had been removed. These three cats were about eight months old and their dentine would normally show a slow increase in calcium from time to time (see control cat—Cat 1). Cat 3 was an older cat. The pulp chamber was reduced to the small dimensions of a fully adult cat, and the calcium content of the dentine would not vary appreciably under normal circumstances in a period of two months (the duration of the experiment). All the cats were females. The following experiments were performed. Cat 1 had a lower canine extracted and two months later was killed and the other canine was extracted. Cat 2 had a lower canine extracted and three of the parathyroid glands were removed and identified microscopically. The cat was kept in the same cage as Cat 1, on normal diet, and two months later was killed and the other lower canine was extracted. Cat 3 had a lower canine extracted and three parathyroids removed and identified microscopically. Two months later the cat was killed and the other canine removed. All three cats recovered from the operation without incident and appeared to be in perfect health throughout the experiment.

On analysis the calcium content of the dentine was as follows:—

Cat No.	Operation	Per cent. CaO in dentine at beginning of experiment	Per cent. CaO in dentine at end of experiment	Difference Per cent. CaO	Difference Per cent. CaO salts
1	Control	35·96	36·25	+ 0·29	+ 0·53
2	Partial parathyroidectomy	37·14	36·65	− 0·49	− 0·89
3	Partial parathyroidectomy	38·35	37·48	− 0·87	− 1·58

It will be seen that there was a loss of calcium in both cats upon which partial parathryoidectomy had been performed, while Cat 1, which was a close control of Cat 2, showed the normal increase in the calcium content of the dentine. Cat 3 might have been expected to remain constant apart from the parathyroidectomy.

A second series of experiments was performed to observe the effect of pregnancy and calcium-deficient diet upon the calcium content of adult dentine. Four different types of operation by way of experiment or control were undertaken. In each case young adult bitches were used, the pregnant ones were primiparæ. The first control was to compare the calcium content of the two upper canines extracted at the same time from one of the bitches; this was the same animal as was used for Experiment V.

			Percentage CaO	Difference Percentage CaO	Difference Percentage Ca-salts
Experiment I	C|	extracted at the same time	35·00	+ 0·08	+ 0·15
	|C		35·08		

The next control was to compare the calcium content of the lower canines of a young adult non-pregnant bitch at an interval of two months (normal duration of pregnancy) on ordinary diet:—

			Percentage CaO	Difference Percentage CaO	Difference Percentage Ca-salts
Experiment II	|C	At beginning of experiment	34·23	+ 1·14	+ 2·07
	C|	Two months later, normal diet	35·37		

The next experiment was to place a young adult non-pregnant bitch on a calcium deficient diet for two months and compare the calcium content of the dentine of the lower canines before and after.

The diet consisted of meat and polished rice boiled in distilled water. The broth was discarded and the "pith" moistened with fresh distilled water. Distilled water was given to drink with a trace of NaCl added.

			Percentage CaO	Difference Percentage CaO	Difference Percentage Ca-salts
Experiment III	$\overline{C}\vert$	At beginning of experiment	35.79	+ 0·11 ...	+ 0·20
	$\vert\overline{C}$	After Ca-deficient diet for two months	35·90		

Finally, two young adult pregnant bitches were placed on calcium free diet (as above) from the commencement of pregnancy and the calcium content of the dentine of the lower canines compared by extracting one at the commencement of pregnancy and the other at the end of pregnancy:—

			Percentage CaO	Difference Percentage CaO	Difference Percentage Ca-salts
Experiment IV	$\overline{C}\vert$	At beginning of pregnancy	35·76	+ 0·60 ...	+ 1·09
	$\vert\overline{C}$	At end of pregnancy, with Ca-deficiency	36·36		

			Percentage CaO	Difference Percentage CaO	Difference Percentage Ca-salts
Experiment V	$\vert\overline{C}$	At beginning of pregnancy	35·68	− 0·32 ...	− 0·58
	$\overline{C}\vert$	At end of pregnancy, with Ca-deficiency	35·36		

The bitches kept perfectly healthy on the diet except that one (a pregnant one) appeared rather lean at the end of pregnancy, although she fed the puppies for two weeks while still on the deficient diet, All the puppies were born alive at full term.

