Section of Odontology

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[*April* 27, 1936]

An Experimental Investigation of the Lymphatic System of the Teeth and Jaws

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ABSTRACT.—A review of the literature is given, followed by a consideration of the available methods of demonstrating the lymphatic system in the area of the teeth and jaws.

A new method of demonstrating this system by the injection or application of lead acetate *intra vitam*, is described, and the technique is explained. The method can be employed to reveal macroscopic or microscopic lymph channels in any part of the body, and is especially of value where decalcification of the hard tissues has to be carried out in the preparation of the sections.

The various types of experiment which have been performed are described, and the macroscopic and microscopic results dealt with separately.

Among the macroscopic results, the lymphatic drainage of various parts of the jaws is described, and the large amount of anastomosis and cross anastomosis between the vessels is shown. A comparison of the lymphatic system in this region in the guinea-pig, cat, dog, and monkey is given, and it is demonstrated that the guinea-pig and monkey possess submental and supraclavicular lymph nodes which assist in the drainage of this area in addition to the submaxillary and cervical groups of nodes possessed by the cat and the dog.

Among the microscopic results, the way in which the mass makes its way from the gingival tissues through the bone, and is found in the pulp, dentine, and cementum of the tooth, even where no pressure is applied, is described. The communication of the lymphatic vessels of the pulp with those of the periodontal membrane and the path of the mass down the periodontal membrane from the gingival trough, and its entry into the alveolar bone from this situation are demonstrated, and the way in which the mass reaches the pulp, dentine, and cementum of the tooth from the gingival tissues is discussed.

The significance of various concentrations of the mass in the tissues, particularly the dentine, is also discussed. Control experiments are described, the conclusions which have been reached are given, and the lines on which further experiments are being continued are indicated.

Finally, the application of the results to the pathology of infection in this region, particularly paradontal disease, is given, and also their application to the phenomena of injection anæsthesia.

RÉSUMÉ.—Revue de la littérature, suivie d'un examen des méthodes qui existent pour démontrer le système lymphatique dans la région des mâchoires et des dents.

Description d'une nouvelle méthode pour démontrer ce système par l'application ou l'injection *intra vitam* d'acétate de plomb, et explication de la technique employée. Cette méthode peut servir pour montrer les canaux lymphatiques macroscopiques et microscopiques de n'importe quelle partie du corps, et elle est surtout utile quand il est nécessaire de décalcifier les tissus en préparant les sections.

Description des différentes expériences qui ont été faites, et discussion séparée des résultats macroscopiques et microscopiques.

Parmi les résultats macroscopiques l'auteur décrit le drainage lymphatique des différentes parties des mâchoires, et le grand nombre d'anastomoses entre les vaisseaux. Les systèmes lymphatiques de cette région chez le cobaye, le chat, le chien, et le singe sont comparés, et il est démontré que le cobaye et le singe possèdent des glandes sous-mentales et sus-claviculaires aidant au drainage lymphatique de cette région aussi bien que les glandes sous-maxillaires et cervicales qui se trouvent aussi chez le chien et le chat. Parmi les résultats microscopiques, l'auteur décrit comment la masse passe du tissu gingival à travers l'os, et se retrouve dans la pulpe, dans la dentine et dans le cément des dents, même sans l'emploi d'aucune pression. La communication entre les vaisseaux lymphatiques de la pulpe et ceux de la membrane périodontale, ainsi que le chemin de la masse le long de cette membrane depuis le creux gingival, et son entrée dans l'os alvéolaire depuis ce point sont démontrés, et le chemin par lequel elle pénètre dans la pulpe, dans la dentine et dans le cément est discuté. La signification des différentes concentrations de la masse dans les tissus, et surtout dans la dentine, est discutée. Description d'expériences de contrôle ; conclusions ; description du genre d'expérience qu'il est proposé de faire.

Finalement l'auteur discute l'application des résultats obtenus à la pathologie des infections de cette région, et surtout des maladies parodontales, ainsi que leur application à l'anesthésie par injection.

ZUSSAMMENFASSUNG.—Nach einer Literaturübersicht bespricht Verf. die Methoden, die zur Darstellung der Lymphbahnen des Zahn- und Kiefergebietes dienen können.

Eine neue Methode zur Darstellung dieses Systems, durch intravitale Applikation oder Injektion von Bleiacetat wird geschildert und die Technik beschrieben. Diese Methode kann zur Darstellung der makroskopischen und mikroskopischen Lymphbahnen irgendwelchen Teiles des Körpers verwendet werden, und ist besonders wertvoll, wenn die Entkalkung harten Gewebes bei der Herstellung der Schnitte notwendig ist.

Verf. beschreibt die verschiedenen Arten von Versuchen, die ausgeführt wurden und behandelt die makroskopischen und mikroskopischen Ergebnisse einzeln.

Unter den makroskopischen Ergebnissen wird die Lymph-Drainierung von verschieden en Teilen der Kiefer beschrieben. Die reichlichen Anastomosen zwischen Gefässen der gleichen Seite und mit solchen der Gegenseite werden dargestellt. Die Lymphsysteme dieses Gebietes bei Meerschweinchen, Katzen, Hunden und Affen werden verglichen, wobei es sich nachweisen lässt, dass Meerschweinchen und Affen submentale und supraklavikuläre Lymphdrüsen besitzen, die in der Drainierung dieses Gebietes helfen, ausser den sub-maxillären und cervikalen Lymphdrüsen, die sich auch bei Katzen und Hunden befinden.

Unter den mikroskopischen Ergebnissen beschreibt Verf., wie die Masse aus den gingivalen Geweben durch den Knochen gelingt, und in der Pulpa, dem Dentin und dem Zement der Zähne wiedergefunden wird, auch wenn kein Druck ausgeübt wird. Die Verbindung der Lymphgefässe der Pulpa mit denen des Periodonts, der Weg der Masse durch das Periodont vom Zahnfleischtrog aus und ihr Eintritt in den Alveolus werden dargestellt, ferner wird ihr Weg in Pulpa, Dentin und Zementum aus den gingivalen Geweben besprochen. Die Bedeutung der verschiedenen Konzentration der Masse in den Geweben, besonders im Dentin, wird besprochen. Verf. beschreibt seine Kontrollversuche, gibt die Schlüsse die er aus dieser Arbeit zieht, und weist auf die Richtung der Fortsetzung seiner Versuche hin.

Endlich bespricht er die Bedeutung dieser Ergebnisse für die Pathologie der Infektionen dieses Gebietes, insbesondere für paradentale Erkrankungen, und für die Injektions-anästhesie.

SUMMARY OF LITERATURE

Sappey in 1874 appears to have been the first investigator to describe the course of the lymphatics of the mouth, cheek, and lips. His descriptions and illustrations have been extensively copied into the textbooks, and it is a tribute to Sappey that his illustrations remain practically unchanged in the anatomical treatises of to-day. Sappey's work was carried out on dead human and animal bodies, but as he was primarily investigating the gross lymphatic drainage of the mouth and cheeks, there is no detailed description of the dental region.

Schweitzer in 1907 and later in 1909 was the first to investigate the lymphatic system of the dental region. Using dead premature fœtuses from six months up to

full term, with a few dead adults as controls, he succeeded in demonstrating lymphatic vessels coursing from the gums to the lymphatic nodes. Using the so-called Gerota mass (*vide infra*) and other masses of similar composition, but substituting different oil pigments, he endeavoured to map out the differential lymphatic drainage of various regions of the upper and lower jaw, these results are, however, incomplete. Schweitzer also demonstrated what appeared to be lymphatic vessels in the pulps of several teeth, being the first to do so.

Noyes in 1917, and Dewey and Noyes in 1928 investigated the lymphatics of the dental region in more detail, working on dead dogs and using Gerota fluid as their injection material. By subperiosteal injection on the sides of the jaws and by direct injection into the pulp of the tooth under pressure, they confirmed the results of Schweitzer and again demonstrated what appeared to be lymphatic vessels and spaces in the dental pulp. They also described and illustrated with drawings the course of the lymphatics from the upper canine via the infra-orbital canal to the submaxillary lymph nodes, and from the lower canine also to the submaxillary nodes via the mental foramen. Another interesting point made by these observers is that the lymphatic vessels of the pulp appear to anastomose with those of the periodontal membrane as they leave the apical foramina. They suggest that the lymphatic drainage of the outer and inner slopes of the gum is separate from the vessels of the periodontal membrane and the pulp of the tooth. The latter system they liken to a funnel draining downwards from the gingival trough and pulp of the tooth and narrowing as it reaches more deeply into the bone.

On the subject of lymph flow in the dentinal tubules much work has been done and the results are extremely contradictory. As far back as 1837 Linderer, and Retzius in the same year, considered that the dentinal canals transported a fluid; this theory was supported by Lessing, Krukenberg, Tomes, Magitot, and others. Black in 1886 noted the fact that staining of the dentine sometimes followed hæmorrhages into the pulp and suggested that this might be due to a flow of fluid up the dentinal tubules. Morgenstern in 1896 somewhat surprisingly stated that he had succeeded in staining endothelial cells in the dentinal tubules and that they were therefore true lymphatic vessels.

Hanazawa in 1917, succeeded in injecting the dentinal canals with a soot-gelatine injection mass under a pressure of 10 to 12 atmospheres and assumed in consequence a stream of fluid *in vivo* between the fibril and walls of the tubule. The enormous pressure used would, however, appear to vitiate entirely any such assumption.

Boedecker in 1922 postulated an efferent lymph stream in the dentinal fibril and an afferent stream in the circumfibrillar space; of this, however, he gave no experimental evidence.

Fish in 1926 and 1927, using live anæsthetized animals, showed that particles of Indian ink inserted into the pulp were carried along the dentinal tubules in a space between the fibril and the wall of the tubule, and gave evidence of an ebb and flow of lymph along this path and also of a flow of lymph up and down the tooth by the marginal lymph plexus.

Another school of thought holds that the space between the fibril and the wall of the tubule along which dyes travel is an artefact caused in fixation, and that *in vivo* the fibril fills the tubule completely. Walkhoff in 1893 also gave this opinion. Lams in 1921 reported that the fibril completely fills the canal in the predentine but shrinks in the dentine to the centre of the lumen of the canal, where in the fixed sections it appears to be surrounded by a space.

Meyer, in 1926, described the dentinal fibre shrinking strongly on fixation, and considered that this shrinkage gave rise to the space between the fibril and wall of the tubule. He repeats this view in 1931. Weidenreich in 1929, and Korte in 1932 also agree with this view. More recently Berkelbach van der Sprenkel in 1935

argues on hypothetical grounds that the fibril probably shrinks away from the wall of the tubule during fixation and that the space is therefore an artefact. This author also assumes the transport of fluid material via the fibril itself, of this, however, he gives no proof.

The view that the space between the fibril and the walls of the tubule is an artefact would seem to be fallacious, since in Fish's experiments solid granules of Indian ink were transported along this space, and it would appear at least unlikely that these particles could move from place to place in a dead tissue.

It would also appear to be improbable, on theoretical grounds, that the living fibril of the odontoblast should be enclosed in the matrix of the dentine, which is apparently impermeable to fluids, with no surrounding lymph through which an interchange of oxygen, products of metabolism, &c., could take place.

The present writer also holds the view that this space is not an artefact but is present *in vivo*, and considers that experiments to be described later, support this contention.

METHOD

At the beginning of this investigation, numerous experiments were carried out in an endeavour to find a suitable injection mass. Since the object of the experiments was to find the paths taken by the lymphatics around and within the teeth, in addition to the course taken by the larger lymphatic channels to the lymph nodes, it was necessary to prepare sections of the hard and soft tissues together. Of the two available methods it was found that with the Koch-Weil technique, in which the sections are eventually prepared by grinding, the soft tissues were liable to be badly damaged. The second method, in which the hard tissues are decalcified, was therefore adopted.

A large number of possible injection materials was thus eliminated, as any injection mass that was not acid-fast, would be attacked by the acid during the process of decalcification, and fail to show in the microscopical sections.

Experiments were made, using water-soluble, acid-fast dyes, such as erythrolitmine and trypan blue. These dyes, however, diffused out into the tissues, and although the appropriate lymph node to which the lymphatic vessels of the area drained was found to be stained by the dye, the paths by which the dye had travelled were not revealed.

The work of Wislocki on the staining of amphibian larvæ with benzidine dyes such as trypan blue, in which he showed that these dyes appeared to possess a particular affinity for lymphatic endothelium in the amphibian larvæ, suggested the possibility that they might also possess a similar affinity for lymphatic endothelium in the adult mammal. This, however, was not found to be the case. The lymphatic endothelium did not appear to be stained to a greater degree than the other tissues in which the dye had diffused, and it was certainly not sufficiently selective to make the lymphatic channels at once apparent.

Mercury, used by the older anatomists in their investigations of the larger lymphatic trunks, was impracticable, since the channels in the paradontal region were too fine to permit of its introduction into them by means of a needle.

Indian ink was tried, but was found to be disappointing in use, since the particles tended to aggregate at the point of injection, preventing the mass from entering freely into the tissue spaces. The injection masses used by most other workers in their investigations of the lymphatic system were the oil-soluble masses of the type known as Gerota mass. This mass consists of Prussian blue (2 parts), turpentine (3 parts), and sulphuric ether (15 parts). Originally advocated by Gerota, it was used by Schweitzer in his experiments, and later used by Noyes, Dewey and Noyes, and Jamieson and Dobson—by the latter workers in their investigation of the lymphatic drainage of the tongue.

This injection mass was tried in the present series of experiments, using the original Gerota mass and also masses of similar type, but substituting different oil colours in an endeavour to map out the regional lymph drainage of various parts of the gums and teeth. These masses, while revealing the grosser lymphatic channels, suffered from the defect that the oil was immiscible with the body fluids in the tissues. A further objection was found to be that the oil solvents, such as xylol, used in preparing the microscopical sections tended to dissolve out the oil-soluble dyes from the tissues.

The available methods of injecting the lymphatic system did not appear to be very satisfactory, and accordingly further experiments were made in the endeavour to improve upon them.

X-ray-opaque salts, such as potassium iodide, were injected and the specimen was X-rayed, but it was found, as expected, that while larger channels could be seen, the trabeculæ of the bone interfered with the interpretation of the results, and the method was abandoned.

