# DENTAL CARIES 1933.

## THESIS

Presented by.....

G. GRAHAM MACPHEE.

M.A., M.B., CH.B., L.D.S.

HONORARY CONSULTING DENTAL SURGEON.
*Liverpool Stanley Hospital.*

HONORARY ASSISTANT SURGEON
*Liverpool Dental Hospital.*

HONORARY DENTAL SURGEON IN CHARGE.
*Pathological Laboratory School of Dental Surgery.*
*University of Liverpool.*



ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

# D E N T A L  C A R I E S,  1933.

Dental caries is a disease almost universally prevalent among modern civilized human beings. Although not in itself immediately dangerous to life, its importance, in view of its remote effects upon the body, can scarcely be over-estimated. Any step, however small, in the direction of solving the problem of its ultimate cause, which is at present unknown, must be regarded as valuable not only from a theoretical but from a practical standpoint. So long ago as 1778, John Hunter (116) recognised this and wrote "The importance of teeth is such that they deserve our utmost attention, as well with respect to the preservation of them, when in an healthy state, as to the methods of curing them, when diseased. They require this attention, not only for the preservation of themselves, as instruments useful to the body, but also on account of other parts with which they are connected; for diseases in the Teeth are apt to produce diseases in the neighbouring parts, frequently of very serious consequences; as will evidently appear in the following Treatise." In order to treat the disease rationally and, better still, prevent its occurrence, it is necessary or at least desirable to

understand its aetiology.

Dental caries has been described as pre-eminently the "curse of civilization", and it was almost unknown in the earliest times. It is true that Colyer and Sprawson (1931) (51) stated that"the Rhodesian skull... shows caries of many teeth". Sprawson wrote (1933) (231) that when the skull was first exhibited in this country in 1921, nobody doubted that the teeth showed evidence of dental caries. A. Smith Woodward (1921) (267) and J. Thornton Carter (1928) (41) described the condition as caries, and R. Schwarz(1933) (273) referred to it as such. Colyer, however, wrote (1933) (48) that in his opinion the "caries" in this skull "did not start in the enamel, but in the dentine following exposure of the dentine", and he stated that he "knows of no skulls of prehistoric man that show caries."

The teeth of other prehistoric skulls - Heidelburg, Krapina, Spy, Neanderthal, Galley Hill, Dordogne, Java, etc. have been noticed by Bryce (1913) (33), Hopson (1915) (109) and numerous other observers, and no dental caries was mentioned, though signs of attrition were common in many. This was confirmed in Pithecanthropus erectus (the Java skull) by Pearsall (1907) (193), and Bryce (33) observed that "in none of those specimens are there any signs of caries of the teeth".

The Piltdown skull, Eoanthropus dawsoni, one of the oldest human relics in existence, had two lower molars "both quinquicuspid and much worn on the surface" (Humphreys, 1914) (113). American writers, however, (Miller, Gregory, 1924) (93) denied that the mandible belonged to Eoanthropus and said it was the jaw of a chimpanzee, and Hopson in 1915 (109) commented on "the simian character of the jaws and teeth. The latter approach very closely those of a female chimpanzee and perhaps more closely still those of a young chimpanzee", so it is doubtful if the teeth can be regarded as human. However, Underwood (1915) (244) stated definitely that there is attrition of the teeth, no caries.

Most of these skulls had a high dental index (megadont) and a low palatal index (leptostaphyline) while Homo heidelburgensis has been described by Wilder (1926) (260) as having "human teeth in an ape jaw".

Thus it is seen that even including the Rhodesian skull, we may say that dental caries, if not entirely absent, was rare among prehistoric men, if any conclusion can justifiably be drawn from the small number of such skulls available for examination.