The observations one may make are as follows :—

(*i*) The calcium content of the symmetrical teeth extracted at the same time in Experiment I shows so little variation that it seems reasonably safe to use symmetrical teeth as controls for experiment (see also Nos. 18, 19, and 20, 21 and 28, 29 in the table of human analyses). There is of course occasionally some possibility of error here.

(*ii*) The increase of CaO percentage of the dentine in Experiment II shows that this normal increase due to lapse of time in young adult bitches must be taken into account in all experiments. (See similar increase in table of human analyses referred to above.)

(*iii*) The effect of the calcium deficient diet in Experiment III appears to be to reduce the increase in calcium which normally occurs.

(*iv*) Experiments IV and V, in which pregnant bitches were fed on a calcium free diet, may be considered separately. The animal in Experiment IV shows a definitely smaller increase in the calcium content of the dentine than the control dog in Experiment II. In Experiment V the bitch appears to have actually withdrawn calcium from its dentine. This loss is of considerable interest, in view of the increase observed in the control (Experiment II), and in view of the fact that it indicates the physiological possibility of calcium being withdrawn from the dentine under stress of calcium starvation.

In presenting these preliminary experiments on the influence of parathyroidectomy and pregnancy on the calcium content of the dentine, it is only intended to indicate the lines upon which the investigation is proceeding. The results so far obtained, however, are so definite that it was felt to be of interest to present them in conjunction with the earlier sections of this paper.

In conclusion, I wish to thank Professor D. T. Harris and Professor J. C. Drummond for their advice and help in the investigations, and to express my thanks to the Dental Board for their grant towards the expenses of this research.

SUMMARY.

(1) Indian ink introduced without pressure into the dental pulp during life is carried up the dentinal tubules into the middle third of the dentine in one hour. From this it is evident that there is a stream of lymph of considerable volume along each tubule, and transverse sections of the ink-bearing tubules make it clear that this stream occupies a space between the fibril and the wall of the tubule (figs. 3 to 6).

(2) When a solution of ferrous ammonium citrate is introduced into the pulp instead of indian ink, the solution may be traced throughout the dentine and detected by staining with potassium ferrocyanide, in a zone just under the cement where the terminal branches of the tubules occur. These branches appear to form a plexus from which the greater part of the lymph finds its way back along neighbouring tubules to the lymphatics draining the pulp (figs. 7 and 8).

(3) This circulation is presumed to be maintained by means of the controlled action of the Rouget cells alternately contracting and expanding the capillaries of the vascular network at the periphery of the pulp.

(4) Lymph vessels in the apical portion of the pulp have been demonstrated and are shown, heavily charged with ink, making their exit through the wide apical canals of the tooth (fig. 9).

(5) A series of sixty-four calcium estimations on human dentine show that the calcium content (estimated as calcium oxide) of dried dentine may vary from 34·30 per cent. to 41·05 per cent., which represents a range in the percentage of calcium salts of from 62·43 per cent. to 74·71 per cent., that is, 12·28 per cent difference, and is almost 20 per cent. of the total calcium in the less highly-calcified tooth.

(6) There is a rapid increase in the percentage of the dentine calcium during the first few years after the eruption of the tooth, due to normal physiological processes.

(7) The dentine of carious teeth, compared with that of healthy teeth, does not appear to be deficient in calcium.

(8) There appears to have been a definite loss of calcium from the dentine in isolated cases of old age and marked chronic periodontal disease, though this was not generally observed.

(9) There does not appear to be any clearly defined difference between the calcium content of the different varieties of teeth—the molars, premolars, canines, and incisors.

(10) Partial parathyroidectomy in cats appears to cause a withdrawal of calcium salts from the dentine.

(11) A calcium deficient diet in young dogs appears to arrest partially the normal increase in the calcium content of the dentine.

(12) A calcium deficient diet in young pregnant bitches appears in one case to have partially arrested the normal increase in the calcium content of the dentine and in another to have caused an actual withdrawal of calcium salts from the dentine.

Note.—The Discussion on the foregoing paper has been postponed to next Session.

Corrigendum.—In the remarks in Discussion by Mr. J. Lewin Payne (President) on Sir Frank Colyer's paper ("Abnormally Shaped Teeth from the Region of the Premaxilla"), p. 56, line 4 from bottom, and p. 57, line 18 from top, for "germinated" read "gemminated."