Trial was then made with salts which would produce a fine precipitate when brought into contact with the body fluids, and then circulate through the tissues with these fluids in the form of a suspension, and experiments were continued on these lines. Of these salts, the best was found to be lead acetate. This salt, used in qualitative organic chemistry as a reagent for precipitating proteins, forms a fine white precipitate remaining for some time in suspension on being brought into contact with mammalian serum. It was found that a strong solution of this salt used as an injection mass gave excellent results. It spread well from the point of injection, and the lymphatic vessels were shown clearly as channels filled with the white suspension.

Lead acetate was particularly suitable for use in these experiments, since the white precipitate in the tissues could be converted into the black insoluble lead sulphide by bringing sulphuretted hydrogen into contact with it, and the injection mass thus fixed in an insoluble and recognizable form. By this process the distribution of the injection mass in the tissues could be observed post mortem in the microscopical preparations.

In order to find out the nature of the precipitate which was formed by the solution of lead acetate on contact with the body fluids of the animal, investigations were carried out *in vitro*, using human and animal serum.

Addition of the solution of lead acetate to the serum caused the immediate deposition of the precipitate even when the lead salt was present in high dilution, showing that the precipitate could not be merely the lead acetate coming out of solution owing to an alteration in the amount of available solvent.

The precipitate was very fine and remained in suspension for a considerable time. It could not be separated from the solution by ordinary methods of filtration as it passed through fine filter-papers, and in consequence had to be separated from the solution by the centrifuge. The precipitate was collected after being washed and centrifuged several times to remove any soluble contaminants, and was then analysed. The main features of this analysis were that lead was present, showing that the precipitate was a lead compound and not merely an organic substance precipitated out of solution by the presence of the lead.

Further investigation of the exact nature of the precipitate is being carried out, but it would appear that the precipitate is very fine, passing through filter-papers with ease, and that it probably consists mainly of a complex compound of lead and the proteins present in the body fluids, of a type such as lead albuminate.

The method is open to several objections, which will now be considered. Firstly, lead acetate is a toxic substance and may cause damage to the cells with which it comes in contact. In estimating the value of this objection, it is necessary to consider how far the flow of lymph is likely to be altered by death of the cells in

the path of its flow. The main flow of lymph takes place along the intercellular spaces and the lymphatic vessels, and since the cells along this route are not thought to play any very active part in regulating this flow, the motive force being provided by a *vis a tergo*, it is unlikely that the cells, even though damaged, would hinder the flow, unless that damage were so gross as to cause actual obstruction.

In the microscopical preparations no apparent damage to the cells could be observed, apart from that to the cells in the immediate vicinity of the point of injection, due to trauma. This apparent immunity of the cells to damage may be due to the suspension having been already formed by the injection mass coming into contact with the body fluids surrounding the point of injection, so that the fluid circulating past the cells is no longer toxic to them. The short time for which the injection mass is left in contact with the tissues, before the death of the animal, may also be a factor in this apparent lack of visible damage to the cells. In view of these findings it is not considered that the toxicity of the injection mass has affected the results to any serious or appreciable extent. A second objection lies in the fact that while experiments *in vitro* have shown that the lead salt is precipitated on contact with serum, and it is assumed that this precipitation is the same with lymph as with serum, it is impossible to say to what degree the salt is precipitated in the tissues of the animal. If the precipitation were complete around the point of injection, it would mean that any of the injection mass found at a distance from the point of injection must have been transported there by an active flow of the body fluids. If, however, the precipitation is incomplete, and some of the lead salt is left in solution, the possibility of a part of it travelling by diffusion must be considered.

The precipitation of the lead salt can be observed in the tissues around the point of injection and in the larger lymphatic vessels. It would seem probable that any salt in solution would be precipitated as it travelled away from the point of injection, on contact with the body fluids in which the concentration of lead was not so high. The injection mass in the larger lymphatic vessels did not appear to have diffused out from them, so far as could be judged macroscopically, after the application of sulphuretted hydrogen.

Inspection of fig. 18 which shows dentinal tubules, injected from a distance, cut in transverse section, reveals these tubules as black rings due to the granules of lead sulphide being at the periphery. This appearance is presumably due to the injection mass having travelled along the tubules in the space between the wall of the tubule and the dentinal fibril. If the injection mass were still in a diffusible form in the dentinal tubules, it would seem probable that it should diffuse into the protoplasm of the dentinal fibril. Since, however, it is entirely at the periphery of the tubules, and there is none in the dentinal fibril, it would argue that at this point the injection mass is not in a diffusible form.

Consideration also of the perivascular lymphatic vessels of the pulp shown in transverse and longitudinal section in figs. 12 and 16, shows that the injection mass is entirely contained within these vessels and has not diffused into the surrounding tissues. This fact again argues that the injection mass at this point was not travelling in a diffusible form.

The problem is an exceedingly difficult one, and while it appears probable that the injection mass travels through the tissues at a distance from the point of injection in a precipitated and non-diffusible form, it is not possible entirely to neglect the rôle that diffusion may play in the experimental findings.

A further objection which may be raised against this method, is the possibility that the lead salt may have reacted with the phosphates present in the hard tissues when circulating *in vivo*, and thus be shown in artificially large quantities in these tissues. On reviewing this possibility, it would at once appear evident that this objection can only be raised against the quantity of injection mass found micro-

scopically in the hard tissues, since the fact that it is found there at all, shows that it reached these tissues, revealing a fluid path to, or through, them. It would seem probable that if the injection mass had reacted in any way with the phosphates present in the hard tissues that some would be found in the matrix of these tissues surrounding the channels through them, since it is in the matrix that the phosphates are in greatest concentration. This was not found to be the case. The injection mass was found traversing the lacunæ and canaliculi of the bone, the tubules of the dentine, the lacunæ and canaliculi of the cementum of the apical one-third of the tooth and along the fibres of the periodontal membrane enclosed in the cementum of the coronal two-thirds of the tooth. In no case was it found in the matrix of these hard tissues, even in the immediate vicinity of the channels leading through them, and it is therefore assumed that if any chemical reaction does take place during the short time in which the injection mass is circulating, it does not do so to a sufficient degree to interfere with the interpretation of the experimental findings.

A further possibility of error, caused by the injection mass travelling in the tissues during the processes of fixation or decalcification, would appear most unlikely. Reference to the section dealing with the technique employed shows that the specimen was first subjected to a solution of sulphuretted hydrogen before removal from the animal, after its death. After removal, it was placed in a solution of the gas for some hours, these processes converting the majority, if not all, of the lead salt into the insoluble black lead sulphide. The specimen was then fixed in formol saline, and after fixation was again immersed for a few hours in the solution of sulphuretted hydrogen, in order to convert any of the salt which might be left into the insoluble form. During decalcification a continuous stream of sulphuretted hydrogen gas was blown through the bottles in which the specimens were being decalcified, in order to keep the sulphide fixed. With these precautions it would seem extremely improbable that any of the injection mass could move from place to place in the tissues at any time after removal of the specimen from the animal. In addition ground sections showed the mass in the same position in the tooth as in the decalcified sections.

Control experiments were carried out on pieces of normal tissue from the same regions as those which were taken for microscopical sections, but from an animal into which no lead acetate had been injected. These were subjected to the same processes, immersion in a solution of sulphuretted hydrogen gas, &c., as the other specimens with the object of seeing whether the process might cause any granules to be revealed or any granules to be deposited, which might be mistaken for the granules of lead sulphide. This was not found to be the case.

While it would not appear probable that sufficient of the lead salt could escape from the area of injection into the blood-stream to be redeposited in the same area to any extent by these means, investigations were made to control this possibility. A specimen of blood and a specimen of urine were taken from an animal one hour after an injection had been made, and were roughly analysed for the presence of lead. No lead was found in these specimens. Finer methods of analysis, such as the microchemical method employed by Aub and his co-workers in cases of lead-poisoning, would possibly have revealed its presence, but its absence in ordinary analysis showed that it was not present in amounts which could affect the results by redeposition from the blood.

Technique

The experiments were performed mainly on dogs and cats. Monkeys, although preferable, were too expensive for continued experiments. Two monkeys were used as controls to the main body of the experiments, and a few guinea-pigs were also used in order to obtain a comparative series of macroscopic results.

The majority of previous workers in this field, viz. Schweitzer, Dewey and Noyes,

&c., had carried out their experiments on dead animals. It is, however, clearly preferable in this type of experiment to inject substances into the live animal while the body fluids are circulating, since the direction of flow of these fluids might enhance or retard the flow of the injection mass in a specified direction. Accordingly, the experiments were always carried out on live anæsthetized animals, with the exception of two animals injected after death as controls.

Cats were anæsthetized with chloroform and ether, and chloralose; and dogs and monkeys with the same anæsthetics with the addition of a preliminary injection of morphia. Except in one group of experiments where the salt was left in contact with the tissues over periods up to four days, the animals were always killed while still under the anæsthetic. In this group of experiments where the salt was left in contact with the tissues for longer periods, the application of the salt was made under chloroform and ether anæsthesia, and the animal kept continuously under large doses of morphia until death. A strong solution of lead acetate was used as the injection mass. In the earlier experiments a specimen of commerical lead acetate was used. It was found, however, that the salt was very sparingly soluble and that it contained a large quantity of insoluble lead carbonate. Purer specimens of the salt of 10% and 25% solubility were tried and found to give better results. It was also found during the course of these investigations that the basic lead acetate gave better results than the ordinary lead acetate, and this salt has been used in the later experiments.

An experiment was performed in which the submaxillary lymphatic node of a cat was carefully exposed by dissection, after which an injection was made into the gingival tissues of the same side. This experiment showed that the first traces of the salt appeared in the larger lymphatic trunks entering the node, in four minutes. Accordingly, from half an hour to two hours was usually allowed between the time of the injection and the death of the animal, in order to allow ample time for the injection mass to travel.

In the majority of the experiments the animal was made to inhale a large dose of amyl nitrite just before death, in order to dilate the peripheral blood-vessels, and thus facilitate their injection with a gelatin mass to differentiate them from the lymphatic vessels. After the death of the animal the blood-vessels were injected with carmine gelatin, via the carotid arteries. In some of the later experiments, when the relationship of the lymphatic vessels to the blood-vessels had already been established, injection of the blood-vessels was not carried out.

The connective tissue in which the larger lymphatic vessels of the jaws run is extremely delicate and the walls of the vessels themselves are very thin. Great care had to be exercised in the dissection, which was of necessity difficult and tedious. The larger lymphatic vessels were shown up clearly by the white precipitate contained within them. Dissection was usually carried out at this stage, since the application of the solution of sulphuretted hydrogen revealed so many fine anastomotic channels that it caused difficulty in the dissection of the main channels.

After the dissection had been completed and photographs taken where necessary, the solution of sulphuretted hydrogen was applied on wool to the outside of the dissected area in order to reveal any vessels that had not already been observed. Fine channels could often be seen after this application where they were not apparent before. Those portions of the tissues which were required for the preparation of microscopical sections were then removed, placed in the solution of sulphuretted hydrogen for some hours in order to convert the lead salt in the tissues into the insoluble lead sulphide, and thus fix it in a recognizable form in the tissues. These specimens were then placed in formol saline to fix the tissues, and again into the solution of sulphuretted hydrogen for twelve or more hours. They were later observed under the dissecting microscope and then left in formol saline until required for the preparation of microscopical sections. The specimens were decalcified with

7% nitric acid, sulphuretted hydrogen gas being blown through the bottles in which the decalcification was taking place by means of a Kipps' apparatus, in order to keep the injection mass fixed as the sulphide during the process. The sections were prepared by the paraffin technique and in each series the sections were alternately stained with van Gieson's stain, and left unstained, so that a comparison could be made. A few sections were stained with hæmatoxylin, or by Mallory's method where it was required to know whether certain cells were alive, to show vessel walls, &c.

The experiments were divided into six main groups as follows:—

Group I.—Injections were made under pressure into the exposed pulp of the tooth.

Group II.—Injections were made into the gingival tissues with a hypodermic syringe, approximately half-way between the gingival crest and the buccal or lingual sulcus.

Group III.—Injections were made, with a hypodermic syringe, through the base of the gingival trough into the periodontal membrane.

Group IV.—Pledgets of cotton-wool were soaked in the injection mass and inserted into the gingival tissues through incisions approximately half-way between the gingival crest and the buccal or lingual sulcus.

Group V.—Pledgets of cotton-wool were soaked in the injection mass and inserted into the tissues of the periodontal membrane through an incision into the base of the gingival trough.

Group VI.—Control experiments: (*a*) Ground sections; (*b*) using dental tissues not injected but subjected to processes undergone by the injected specimens; (*c*) using dead animals.

The experimental technique used in these different groups will now be briefly described.

Group I.—In the experiments of this group an endeavour was made to force the injection mass into the pulp of the tooth with pressure, and thence to trace the lymph drainage in the pulp and the course of the main lymphatic vessels on leaving the pulp to their termination in the nodes. Young cats and dogs, the apices of whose teeth were still open, were chosen for these experiments, and the canines were selected as the best teeth for the purpose, owing to their size and ease of access.

The animal was first anæsthetized by the method previously described, and the tips of the canines cut across with a bur in the dental engine at the junction of the gingival two-thirds and coronal one-third of the crown. The pulp was then exposed by drilling straight down the centre of the tooth from the cut end towards the root until the pulp was reached. The outside of the tooth was roughened and a few superficial grooves cut on the tooth to give retention to a copper cylinder of appropriate size, which was then cemented on to the tooth with oxyphosphate cement, forming an air-tight and water-tight joint between tooth and hand. The cement was allowed to harden, and the exposed part of the canal was gently mopped with cotton-wool in order to remove any blood that had oozed out of the exposed pulp. The copper cylinder was then filled with the injection fluid and a thick rubber tube from the injection apparatus, also filled with the fluid to prevent air-locks, was placed over the copper cylinder and wired in position.

A pressure apparatus was used, in which a pulsating pressure could be obtained in addition to constant pressure. The amount of constant pressure, and the frequency and amount of the added pulsating pressure were all variable. The optimum pressures were found to be about 80 mm. Hg of constant manometric pressure with an added pulsating pressure of about 10 to 20 mm. Hg every half second. Injection of the tooth was continued from half to one hour.