The skulls of very ancient Egyptians show little sign of dental caries. "Both in Nubia and Egypt the ordinary form of dental caries is exceedingly rare in pre-dynastic and proto-dynastic people, and among the poorer classes it never became at all common until modern times... But dental caries, although extremely rare before the Pyramid Age, became common as soon as people learned luxury. In the cemetery of the time of the Ancient Empire, excavated by the Hearst Expedition at the Gizeh Pyramids, more than five hundred skeletons of aristocrats of the time of the pyramid-builders were brought to light, and in these bodies it was found that tarter-formation, dental caries and alveolar abscesses were at least as common as they are in modern Europe to-day. And at every subsequent period of Egyptian history one finds the same thing - the wide prevalence of every form of dental disease among the wealthy people of luxurious diet, and the relative immunity from it among the poorer people who lived mainly on a coarse uncooked vegetable diet." (G. Elliot Smith and Warren R. Dawson, 1924) (224). As the poorer people "ate coarse food mixed with a considerable amount of sand, the teeth rapidly wore down, and as a result the pulp-cavities became opened up;... hence it is common to find alveolar abscesses without

dental caries, but some of the royal mummies suffered from both". (224)

The pre-dynastic period was probably much older than 4,000 B.C. (43). In 1908, G. Elliot Smith wrote (222) that "5000 years ago, in the time of the pyramid builders, dental caries and alveolar abscesses were at least as common amongst the adult aristocracy of Egypt as they are in Europe to-day, although dental caries was rare among people of lower social status, and almost unknown among children. Among several thousand children found in ancient Egyptian graves, I have seen only three instances of caries in deciduous teeth."

In 1932 he wrote (223), as a result of further discoveries, that "among the predynastic and early dynastic Egyptians dental caries was extremely rare, but alveolar abscesses due to the wearing down of the teeth (from the admixture of sand with food) were extremely common. Suddenly in the Pyramid Age in Lower Egypt, when a large number of alien people of so-called Alpine (Armenoid) race had come into Egypt, dental caries in the adult population became so common that more than 80 per cent. of the people had caries, often in extreme forms. A remarkable fact is that the deciduous teeth were wholly immune from caries. In fact it was not until the fifth century of

the Christian era that caries made itself manifest in the deciduous teeth." Also, "It was only when this alien population became more numerous in the third, fourth and fifth dynasties that dental caries became extremely common; so common, in fact, as to suggest that the alien people belonging to the so-called Alpine race were more susceptible to caries than the indigenous people of Mediterranean race."

Derry, writing in 1933,(58) rather disagreed with these observations, and reported that "in 129 skulls of people belonging to the Badarian and early pre-dynastic people ... we only get 5.4% of carious teeth." In skulls of the pyramid age, of the fourth, fifth and sixth dynasties, he found only 17.6% exhibiting caries, or, excluding caries secondary to enamel attrition, only 5.9%. As these figures were based on only 17 skulls, their statistical value is questionable. He doubted even that it was not until the Ptolemaic-Roman period in Egypt that dental caries became common, and stated that "at the present moment, good teeth are the rule among the country people", and "while there appears to be a large number of persons affected with dental caries in the town, the consensus of opinion, in the absence of statistics, is that the condition of the teeth in the

general run of fellahin is excellent."

At a later period, Coptic bodies showed a marked contrast to the pre-dynastic Egyptian skulls, as the teeth were dirty, yellow, and covered with tartar, while attrition was not marked. "It would be difficult to find anywhere so many diseased teeth as in these Coptic bodies. Practically every skull ... had some serious dental defects." (Ruffer, 1921) (211). Caries was present in many cases.

Other ancient skulls had comparatively little caries; G.M. Morant, (1933) (175) wrote, "It is a matter of common experience that the teeth in ancient skulls are, on the average, far better preserved than those of a cemetery population to-day can be": and in twelfth and thirteenth dynasty Nubians he gave in a series of 193 adult skulls with complete palates, 64% males and 69.5% females as having no teeth lost before death.

In 1870, J.R. Mummery (182) published an account of his investigations of ancient British skulls, chiefly from the ancient British tumuli of Wiltshire. Nearly 3,000 skulls were examined, and he tabulated 1,658 of them, "rejecting those of which the authenticity was doubtful, or from which too many teeth had fallen out."