The main difficulty with this technique was found to lie with the copper caps,

which were liable to become detached from the tooth, owing to the cement giving way under the strain of manipulation and pulsating pressure. If further experiments of this type had to be carried out, caps of trephine type fitting the tooth closely, as described by Noyes, would be used.

As, however, the whole method suffered from the defect that a large pressure was being used, and that this might force the injection mass through the tissues in an abnormal manner—apart from such sources of experimental error as the injection mass being forced into open blood-vessels or stopped from entering the pulp at all by a mass of blood-clot jammed into the canal by the pressure—the method was not continued. Sufficient experiments of this type were carried out, however, to confirm those results obtained by Noyes and Dewey in which they showed that the course of the lymphatics from the pulp to the lymphatic nodes in the upper and lower jaws, could be traced by this method.

Group II.—In the experiments of this group, the solution was injected into the buccal and lingual sides of the gums and also subperiosteally, an ordinary hypodermic syringe with a fine needle being used. The injections were made usually in one, and occasionally in two, places opposite the premolars or molars of one side of the maxilla or mandible. The buccal side was usually chosen for convenience but injections were also made on the lingual side to see if there were any difference in flow and drainage.

After injection of a quantity varying from ♏i to ♏ii in a small animal, to ½ c.c. in a larger one, the area was gently massaged with the finger tips. The time between injection and the death of the animal varied from approximately half an hour to one hour and a half.

Group III.—In this group, injections were made directly into the periodontal membrane through the floor of the gingival trough, a Record syringe with a fine needle being used. The injections were made by sliding the needle down the side of the tooth, and piercing the floor of the gingival trough close to the tooth. This was done beside various teeth of upper and lower jaws, about ♏i to ♏ii being injected into each periodontal membrane very slowly in order to minimize the pressure, which must of necessity be great, owing to the unyielding nature of the alveolar bone and the tooth.

Macroscopic Results

Group I.—Injection of the upper canine tooth of the cat and dog, by means of the apparatus and technique previously described, reached the antero-lateral submaxillary lymphatic node by one or more vessels emerging from the infra-orbital foramen, and following the course of the external maxillary (facial) blood-vessels (*see* fig. 1). Lymphatic vessels were also found emerging from the smaller foramina in the vicinity of the infra-orbital foramen, anastomosing with the larger vessels emerging from this foramen. From the canine tooth of the lower jaw, the injection reached the antero-medial node mainly by vessels emerging from small foramina in the mandible posterior to the mental foramen, travelling backwards in the submucosa of the outer aspect of the mandible to the node. Only occasionally were vessels observed emerging from the mental foramen.

The results obtained in the upper jaw confirm those obtained by Dewey and Noyes in 1928. The results obtained in the lymphatic drainage of the lower canines in the dog are not, however, in agreement with their observations. These authors state that from the lower canine tooth in the dog lymphatic vessels emerge from the mental foramen, and thence run backwards to the submaxillary lymph nodes. In these experiments and also those of Group III (*vide infra*) it was found that the lymphatic vessels emerged from the mandible mainly by small foramina posterior to the mental foramen, and only rarely through the mental foramen itself.

Group II.—The two most striking facts observed macroscopically in this group were the very free lymphatic drainage from the gingival tissues and the large amount of anastomosis in the whole system of this region.

Injection into the upper jaw in the premolar or molar region of the cat or dog showed, on dissection, that the injection mass had been taken up and carried in the lymphatic vessels to the submaxillary lymph nodes. Usually about four large vessels were seen, with smaller vessels, some superficial to the blood-vessels and some deep. These vessels were connected by anastomotic branches and pursued a more or less parallel course to the node, the size and number of these vessels varied

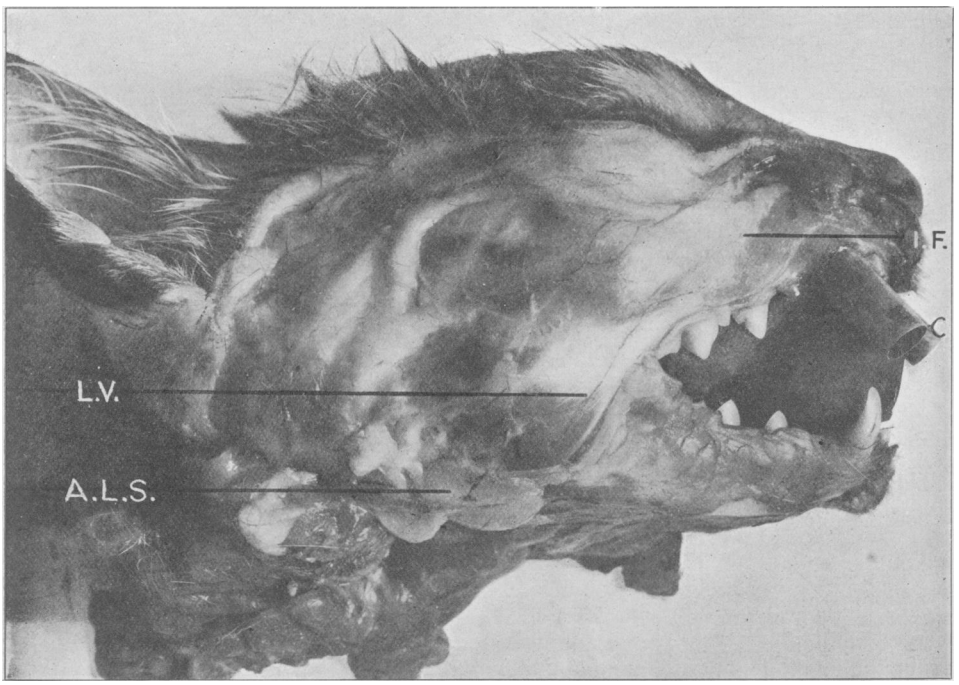

FIG. 1.—Group I. Cat. The injection mass was forced into the pulp of the canine tooth by pressure through the copper cylinder (C) cemented to the tooth. The mass was carried to the antero-lateral submaxillary lymph node (A.L.S.) by lymphatic vessels (L.V.) emerging from the infra-orbital foramen (I.F.).

with the genus of animal used. From the upper jaw these lymphatic vessels followed the course of the external maxillary (facial) blood-vessels to some extent, but diverged from them to a larger degree than is usually described. This is seen clearly in fig. 3. The diameter of these larger lymphatic channels appeared approximately the same from their beginning at the point of injection, to their entry into the lymph node. A beaded appearance was constantly seen in the lymphatic vessels after injection, due to the injection material being held up at the numerous valves. This is also seen in fig. 3. In the dog and the cat three lymph nodes were always found on each side at the bifurcation of the external jugular veins, and the main drainage from the maxilla took place into the outer one or two of these nodes. Often, however, solitary branches from the upper jaw were seen entering the medial submaxillary node in company with the lymphatic vessels from the lower jaw.

In one animal (a dog) an anastomotic channel travelling to the auricular lymph node was revealed after the application of t he solution of sulphuretted hydrogen. In this case the injection had been made int o the buccal side of the gingival tissues in the premolar region in the ordinary way.

One or more lymphatic vessels were constantly observed emerging from the infra-orbital foramen, and occasionally from smaller foramina in that neighbourhood.

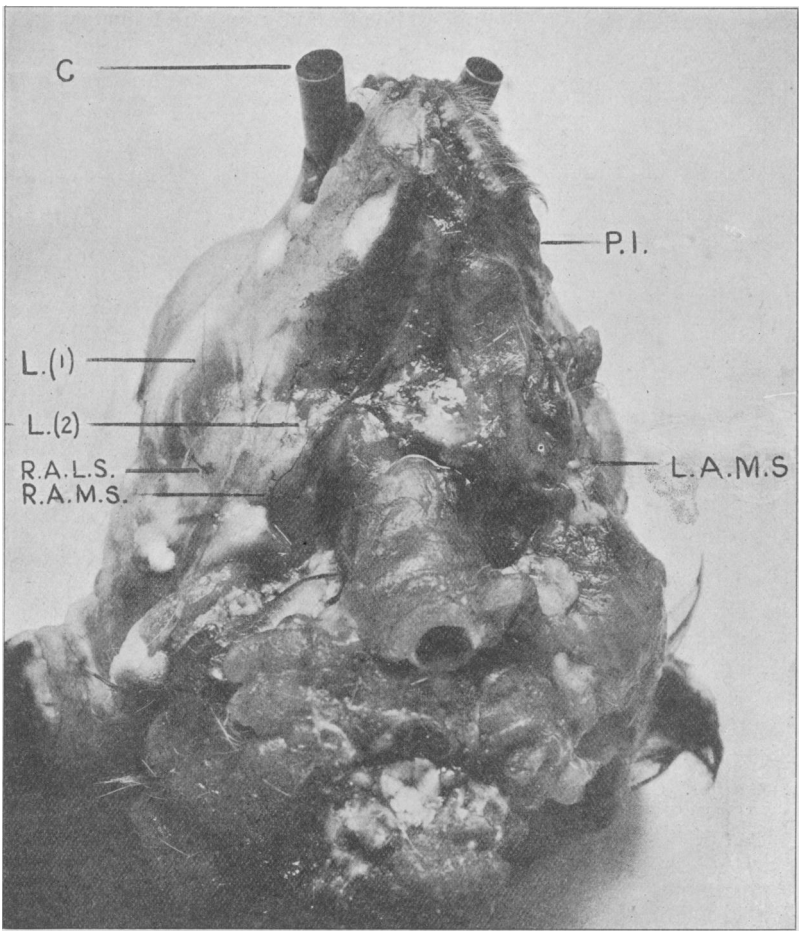

FIG. 2.—Groups I and II. Cat. On the right side the upper canine tooth was injected through the copper cylinder (C). The injection mass reached the antero-lateral submaxillary lymph node (R.A.L.S.) by the lymphatic vessels (L. (1)). On the left side an injection was made into the gingival tissues in the premolar region (P.I.) of the lower jaw. The main drainage from this point took place to the left antero-medial submaxillary lymph node (L.A.M.S.) but anastomotic channels (L. (2)) spread across the floor of the mouth to reach the right antero-medial submaxillary lymph node (R.A.M.S.).

These vessels bent backwards on leaving the foramina, and travelled to the submaxillary lymph nodes in company with the other vessels in the course of the external maxillary blood-vessels. Comparison with the results of Group III shows that these vessels which emerged from the bone in the infra-orbital region were

probably carrying the injection mass, which had reached the deep tissues of the jaws.

Injection into the gingival tissues of the lower jaw of the dog and cat reached the inner of the three submaxillary lymph nodes by vessels which ran parallel to the blood-vessels of the lower jaw, in a similar manner to those of the maxilla. Vessels were also found on the inner side of the mandible, travelling backwards with the blood-vessels and emerging from the muscular tissue of the floor of the mouth to reach the inner of the three submaxillary lymph nodes.

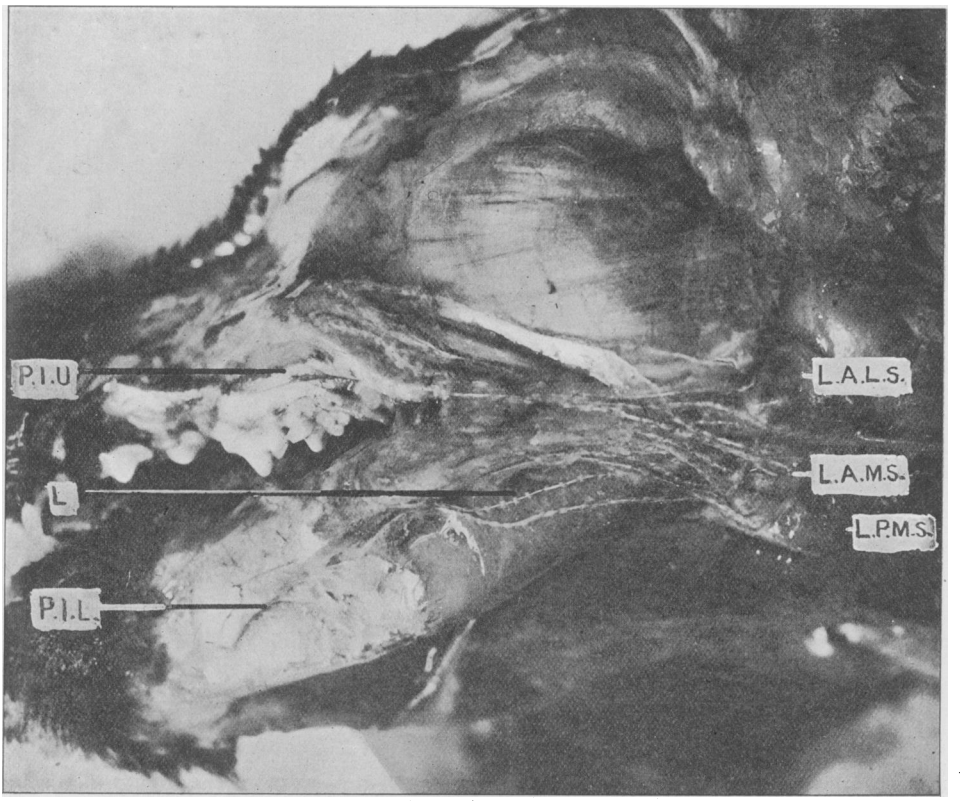

FIG. 3.—Group II. Dog. Injections were made into the gingival tissues of the upper and lower jaws at the points (P.I.U.) and (P.I.L.). Lymphatic vessels are seen running backwards to the antero-lateral submaxillary lymph node from the upper jaw, and to the antero-medial lymph node from the lower jaw (L.A.L.S.) and (L.A.M.S.). The postero-medial lymph node (L.P.M.S.) has not been reached. Some vessels from the upper jaw are seen to enter the antero-medial lymph node (L.A.M.S.). The beaded appearance of the lymphatic vessels due to the mass being held up by the numerous valves is shown at (L.). (Before the application of sulphuretted hydrogen.)

Anastomotic channels to the opposite side of the mouth were common, and injection into the gingival tissues of the lower jaw, in whatever position they were made, usually resulted in the injection of the lymph nodes of the opposite side, as well as the same side, to some degree (*see* fig. 2). Careful dissection of the soft tissues attached to the bone of the maxilla and mandible revealed numerous small channels travelling from the gingival tissues into the bone. When the jaws were cut across and the cut ends observed under the dissecting microscope, these channels

passing from the soft tissues into the bone were seen clearly, and appeared to communicate with the canals and lacunæ of the bone. These channels are seen in fig. 13.

These small channels were present in large numbers in both the mandible and the maxilla, but appeared to be more numerous in the latter. The importance of these channels communicating with the bone will be noted when a study is made of the microscopical results (*vide infra*).

A search has been made on many occasions, without success, for any lymphatic vessels emerging from foramina at the posterior end of the maxilla or mandible, after

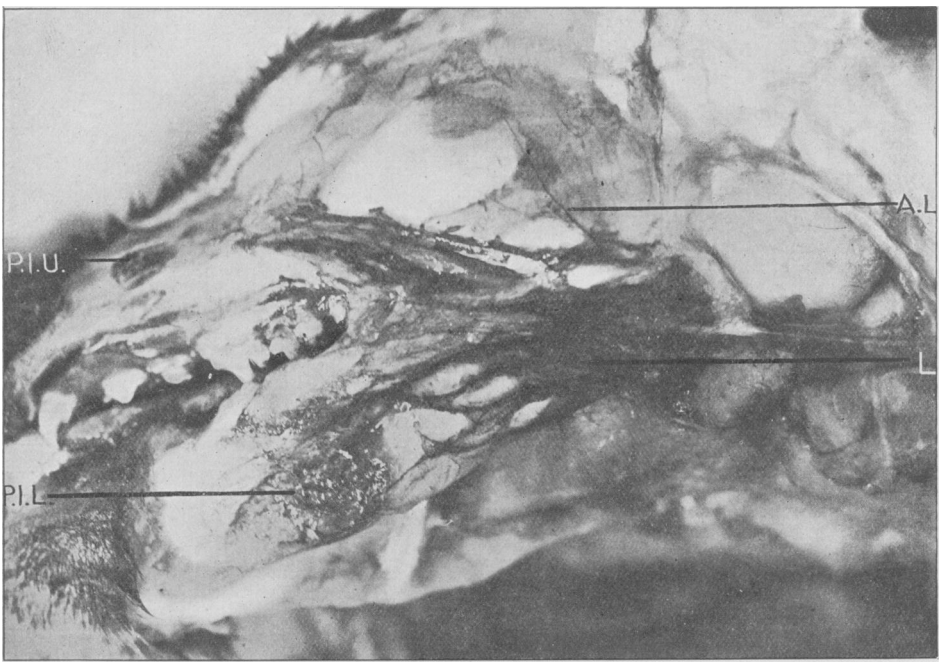

FIG. 4.—Group II. Dog. The same specimen as shown in fig. 3 is here shown after the application of sulphuretted hydrogen. It is seen that the small channels revealed after this application mask the main channels and that the lymphatic vessels travelling backwards from the points of injection (P.I.U.) and (P.I.L.) to the lymph nodes (L) cannot be dissected or shown as clearly in most cases as before the application. The sulphuretted hydrogen does however reveal small channels, e.g. A.L., which could not previously be seen.

injection into the gingival tissues. Lymphatic drainage from the gingivæ of both jaws appeared to take place mainly by the lymphatic vessels accompanying the blood-vessels travelling backwards in the soft tissues, but these small vessels passing into the bone from the gingival tissues were in connexion with channels in the bone. These channels were, in turn, in connexion with vessels in the larger spaces and canals of the bone. These vessels travelled backwards from the anterior teeth, and forwards from the posterior teeth, to emerge from the bone again through foramina in the infra-orbital region in the maxilla, and the mental region in the mandible, and thence backwards to the lymph nodes with the lymphatic vessels travelling in the soft tissues previously described.

Little appears to be known of the lymphatic drainage of this area in animals, and except for the work of Dewey and Noyes on dogs, there is no literature on the subject as far as can be ascertained. It was therefore thought that a comparison of the lymphatic drainage of the jaws and gingival tissues in the different types of animal investigated might prove of interest.

The main macroscopic results of this group are as follows: The guinea-pig and the monkey possess more numerous lymph nodes than the dog and the cat, although

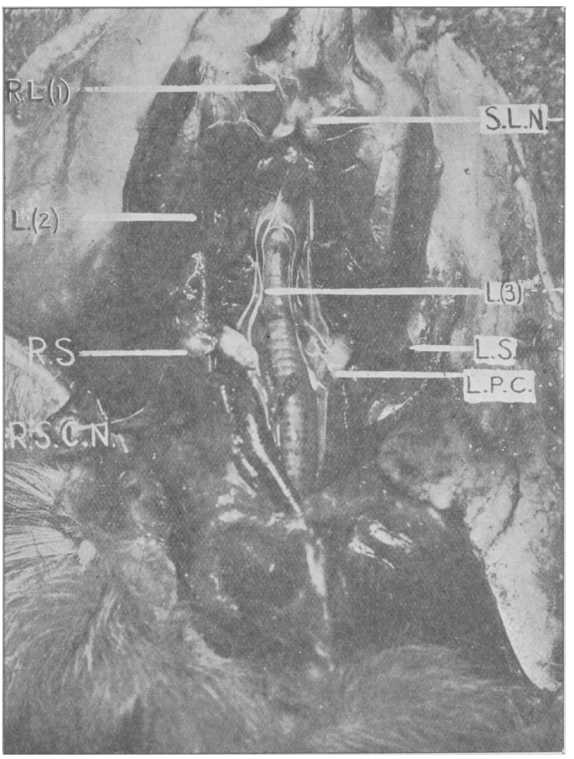

FIG. 5.—Group II. Guinea-pig. Injections were made into the gingival tissues of the upper jaw on the right side, and into the lower jaw on the left side. On the right side lymphatic vessels (R.L.(1)) are seen running to the submental nodes (S.L.N.), and also directly from the point of injection to the submaxillary node (R.S.) by the vessels (L.(2)), and thence to the supraclavicular nodes (R.S.C.N.). (The latter node is pulled out of position on a pin for focusing purposes.) On the left side vessels are seen travelling from the submental nodes (S.L.N.) to the paratracheal cervical nodes (L.P.C.) of the same side, and by the vessels (L.(3)) to the opposite paratracheal group of cervical nodes. The left submaxillary node (L.S.) and the left supraclavicular nodes have not been reached by the injection into the lower jaw. (Before application of sulphuretted hydrogen.)

they are smaller in comparison with the size of the animal, especially is this so in the monkey. Submental lymph nodes, and supraclavicular lymph nodes were always found to be present in the monkey and in the guinea-pig, whereas these nodes were never found in the dog or the cat.

Injection into the inner and outer aspects of the gingival tissues of the cat and the dog, reached the antero-lateral submaxillary lymph node in the case of the maxilla, and the antero-medial lymph node in the case of the lower jaw. Anastomotic channels between the vessels and nodes of the same side were common, as also was

cross-anastomosis to the opposite side. The postero-medial submaxillary lymph node was not reached save by anastomotic channels from the anterior submaxillary lymph nodes, and probably drains a posterior region of the head. From the submaxillary lymph nodes vessels were found leading to a paratracheal group of cervical lymph nodes, usually consisting of one large node on each side. No vessels were found travelling straight to these nodes from the gingival tissues, and drainage from the latter always appeared to take place primarily to the submaxillary nodes (*see* fig 3).

In the guinea-pig, drainage from the gingival tissues of the upper jaw took place mainly to the submaxillary group of lymph nodes and thence to the supraclavicular

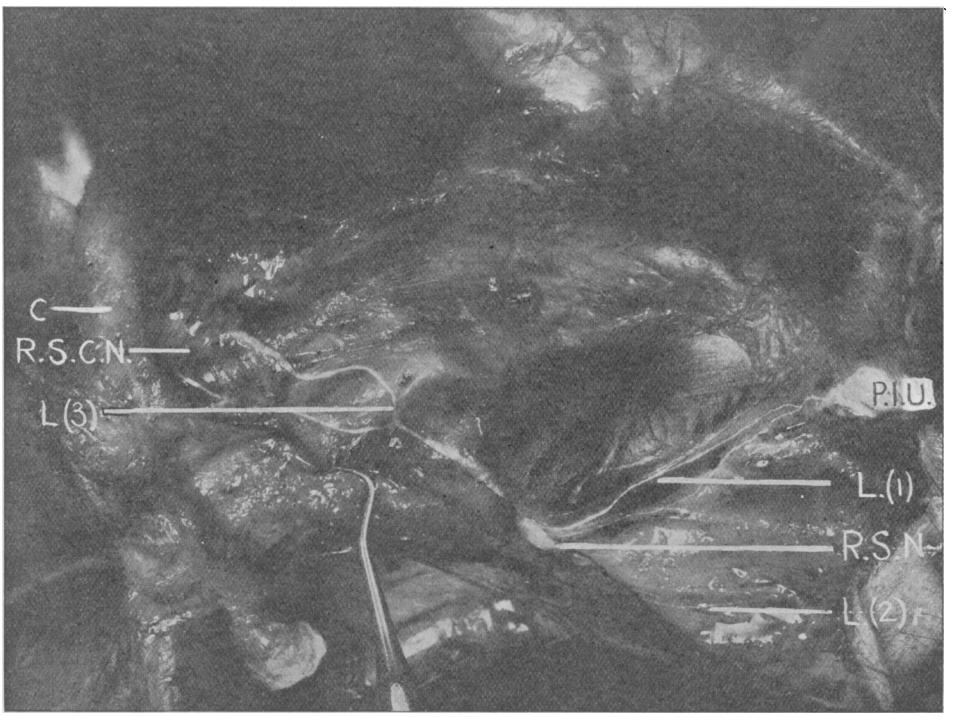

FIG. 6.—Group II. Monkey. Injection into the gingival tissues of the upper jaw at the point (P.I.U.) revealed lymphatic vessels (L. (1)) travelling to the submaxillary groups of nodes (R.S.N). From this group of nodes vessels (L.(3)) travelled backwards to the supraclavicular nodes (R.S.C.N.) lying just above the clavicle (C). A small vessel travelling over the buccal pouch is seen at (L.(2)). (Before the application of sulphuretted hydrogen.)

group. The submental nodes and the cervical nodes were also usually reached, but to a lesser extent. Cross-anastomosis to the nodes of the opposite side was common. Injection into the gingival tissues of the lower jaw reached the submental nodes, and thence the paratracheal nodes, in large quantity. The submaxillary nodes were rarely reached, and the supraclavicular nodes only reached after the injection of a larger amount of the solution than usual (*see* fig. 5).

Injection experiments were performed on the upper jaw of one monkey, and on both upper and lower jaws of another. The macroscopic results, though not sufficient to enable any definite conclusions to be formed, are yet interesting to

compare with those in the other animals of the series, particularly owing to the closer similarity of their structure to that of man.

Injection into the gingival tissues of the upper jaw in each case reached the antero-lateral submaxillary lymph node by small vessels travelling posterior and deep to the buccal pouch, accompanying the external maxillary blood-vessels. The submaxillary lymph nodes were found to be three in number on each side, and arranged as in the dog and the cat; the nodes were not, however, as large as in the latter animals. In one animal a small vessel was found travelling over the buccal pouch to reach the antero-medial lymph node. In both animals vessels were found leading from the antero-lateral submaxillary lymph node to the supraclavicular group of nodes. A small submental lymph node was found on each side, just anterior and internal to the submaxillary group of nodes. The submental nodes and the paratracheal deep cervical nodes were not reached by injections into the gingival tissues of the upper jaw, nor were any vessels found leading to them (*see* fig. 6).

In the lower jaw, drainage from the gingival tissues took place mainly by vessels leading to the submental node of the same side, and by cross-anastomosis to the submental node of the opposite side. From these submental nodes, vessels were found leading to the paratracheal group of deep cervical nodes of the same side, and to a lesser extent to the group of the opposite side. The supraclavicular group of nodes was not reached by injections into the gingival tissues of the lower jaw, nor were any vessels found leading to them.

The greater degree of variation in drainage found in the guinea-pig compared to the monkey, may possibly be explained by a greater amount of anastomosis between the vessels and nodes of the former animal, or by a larger quantity of solution in ratio to the size of the animal having been injected.

Group III.—In this group where an attempt was made to inject directly into the periodontal membrane via the gingival trough, it was found that drainage took place entirely to the submaxillary nodes (dog). The antero-lateral node was reached by vessels from the upper jaw, and the antero-medial node by vessels from the lower jaw (*see* fig. 7). It will be seen from this photograph, which was taken after injections had been made through the gingival troughs of the upper and lower second and third incisors, canines, premolars, and carnassials of the left side, that several small vessels emerge from the infra-orbital foramen and travel backwards to the node, over the surface of the masseter muscle. A larger vessel is also seen inferior to these vessels travelling from the gingival tissues to the same node.

In the lower jaw of the specimen, vessels were seen to run backwards in the submucosa to the antero-medial node; vessels were also found (not seen in the photograph) emerging from foramina in the bone and joining to form larger vessels running back to the same node. No vessels were found emerging from the mental foramina, but were found emerging from smaller foramina posterior to these. No vessels were found emerging from the posterior end of the inferior dental canal or from any foramina at the posterior end of the maxilla. In the experiments of Group II a few poorly-injected vessels were occasionally found emerging from the infra-orbital foramen or from the small foramina posterior to the mental foramina as found in Group III above. These were not, however, as marked as in Group III, and it seems probable, therefore, that in the Group II experiments, while a large amount of the injection material travelled into the spaces of the bone and into the teeth (*see* account of microscopic results) and thence into channels emerging from these foramina, the main part of the drainage of this area was carried to the nodes by vessels running in the submucosa directly to them. In Group III it would appear that a larger proportion of the injection material travelled into the bone spaces, to reappear in vessels from the foramina mentioned above, and a lesser proportion was carried by vessels in the submucosa directly to the nodes. The two systems were, however, in communication.