The Dolicocephalic type belonged to the Stone Age, in this country approximately 3,000 - 1,000 B.C. (43)

The Brachycephalic type belonged to the Bronze Age, from 1,000 B.C. to the start of the Christian era. These latter were a more civilized race, agricultural, "said by Caesar in the fifth book of his Commentaries to have exactly resembled the people inhabiting Belgic Gaul ... The change of diet and habits accompanying a higher degree of civilization appears to have had a deteriorating effect on their teeth." (182)

| Ancient Races | Number of skulls | Total cases of caries | Not exceeding 2 teeth | Not exceeding 4 teeth | Not exceeding 6 teeth | Extensive caries | On approximal surfaces | Alveolar abscess | Destructive attrition | Absence of 3rd molars | Lower 3rd molar beneath coronoid process | Other irregularities | Width of arch at 1st molar | Percentage of cases of caries | Approximate proportion of cases of caries (One case in ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ancient Britons (Dolicocephalic) | 68 | 2 | 2 | - | - | - | 1 | - | 11 | 1 | - | - | 2 1/8 to 2 5/8 | 2.94 | 34 |
| Ancient Britons (Brachycephalic) | 32 | 7 | 2 | 2 | 3 | - | 6 | - | 10 | - | - | 3 | 2 1/8 to 2½ | 21.87 | 5 |
| Ancient Britons (Canon Greenwell's explorations) | 59 | 24 | 7 | 7 | 1 | 9 | 9 | 13 | 26 | 7 | 8 | 4 | 2 1/8 to 2½ | - | 2 |
| Ancient Britons (Miscellaneous) | 44 | 9 | 3 | 4 | 1 | 1 | 5 | 4 | 20 | 3 | 4 | 2 | 2 1/8 to 2½ | 20.45 | 5 |
| Romano-Britons | 143 | 41 | 5 | 9 | 8 | 19 | 29 | 9 | 6 | 8 | 4 | 5 | 2 1/8 to 2 5/8 | 28.67 | 5 |
| Anglo-Saxons | 76 | 12 | 7 | 5 | - | - | 5 | 1 | 7 | 5 | 1 | 1 | 2 1/4 to 2 5/8 | 15.78 | 6 |
| Ancient Egyptians | 36 | 15 | 2 | 1 | 1 | 11 | 12 | 6 | 26 | 2 | 1 | 2 | 2 1/8 to 2 5/8 | 41.66 | 2 |

From his figures it is seen that the most primitive race (Stone Age) had the best teeth, and the most highly civilized (Romano-Briton) the worst. After the departure of the Romans and the invasion of Britain by more barbarous tribes, the standard of luxury fell and the condition of the teeth improved, as in the Anglo-Saxons, in whom Fullerton, (1913) (79) found attrition but no caries.

Mummery's Egyptian skulls were mostly from mummies, and therefore of the more aristocratic and luxurious individuals, and he "found a great difference in the condition of the teeth in the various groups of skulls."

Patrick (1894) (191) gave the results of an "examination of prehistoric crania" deposited in six of the principal museums of the United States. Out of a total of 46,657 teeth examined, 2,886 were "decayed", a percentage of only 6.1. 11,338 teeth were "diseased", a percentage of 24.3. Owing to the vagueness as to which teeth are regarded as "diseased" (the term seems to include mere malposition) the value of the report is somewhat diminished.

M. Baudouin in 1920 recorded 6 out of 79 Stone Age jaws as showing signs of caries, while 5 out of 130 detached teeth were carious.(8)

J.H. Mummery in 1922 described (179) a third molar of neolithic age from a British barrow, which "showed more numerous impeffections of structure than any apparently sound modern tooth I have examined. ... Despite the fissures and defects in the enamel, there was not a trace of caries."

More recently (1933) I had an opportunity, thanks to the courtesy of Dr. E.E. Henderson, (102) of examining a number of skulls which he had caused to be excavated from a leper colony at Bruce's Well, St. Ninians, near Prestwick in Ayrshire. These remains were supposed to be about 600 years old and were presumed to be from leprous persons of the poorer class. Out of 260 teeth examined, five were definitely carious, four cavities being interstitial and one being on the occlusal surface of a lower molar. This gives about 2% of carious teeth. Almost all the teeth showed signs of more or less marked attrition, and in the older skulls many had heavy deposits of salivary calculus (tartar) with, in some cases, marked absorption of the margins of the alveolar process. In other words, the owners of the jaws apparently suffered from so-called "pyorrhoea".

The mandibles and skulls were mixed and, in many cases fragmentary, so it was not possible to estimate the percentage of individuals afflicted with dental caries.

These findings in a small number of teeth (260) are similar to the condition reported with regard to the poorer classes of ancient Egyptians.