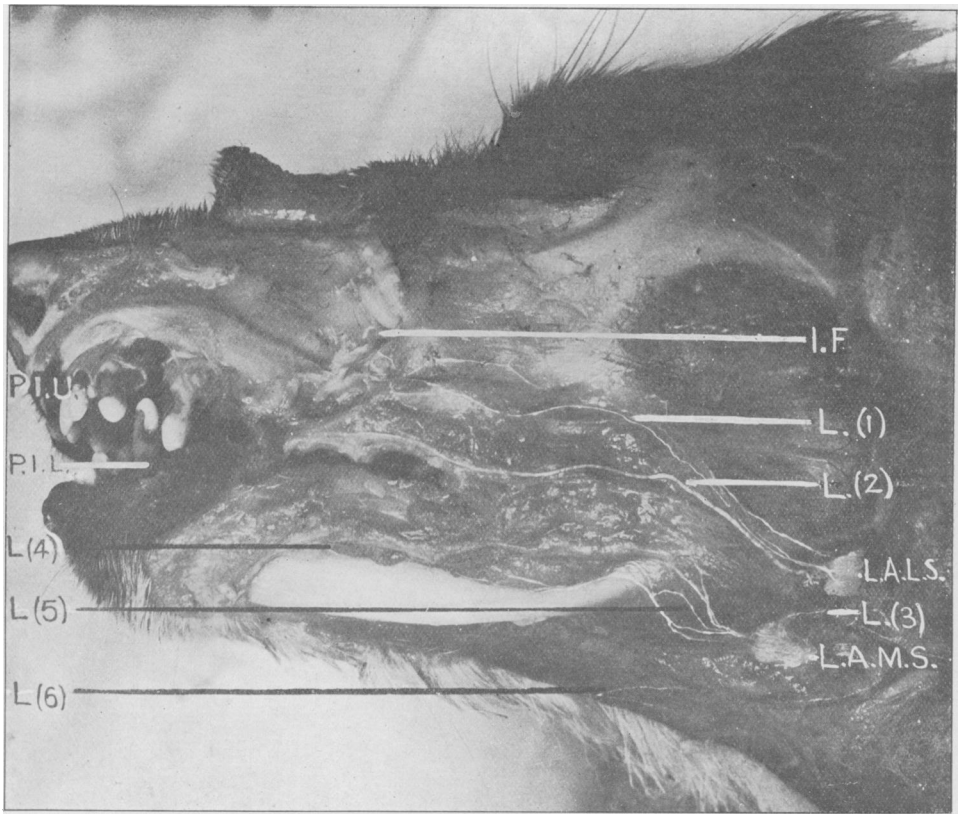

FIG. 7.—Group III. Dog. Injections were made into the periodontal membrane of teeth in the upper and lower jaws at the points (P.I.U.) and (P.I.L.). In the upper jaw part of the drainage took place to the antero-lateral submaxillary node (L.A.L.S.) by superficial lymphatic vessels (L.(2)), and part to the same node by vessels (L.(1)) emerging from the infra-orbital foramen (I.F.). In the lower jaw superficial and deep vessels were found (L.(4)), the latter emerging from small foramina posterior to the mental foramen. These vessels travelled backwards to the antero-medial submaxillary lymph node (L.A.M.S.) A small vessel connecting the two nodes is seen (L.(3)), and an anastomotic vessel travelling across the floor of the mouth from the opposite side is seen at (L.(6)). Characteristic beading of the vessels is well shown at (L.(5)). (Before application of sulphuretted hydrogen.)

Group IV.—Vessels were found leading to the submaxillary lymph nodes (dog), following the same course as the vessels demonstrated in the experiments of Group II. The nodes themselves were seen to have been reached in a few cases.

Group V.—Vessels were found travelling in the same course as those demonstrated in Group III. These vessels were, however, sparse and poorly injected. No lymph nodes could be seen to have been reached.

MICROSCOPIC RESULTS

Foreword.—Before dealing with the microscopic results, it is necessary to consider whether the small vessels found filled with black granules of lead sulphide in the pulp and other soft tissues are true lymphatic vessels or merely spaces or blood-vessels that have become filled with the injection material.

In the macroscopic results there can be no doubt that the large vessels described are true lymphatic vessels. Their course to the lymphatic nodes, the constancy of

their size in their course from tissues to node, the lack of any anastomosis with blood-vessels, and their beaded appearance due to the very numerous valves, a fact quoted by Schafer and Maximow as being typical of lymphatic vessels, make it certain that these are true lymphatic vessels.

With the smaller vessels of the pulp and other soft tissues, only seen on microscopic examination, the same degree of certainty does not exist. The existence of lymphatic vessels in the pulp especially has been a subject of much controversy. The only three organs of the body at present known in which the blood comes into direct contact with the cells of the tissues without the intermedium of lymph, are the spleen, the bone-marrow, and the liver (Samson Wright), all tissues connected with blood formation or destruction. It would seem highly improbable that the pulp of the tooth, which apparently has no such function, should also lack the lymphatic system possessed by all other tissues. The work of Schweitzer and Noyes and Dewey provides positive evidence of the presence of lymphatic vessels or lymph spaces in the pulp.

There is no method of histological staining at present known by which the endothelium of a lymphatic vessel can be distinguished from the endothelium of a blood-vessel, and in consequence the only evidence available is of a circumstantial nature. Again, it cannot be stated with certainty where a lymph space ends and where a lymphatic vessel begins, but as the body fluids in the one are in connexion with those of the other, the point is of academic interest only, since they are all part of the same circulatory system.

In these experiments, the evidence that the spaces and vessels in which the injection material is found are lymph spaces and lymphatic vessels, may be summarized as follows:—

(1) After the injection of the lead acetate solution and its conversion into the sulphide, the animal was killed, and the blood-vessels of the head and neck were well washed out with saline solution via the carotid arteries, as described in the section dealing with the method employed. Carmine gelatin solution was then injected into the blood-vessels. It is probable, therefore, that if the lead salt were in the blood-vessels and not in the separate lymphatic system, it would be washed out by the saline solution. It might be argued that the saline injection had not reached the smaller blood-vessels in which the lead salt could thus remain undisturbed. Yet after the administration of amyl nitrite the peripheral vessels were found to be well dilated and even the smallest blood-vessels were reached by the carmine gelatin injection. Blood-vessels in which there were corpuscles or carmine gelatin were occasionally found to contain a few granules of the salt. These were infrequent, but if found, they were considered as nullifying the evidence of that area.

(2) The smaller vessels containing the granules were found to lie in a network surrounding the small blood-vessels (*see* figs. 12 and 16), a position typical of lymphatic vessels in other parts of the body (Schafer). In fig. 12, where the carmine gelatin mass has shrunk away from the walls of the blood-vessel in fixation, the granules are seen in the walls of the vessel.

(3) After an injection has been made into the tissues, e.g. into the submucosa of the gingiva, the injection material has not been swept away from the infected area as would be probable if it had got into the blood-stream. It has, however, been seen again filling the large lymphatic vessels leading to the lymph nodes draining the area, and since it is found at the end of the system, it must have got into the beginning of it, unless it is assumed that the injection material has primarily got into the blood-stream, and then escaped out again into the lymphatics, before being swept on by the blood-stream. The latter hypothesis would appear extremely improbable, especially in view of the fact that neither the blood nor the urine contained appreciable quantities of lead.

It therefore seems reasonable to assume that the vessels to be described are true lymphatic vessels, and they will accordingly be referred to as such.

Group I. (Injections made directly into the pulp of the tooth under pressure.)—The pulp near the point of entrance of the injection mass in the crown of the tooth showed numerous lymph spaces filled with the mass. Further down the tooth small lymphatic vessels were found, these vessels being mainly perivascular. Near the apex of the tooth the smaller vessels appeared to anastomose and join to form larger vessels, which were now separate from the blood-vessels. The injection mass

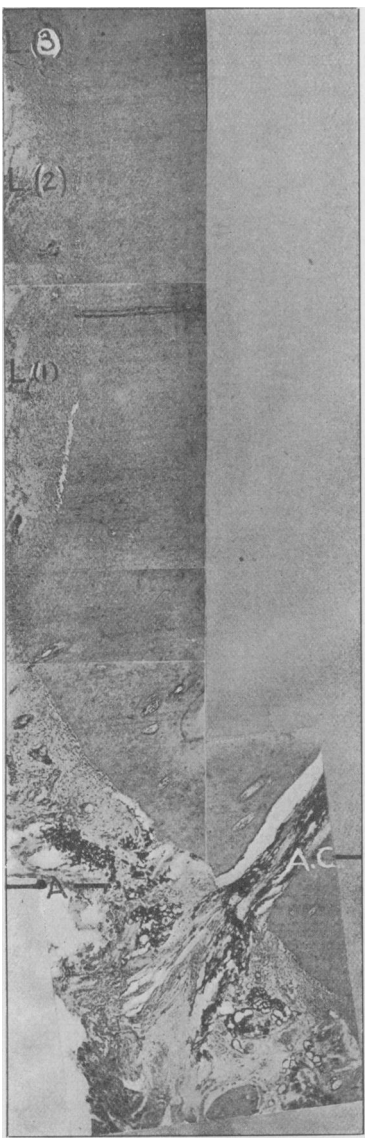

FIG. 8.—Group I. Dog. The injection mass travelled through the pulp of the tooth and emerged in lymphatic vessels through the apical canal (A.C.). These vessels anastomosed with lymphatic vessels of the periodontal membrane (A.), and lymphatic vessels (L.(1)), (L.(2)), (L.(3)), at some distance from the apex are seen to be filled with the injection mass. [× 30.]

did not appear to be entirely collected into these vessels in its flow down the pulp since it was found in lymph spaces far down the pulp towards the apex of the tooth, opposite lymphatic vessels. It was not clear whether the bulk of the injection mass was collected into these vessels, and then travelled from them by side channels into the spaces during its passage down the pulp, or if, in addition to being collected into vessels from the spaces near the point of injection, it travelled from space to space towards the apex of the tooth.

In longitudinal sections, the vessels were found to be widely scattered through the pulp, but were most numerous towards its centre. The injection mass was found to have travelled into the dentinal tubules in considerable quantity during its passage down the tooth, but there did not appear to be any particular concentration of lymphatic vessels around the odontoblasts.

Lymphatic vessels were seen surrounding nerve trunks in the pulp and periapical tissues, thus establishing the existence of perineural lymphatic vessels in the pulp of the tooth. Sections in which these perineural lymphatic vessels were seen were very kindly examined for me by Mr. R. V. Bradlaw, and reported by him in an earlier communication this year.

At the apex of the root the injection mass was found to be collected in large lymphatic vessels, in which it passed out of the apical foramina with the blood-vessels and nerves. After emerging from the apical foramina these vessels anastomosed freely with the lymphatic vessels of the periodontal membrane, and it was observed that the injection mass had travelled in the lymphatic vessels high up the periodontal membrane towards the crown of the tooth (*see* fig. 8).

The fact that these vessels in the periodontal membrane were injected so far from the apical foramina may have been due to the pressure employed in the injection, but the fact emerges that the lymphatic vessels of the pulp of the tooth and the lymphatic vessels of the periodontal membrane must be in communication, thus confirming the observation made by Dewey and Noyes on this point in 1928.

Group II.—In this group, in which injections were made into the sides of the gingival tissues bordering the teeth, it was found that the injection mass travelled more freely through the bone when the injection was made subperiosteally than when it was made into the soft tissues of the gingivæ short of the periosteum. It also travelled more freely through the bone in the upper jaw than in the lower jaw. These facts accord with clinical experience in the administration of local anæsthetics.

The mass spread from the point of injection and travelled through the bone of the jaw by the lacunæ and their canaliculi, spreading from canaliculus to canaliculus and from lacuna to lacuna until it had traversed the bone (*see* fig. 13). In no case was it seen to have entered the matrix of the bone. This would appear to confirm the observations that any fluid exchange in bone must take place through the lacunæ and their processes, and that the matrix of the formed bone is impermeable (Fish). A closer view of the injection mass in the bone is given in fig. 13.

On examining vertical sections through the jaw opposite points at which an injection had been made, it was found that in the majority, the dentinal tubules of the neighbouring teeth contained the injection mass apparently lying in a space between the fibril and the wall of the tubule.

In some cases the mass had apparently travelled into the dentinal tubules from the pulp of the tooth, which had been reached by vessels entering through the apical foramina. Sections were observed in which it could be seen entering via the apical foramina, travelling through the pulp of the tooth by lymphatic vessels, usually perivascular in situation, and entering the dentinal tubules. Such a section is seen in fig. 9, and in this case the course of the injection mass can be clearly traced.

In other specimens it was extremely difficult to follow its course mass to

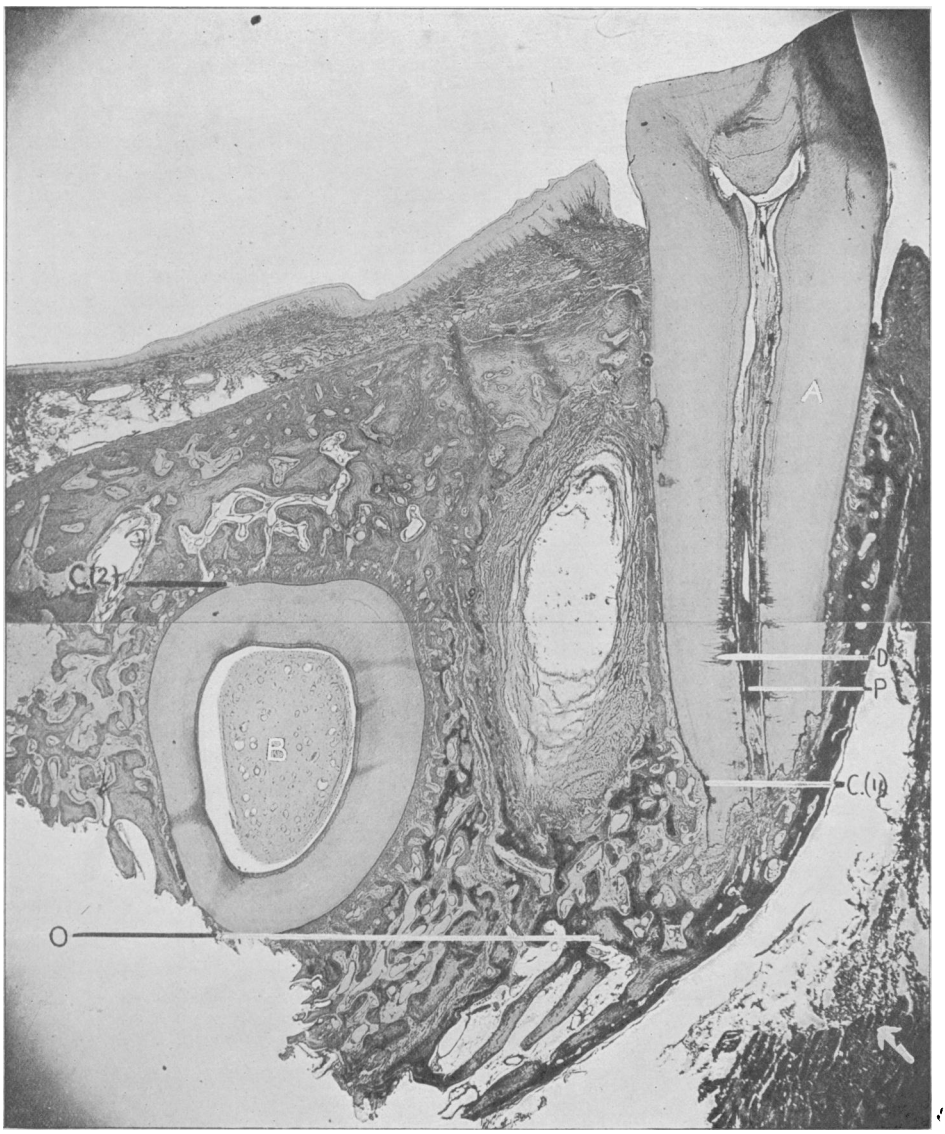

Fig. 9.—Group II. Monkey. The mass injected in the area shown by the arrow travelled through the bone (O) by the lacunæ and canaliculi, and reached the pulp (P) of the deciduous tooth (A) (cut longitudinally) and spread into the dentinal tubules (D) of this tooth. It can also be seen to be concentrated along the cementum (C.(1)) of the same tooth. A concentration of the mass is seen along the cementum of the canine tooth "B" (cut transversely). The mass has also reached the pulp and dentinal tubules of the latter tooth. *See* figs. 10, 11, and 12 for higher magnification of parts of this section. [× 25.]

the dentinal tubules, since, although the injection mass had reached the tubules in considerable quantity, it could not be seen in the pulp at all. Specimens in which this occurred were examined more closely by observation of serial sections of the tooth in question, in order to eliminate the possibility of the section having been taken through the tooth in such a position that the part of the pulp observed was not in reality opposite the particular dentinal tubules under discussion. In some specimens it was still found that no traces of the injection mass could be seen in the pulp, while there was a great deal in the tubules.

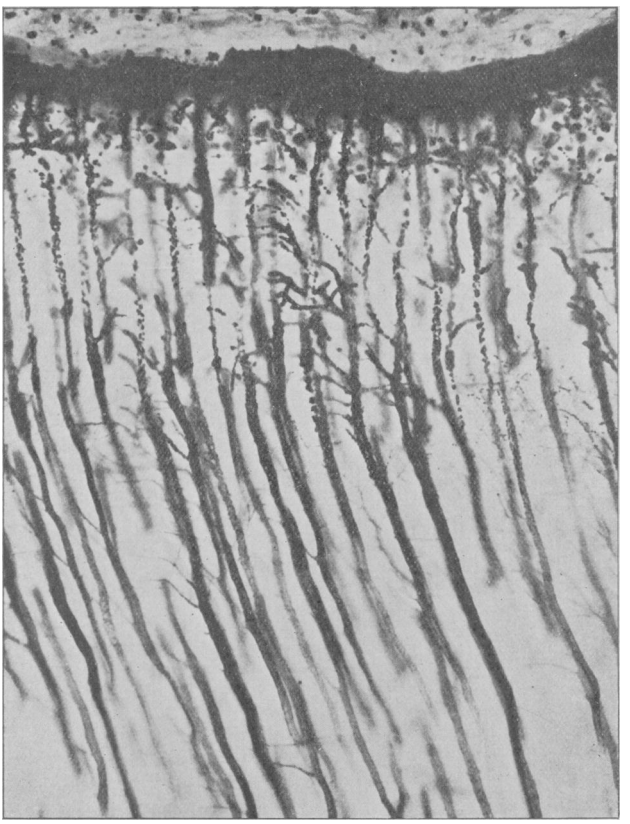

FIG. 10.—Group II. Monkey. Dento-cemental junction of the tooth "A" seen in fig. 9. Granules of lead sulphide are seen filling the dentinal tubules and their side branches, the latter being particularly numerous near the cementum. The mass is seen to be concentrated along the cementum. [× 735.].

To account for this phenomenon there appeared to be three possible explanations. Firstly, that the animal might have been killed at such a time that the injection mass had left the pulp through which it had travelled and had all reached the dentinal tubules, leaving no traces behind it. Against this explanation was the fact that the appearance was seen in animals which had been killed after different time-intervals, the shortest being half an hour. A second possible explanation was

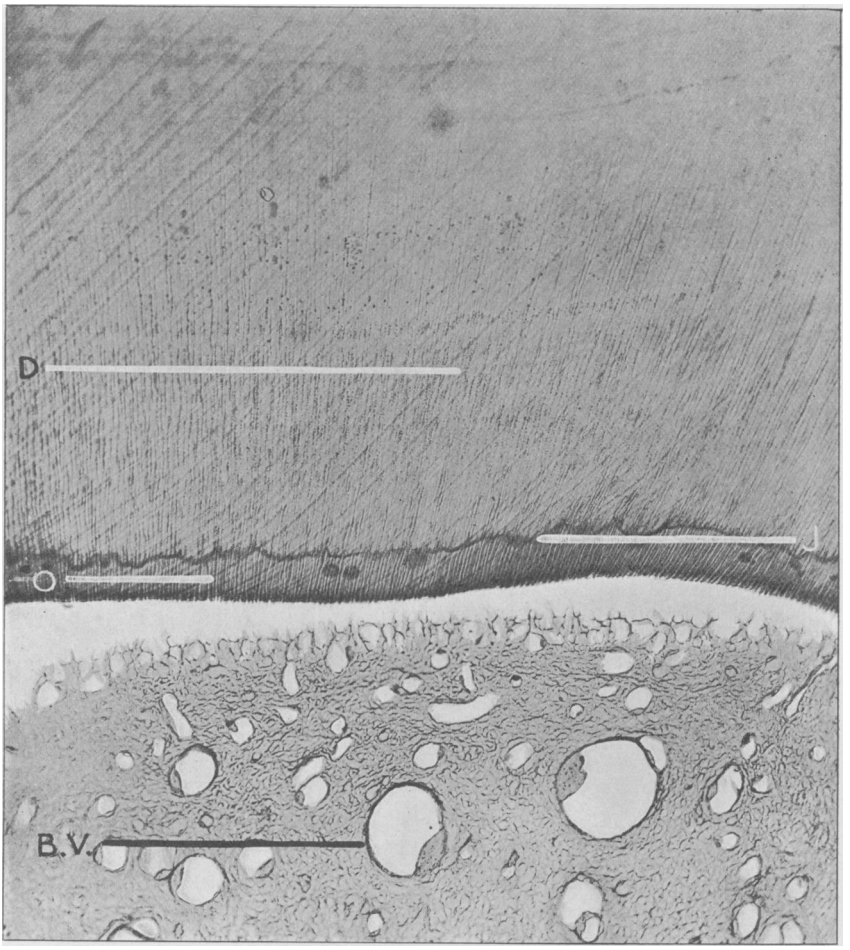

FIG. 11.—Group II. Monkey. Higher magnification of part of tooth "B" seen in fig. 9. Granules of lead sulphide are seen in the dentinal tubules (D), and a concentration of the mass is seen along the junction of the odontogenetic zone and the formed dentine (J). The tubules in the odontogenetic zone (O) are devoid of granules. The mass is also contained in perivascular lymphatic vessels surrounding some blood-vessels (B.V.) in the pulp. *See* fig. 12 for higher magnification of the latter. [× 115.]

that the injection mass had originally been present in the pulp, but had fallen out or been washed out during the preparation of microscopical sections. This explanation did not seem probable, since in many sections it was observed in lymphatic vessels in the pulp from which it had not been lost, and indeed from which it would appear difficult for it to have been lost (*see* figs. 12 and 16). A third possibility was that it had entered the dentinal tubules from the outside by traversing the cementum, and not from the inside via the pulp.

The path by which the injection mass reached the dentinal tubules is not yet clear, and further work is being carried out on this point.

The distribution of the injection mass in the dentine was of great interest. The maximum concentration was found along the zone of terminal branching of the dentinal tubules under the cementum or enamel. This concentration is seen in fig. 15. Another smaller concentration was seen along the junction of the dentine and the odontogenetic zone (*see* fig. 11).

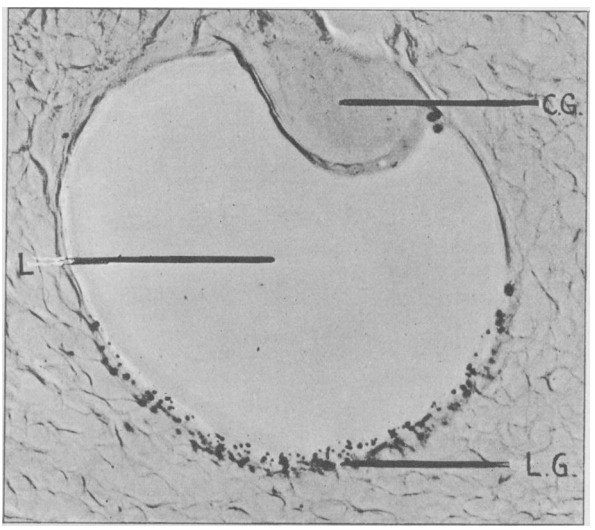

FIG. 12.—Group II. Monkey. Higher magnification of the blood-vessel (B.V.) seen in fig. 11. The carmine gelatin mass injected into the blood-vessels has shrunk away from the walls of the blood-vessel during fixation and is here shown at (C.G.), the lumen (L.) of the vessel being clear. The granules of lead sulphide contained in perivascular lymphatic vessels are seen at (L.G.) [× 250. Red colour filter.]

Further small concentrations of the injection mass were often seen along the so-called " incremental lines " in the dentine. This appearance is seen in fig. 14 and may be of some significance in view of the fact that X-ray examination of human teeth has shown that the dentine is not so highly calcified along these lines as in the remainder of the tissue (Thewlis).

In considering the appearances found in this group of experiments, it must be remembered that the injection mass was under a small amount of pressure due to the distension of the tissues over the site of injection. Consideration of the microscopical results of Group IV, however, shows that the mass still travelled in the same manner, though not in the same volume, without any pressure being applied.

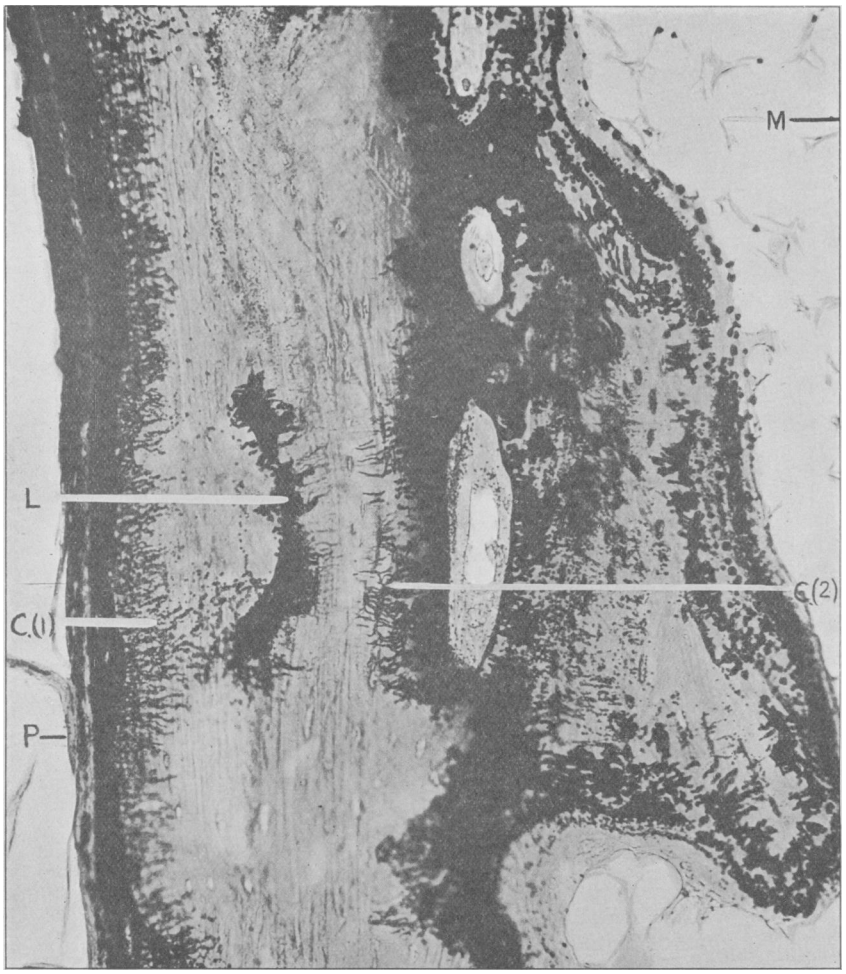

FIG. 13.—Group II. Dog. The mass has travelled through the periosteum of the bone (P) and is seen in the numerous small canaliculi (C.(1)) leading into the bone at right angles to its surface. From these canaliculi it enters lacunæ (L) and by further intercommunication of canaliculi (C.(2)) travels through the bone to escape into the soft tissues (M). [× 200.]

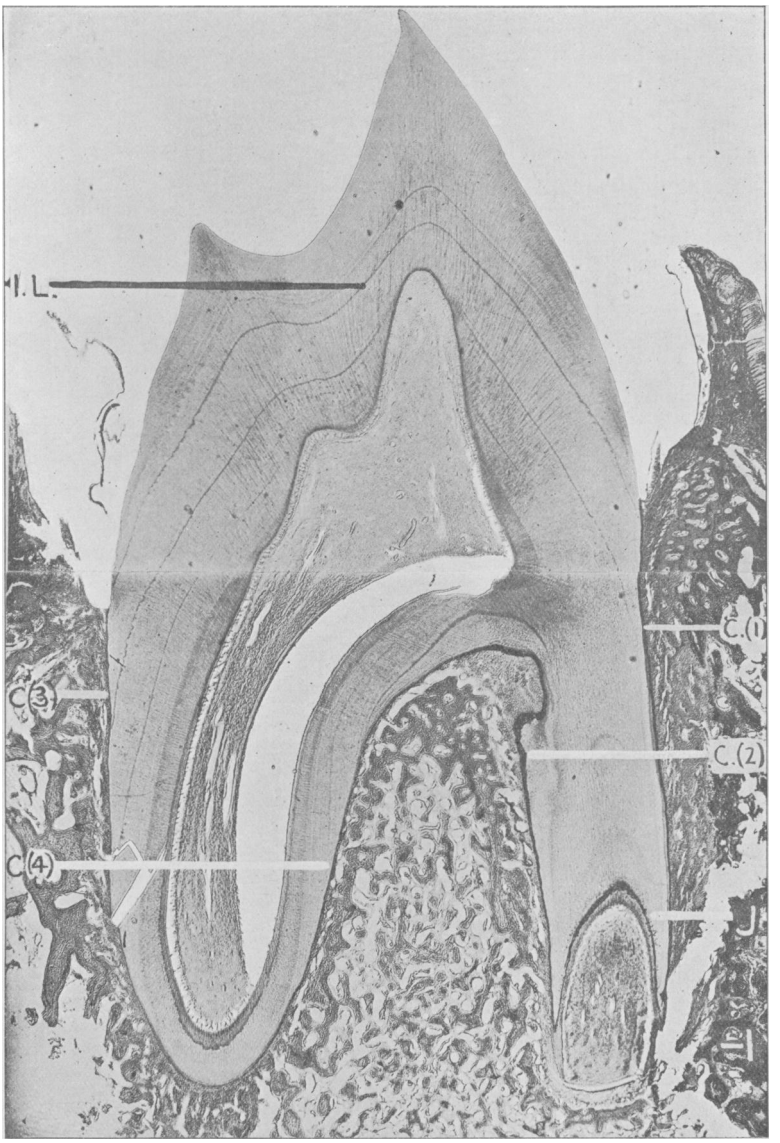

FIG. 14.—Group II. Dog. The mass injected into the gingival tissues in the region of (I) travelled through the bone and is seen to be concentrated along the cementum (C.(1)) and (C.(2)) of the buccal root. It was also found in perivascular lymphatic vessels in the pulp of this root, and the concentration of the mass along the junction (J) of the odontogenetic zone and the formed dentine in this root is well shown. There is no concentration of the mass along the cementum (C.(3)) and (C.(4)) of the lingual root. The concentration of the mass along the so-called "incremental line" is seen at (I.L.) [× 12.]

Group III.—In this group of experiments it was not realized that so much pressure would be engendered by injecting into a soft tissue between two hard tissues, i.e., the alveolar bone and the tooth. The injections were small and made very slowly but even so, some damage was done to the periodontal membrane. In consequence it was not felt that the microscopical results were reliable, and for this reason they are not here quoted.

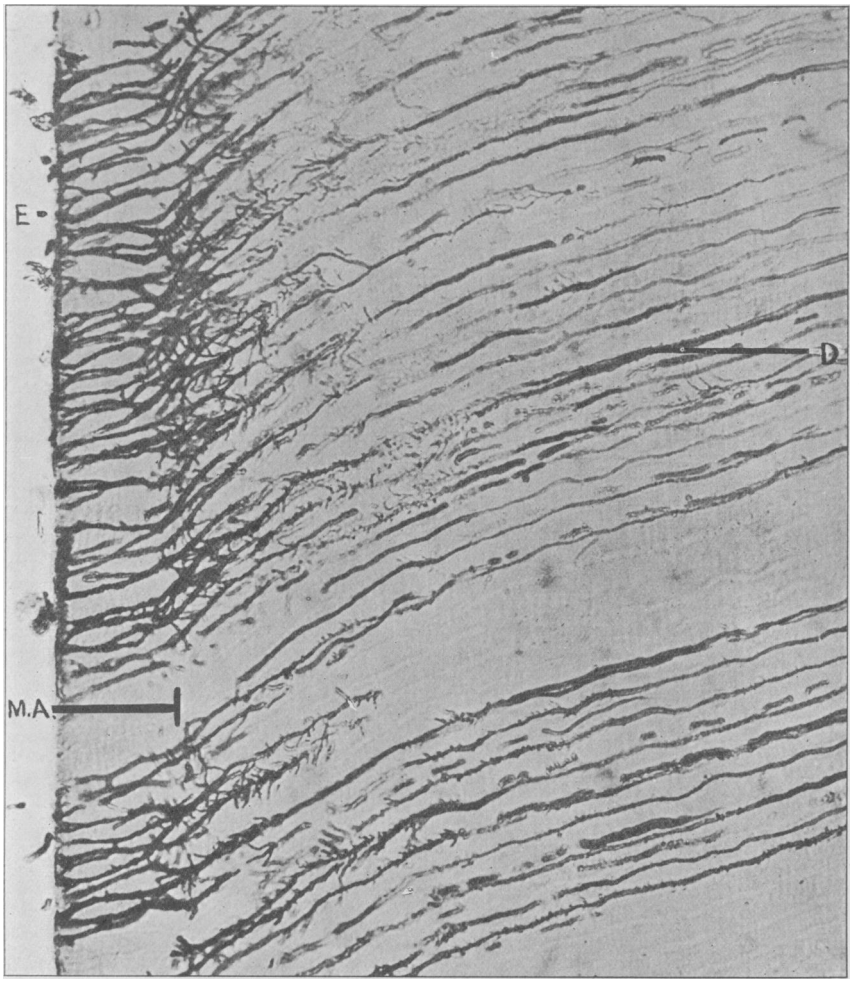

FIG. 15.—Group IV. Dog. Section through the amelodentinal junction of a tooth showing the concentration of the lead mass along the area of maximum branching (M.A.) of the dentinal tubules (D). The position formerly occupied by the enamel (decalcified) is shown at (E). [× 275.]

Group IV.—The appearances of the microscopical sections in this group of experiments, in which pledgets of wool soaked in lead acetate were placed in incisions made into the buccal and lingual sides of the gingivæ, were found to be very similar to those of Group II. The mass was again found traversing the bone by way of the

lacunæ and their canaliculi, and also in the dentinal tubules of the adjacent teeth. In a few sections lymphatic vessels were seen in the pulp of the tooth, as seen in fig. 17, but in no case could any continuity of vessels be traced from the point of insertion of the mass through the apical foramina to the pulp, as described in the results of Group II where the mass was injected. The mass could be seen along the cementum of the tooth on the side of insertion of the pledget, and was particularly marked in the zone of terminal arborization of the dentinal tubules under the cementum. It could also be seen, though to a lesser extent in the corresponding positions on the opposite side of the tooth to that on which the pledget was placed.

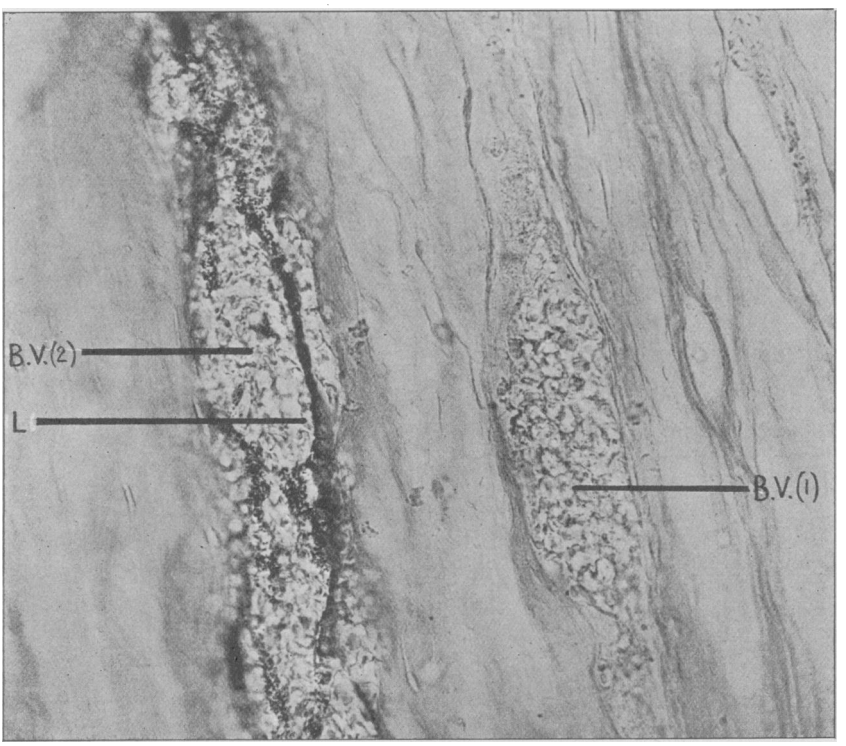

FIG. 16.—Group IV. Dog. Blood-vessels (B.V. (1)) and (B.V. (2)) in the pulp of a tooth, showing perivascular lymphatic vessels (L) arranged in a network over one of them. [× 550.]

The concentration of the lead mass along the junction of the odontogenetic zone and the dentine as described in Group II was again found.

Transverse sections of the dentinal tubules (*see* fig. 18) showed that the mass appeared to be travelling in the space between the dentinal fibril and the wall of the tubule, since these tubules containing the mass appeared as rings, owing to the centre of the tubule being taken up by the fibril, and consequently not being reached by the lead salt.

The fact that the fibril did not contain any of the mass pointed to the important conclusion mentioned elsewhere that the mass was apparently not in a diffusible form at this point. If it is accepted that it was travelling in a particulate form since it did not diffuse, it must follow that it must have reached this point

by a flow of lymph *intra vitam* along the tubules. It can also be argued, though with less justification, that since the mass is apparently not in a diffusible form in the dentinal tubules, it may also not be in this form in the other tissues at a distance from the point of insertion of the pledget. If this were the case, since no pressure was used in this group of experiments, the flow of the mass through the peridental tissues and along the tubules was a vital phenomenon due to flow of lymph along these paths.

The path by which the mass had reached the pulp and the dentinal tubules still remained obscure. Ross in 1933, and Stones in 1934, have pointed out that both the primary cementum of the coronal two-thirds of the root, and the secondary cementum of the apical one-third of the root are permeable to dyes in young animals, so that it is possible that the mass may have reached the pulp and dentinal tubules by travelling through the cementum. It was found in both the primary and secondary cementum in the present experiments, particularly in the lacunæ and canaliculi of the latter. No concentration of the mass was found in the apical area of the root, and only a few lymphatic vessels were found in the pulp. This, however, does not rule out the possibility that the mass may have all been swept into the dentinal tubules and cementum after traversing the pulp from the apical foramina, without leaving any traces of its course in the apical region, and the evidence as to its path of entry is therefore inconclusive.

The appearance, described in Group II, of a continuity of injection mass from the point of injection through the apical foramina to the pulp and dentinal tubules of the tooth, seen in some sections, may have been due to the pressure caused by the injection, since, as mentioned previously, no corresponding appearance was found in the sections of Group IV. The similarity of the remainder of the microscopical results in Group IV to those obtained in Group II, and the way in which the mass traversed the bone and reached the dentinal tubules and the pulps of adjacent teeth, showed that the results obtained in Group II were not entirely due to the pressure used in the injection of the mass.

There was little difference in the amount of the mass which had traversed the bone in the maxilla and the mandible, although it appeared to be held up to a greater degree in the lacunæ and canaliculi of the thicker bone of the mandible. Whether it took longer to travel through the bone of the maxilla or mandible was not ascertained.

Group V.—In this group of experiments, in which pledgets of wool soaked in lead acetate were placed in incisions made in the base of the gingival trough, it was found that the mass travelled down the periodontal membrane, and could be seen in lymphatic vessels in the latter. The mass could be seen in the lacunæ and canaliculi of the alveolar bone into which it had travelled from the periodontal membrane, and also in the dentine, cementum, and lymphatic vessels of the pulp of the tooth.

It was present in a greater amount in the dentine and cementum on the side of the tooth on which the pledget had been placed, but also appeared to have travelled laterally around the tooth in the periodontal membrane, since it could be seen, though in lesser quantities, in the alveolar bone, dentine, and cementum of the opposite side of the tooth to that on which the pledget had been placed.

A few definite lymphatic vessels as shown in fig. 18, were seen in the pulp of the tooth, but no traces of the mass could be seen in lymphatic vessels in the periapical tissues, or in lymphatic vessels in the apical foramina.

In the dentine, the mass could be seen in the tubules, particularly again in the terminal branches under the cementum as previously described. The concentration of the mass along the junction of the odontogenetic zone and the formed dentine, described in the microscopical results of Group II, was not found in this group to the same extent as in Group II and Group IV. In some of the sections of this group and also of Group IV, the mass was seen in the terminal branches of the

dentinal tubules under the enamel in the crown of the tooth, in a corresponding situation to that already described under the cementum. In the dog the maximum branching took place just before the termination of the tubules (*see* fig. 15). The way by which the mass had reached this position was not

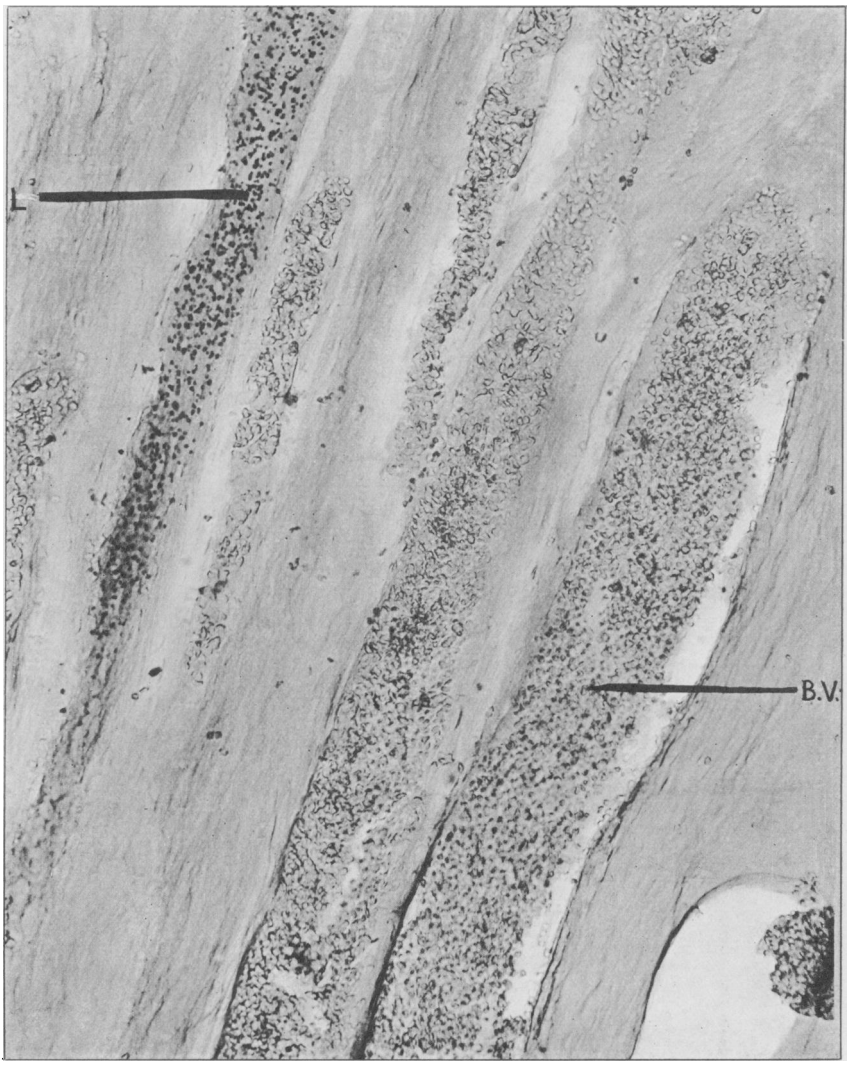

FIG. 17.—Group V. Dog. Section of the pulp of a tooth showing blood-vessels (B.V.) and a large lymphatic vessel containing the granules of lead sulphide (L). [× 300.]

clear. In many of these sections it could be seen for some distance along this zone under the enamel, although the tubules centripetal from this zone appeared to be devoid of it. This suggested the possibility that the mass might have travelled along this zone by lateral flow along the side branches of the tubules, which are

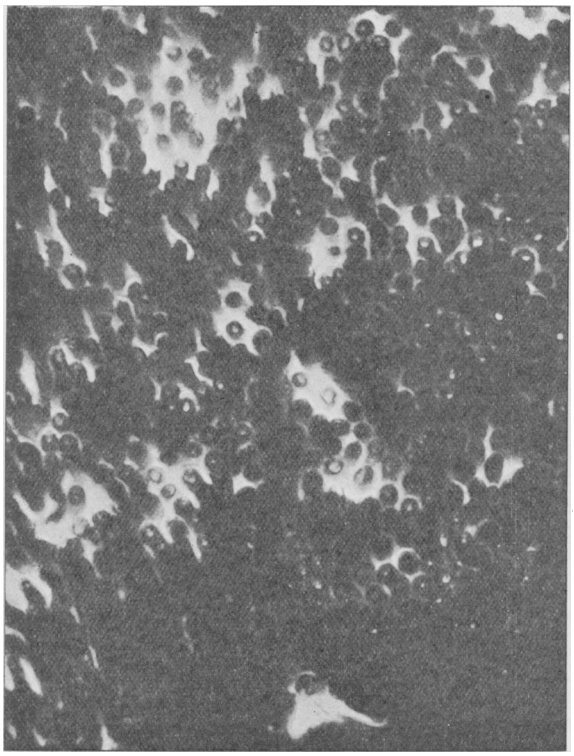

FIG. 18.—Group II. Dog. Transverse section of the dentinal tubules, showing that they appear as rings, owing to the injection mass being situated circumferentially in each tubule. [× 600.]

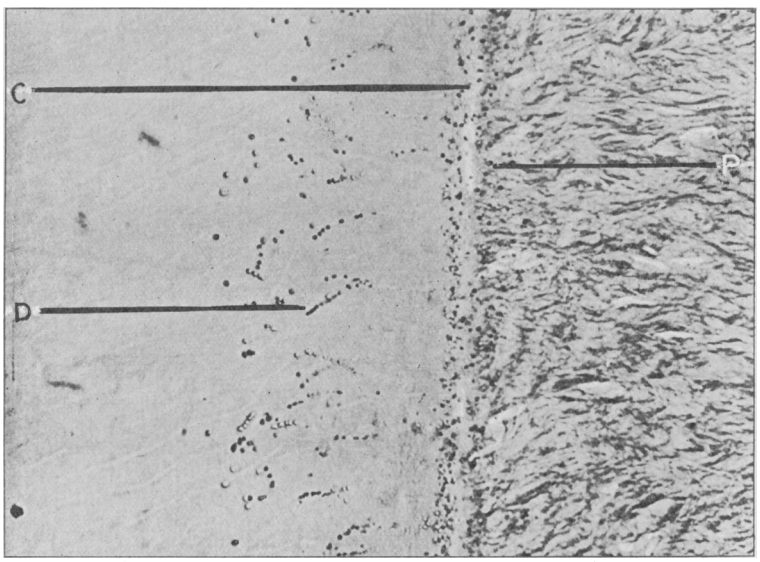

FIG. 19.—Group V. Dog. The mass placed on a pledget of wool in an incision in the base of the gingival trough, has travelled down the periodontal membrane, in which it can be seen at (P), and has reached the dentinal tubules (D) and cementum (C) of the tooth. [× 250.]

particularly numerous at this point, and is of interest in connexion with the clinical observation of the spread of dental caries laterally along the amelo-dentinal junction.

The mass was present in the primary and secondary cementum of the tooth. In the latter it appeared to be travelling along the lacunæ and canaliculi, and in the former gave the appearance of lying in channels, whether these are along the course of the periodontal fibres embedded in the cementum, or not, has not yet been ascertained. The route which the mass had followed in its course to the tissues of the tooth from the periodontal membrane, was still not clear from these experiments.

Group VI.—(*a*) Ground sections: Teeth were taken from experiments of Group II and sections prepared by grinding, in order to control the possibility that the injection mass might have travelled, or become altered in any way during the process of decalcification. The mass was found in the same positions and concentration in the hard tissues of the tooth as in the decalcified sections. In addition, several dentinal tubules crossing the amelo-dentinal junction, and entering the enamel were found to contain the mass (monkey).

(*b*) Uninjected specimens: Sections were prepared from tissues taken from similar positions to those previously described, but from an animal which had not been injected, and subjected to the same processes of exposure to sulphuretted hydrogen, fixation, and decalcification. No black granules similar to those described in the microscopical results, were found.

Thick ground sections were also prepared from teeth from uninjected animals and were decalcified, sulphuretted hydrogen gas being blown through the jars as with the injected specimens. In addition lead acetate was added to the fluid in the jars and the gas blown through before the introduction of the tooth. Granules of free lead sulphide were therefore in movement in the jars while the section was being decalcified. After decalcification the section was observed under the microscope. It was found that the granules had not entered the dentinal tubules or any other part of the tooth.

(*c*) Dead animals: An experiment of the Group II type was carried out on a dead animal (cat). It was found that the injection mass had not penetrated the bone so readily as in the live animals, and was held up to a greater extent on the outside of the bone. The mass was present in the dentinal tubules, though to a far lesser extent than in the sections from animals which had been injected *in vivo*. The injection mass was not found in the pulp.

Further control experiments of this type are being carried out, using pledgets of wool soaked in the mass, as in Groups IV and V, as the pressure used in the injection in this control experiment renders the results less valuable than would be the case were no pressure to be employed.

Conclusions

(1) The guinea-pig and the monkey differ from the cat and the dog in possessing submental and supraclavicular groups of lymph nodes, which assist in draining the area of the jaws, in addition to the submaxillary and cervical nodes possessed by the latter animals.

(2) In the guinea-pig and the monkey drainage from the tissues of the upper jaw takes place mainly to the antero-lateral submaxillary lymph node, and thence to the supraclavicular group of lymph nodes. Drainage from the tissues of the lower jaw takes place mainly to the submental group of lymph nodes and thence to the paratracheal group of lymph nodes.

(3) In the cat and the dog, drainage from the tissues of the upper jaw takes place mainly to the antero-lateral submaxillary lymph node and thence to the paratracheal group of lymph nodes. From the lower jaw, drainage takes place

mainly to the antero-medial lymph node and thence to the paratracheal group of lymph nodes.

(4) The lymph nodes in the guinea-pig and the monkey are smaller, relative to the size of the animal, than in the cat and the dog; they are, however, more numerous.

(5) There is a very large amount of anastomosis between the vessels and nodes of the lymphatic system in this region. Cross-anastomosis to the opposite side is a common phenomenon, particularly in the lower jaw.

(6) Drainage from the deep tissues of the tooth and bone takes place backwards from the anterior portion of the jaw and forwards from the posterior portion to a nodal point, and the lymphatic vessels emerge from the infra-orbital foramen in the maxilla, and from a small foramen or foramina posterior to the mental foramen in the mandible (cat and dog).

(7) The suggestion advanced by Dewey and Noyes—that the lymphatic drainage from the pulp and periodontal membrane of the tooth can be likened to a funnel narrowing as it reaches the deep tissues of the jaw, and that the lymphatic drainage from the slopes of the gingivæ is a separate system—does not appear to be correct. In these experiments the mass was found to travel from the slopes of the gingivæ to the pulp of the tooth, the vessels outside the bone and those inside appeared to be in direct communication.

(8) The lymphatic vessels of the periodontal membrane, and of the pulp of the tooth are in communication.

(9) A fluid path is shown from the gingival tissues through the bone to the pulp, dentine, and cementum of adjacent teeth. The exact path by which the mass reaches the tissues of the tooth from the gingival tissues is not yet clear, but the same appearances were found in the results of Group IV in which no pressure was employed, as in those of Group II in which the mass was injected hypodermically.

(10) Lymphatic vessels are present in the pulp of the tooth. These vessels are often perivascular but larger separate vessels are also present. Perineural lymphatic vessels are present in the pulp of the tooth.

(11) Lymphatic vessels are present in the periodontal membrane, and without pressure the mass was found to have travelled down the periodontal membrane in these vessels, and into the lacunæ and canaliculi of the alveolar bone. The tissues of the tooth were also reached, though the path by which the mass travelled to them from the gingival trough is not yet clear.

General Discussion of Results

While the experimental animal is not always comparable to the human in its structure or reactions, and the demonstration of the path taken by the fluid mass *in vivo* does not necessarily mean that organisms or their toxins follow this path, the results of these experiments may yet have some bearing on clinical problems. Thus the large amount of cross anastomosis found in the vessels draining the teeth and jaws in the monkey, suggests that swollen lymph nodes in the human may not always be due to a focus of infection on the same side of the body.

The fact that the drainage from the upper jaw in the monkey takes place primarily to the submaxillary group of lymph nodes and then to the supraclavicular nodes, suggests that swelling of the latter nodes may be due to a focus of infection in the upper jaw, when no other obvious septic focus can be found.

Since organisms and their toxins travel mainly along the lymphatics, their spread is largely governed by the direction of these vessels, and the distribution of the latter is of importance in placing the pathology of the area on an anatomical foundation.

It is of interest, therefore, that the lymphatics of the periodontal membrane and those of the pulp of the tooth appear to be in communication. The spread of the mass downwards in the lymphatics of the periodontal membrane from the gingival trough, and the communication of these lymphatics with the spaces of the alveolar bone, may prove to be of some importance in the study of paradontal disease, especially in view of the fact that Fish considers that paradontal disease starts as a lymphangitis in the region of the gingival trough. The downward drainage of the lymphatics from the gingival trough in the periodontal membrane towards the apex of the root, suggests that infective processes in the neighbourhood of the apex may be due to organisms which have travelled downwards from the gingival trough via these lymphatics. It may therefore be well, before considering the treatment of a dead or infected tooth by apicectomy or other operation, to make certain that the gingival tissues are healthy, and that they are kept in a healthy condition subsequent to the operation.

The fact that the mass was not found to travel through the bone of the mandible so freely as through the bone of the maxilla, and that subperiosteal injection was more effective than injection into the mucosa short of the periosteum, is in accord with the findings in clinical injection anæsthesia. The existence of perineural lymphatic vessels in the pulp of the tooth and the demonstration of the mass in the pulp of the tooth after injection or application of the mass into the slopes of the gingivæ, suggested the possibility that the anæsthetic solution employed in injection anæsthesia might not be acting on the nerve trunk in the bony canal, as generally thought, but might be travelling further and acting on the actual end-organs of the nerves in the pulp. Further work is being carried out on this point.

The path by which the mass reaches the pulp, dentine, and cementum of the tooth, from the gingival tissues, is not yet clear, and experiments are being continued towards its elucidation. The fact that the mass does appear to reach the pulp, dentine, and cementum of the tooth so freely from the gingival tissues, even when no pressure is used, does not accord with the usually accepted views on this point. In consequence, until all possibility of artefact in this connexion is excluded the results cannot be taken as definitely proven. The control experiments which have been performed, have been described previously, and further control experiments are at present being carried out.

In conclusion the writer would like to express his thanks to Mr. J. G. Turner, and Dr. E. W. Fish for their kindness in reading the manuscript and for their most helpful criticism: to the laboratory staff of the John Hampton Hale Research Laboratory of the Royal Dental Hospital for their assistance with the technical side of the investigation: and to the Medical Research Council for providing a grant, which made the investigation possible.

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