Turning to the dental condition of existing primitive races, it is found that a vast amount of work has been recorded. Much of this is of little value, being often no more than the opinion of explorers based on mere impressions alone. On the other hand, much painstaking labour has been expended to very little purpose. One of the most complete reports was that of J.R. Mummery (1921) who made world-wide investigations. His results are perhaps best given in his tabulated form.

| Modern Races | Number of skulls | Total cases of caries | Not exceeding 2 teeth | Not exceeding 4 teeth | Not exceeding 6 teeth | Extensive caries | On approximal surfaces | Alveolar abscess | Destructive attrition | Absence of 3rd molar | Lower 3rd molar beneath coronoid proc. | Other irregularities. | Width of arch at 1st molar | Percentage of cases of caries | Approx. prop. of cases of caries. 1 in | Food | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Esquimaux | 69 | 2 | 2 | - | - | - | - | - | 22 | 2 | - | - | 2 3/8 to 2 6/8 | 2.89 | 34 | Seal, walrus, whale's flesh and blubber, Reindeer, fish, sea-fowl. | Zygomatic arch very powerfully developed. Secondary dentine filling pulp cavity. |
| North Americans(Coast) | 56 | 2 | 2 | - | - | - | 2 | - | 32 | 1 | 1 | - | 2 3/8 to 2 6/8 | 3.57 | 28 | Salmon, moose, reindeer. | Teeth very extensively worn. Secondary dentine formed No alveolar abscess. |
| North Americans(Interior) | 23 | 2 | 1 | - | 1 | - | - | - | 2 | 3 | 1 | 1 | 2 3/8 to 2 5/8 | 8.69 | 11 | Musk ox, moose, bison, beaver & other fur-bearing animals, wild roots. | Teeth much less worn. Secondary dentine formed. |
| South Americans (Chili) | 19 | 3 | 2 | - | 1 | - | 1 | - | 3 | - | - | - | 2 4/8 to 2 6/8 | 15.78 | 6 | Wheat, maize, beans, figs, grapes, pine-kernels, dried beef. | Teeth considerably worn. Secondary dentine formed |
| Fiji Islanders | 38 | 2 | 1 | - | 1 | - | - | - | 4 | - | - | - | 2 4/8 to 2 7/8 | 5.26 | 19 | Human flesh, pigs, turtle, fish, maize, taro bread-fruit, plantain, cocoanut. | Teeth sometimes considerably worn. No abscess. Caries very slight in degree. |
| Polynesian (various) | 79 | 8 | - | 1 | 3 | 4 | 1 | 8 | 15 | 9 | - | - | 2 3/8 to 2 5/8 | 10.12 | 10 | Pork, fish, cocoanut, yam, plantain, sugar-cane, bread-fruit. | Teeth often extensively worn. Alveolar abscess frequent. Caries more extensive. |
| Sandwich Islanders | 21 | 4 | - | 1 | - | 2 | 3 | 4 | 4 | - | - | - | 2 3/8 to 2 5/8 | 19.04 | 5 | Goat, pig, turkey, cocoanut, bread-fruit, plantain, taro. | Teeth ill-developed. Abscess frequent. A case of honey-combed teeth. |
| New Zealanders | 67 | 2 | 1 | 1 | - | - | 2 | - | 25 | 3 | 1 | - | 2 4/8 to 2 7/8 | 2.98 | 33 | Human flesh, pork, fish, shell-fish, tree-fern roots, maize. | Maxillary arch very expanded. Caries very slight in degree. Teeth worn. Secondary dentine. |
| Australians | 132 | 27 | 8 | 2 | 10 | 7 | 15 | 11 | 28 | 1 | 5 | 3 | 2 3/8 to 2 6/8 | 20.45 | 5 | Kangaroo, opossum, turkey, emu, iguana, ants, frogs, yams, wild roots. | Excessive abscess following attrition. Caries often very extensive. |
| Tasmanians | 33 | 9 | 3 | - | 2 | 4 | 8 | 4 | 6 | 2 | 2 | 4 | 2 3/8 to 2 5/8 | 27.27 | 4 | Kangaroo and other game, roots, shell fish. | Similar condition, in aggravated proportion and degree. |
| Malays | 24 | 3 | 2 | - | 1 | - | - | - | 2 | - | - | - | 2 3/8 to 2 6/8 | 12.5 | 8 | Birds, fish, bananas, cocoanut, yam. | Teeth usually very fine. Incisors often filed on the labial surface. |
| Chinese | 27 | 10 | 1 | - | 3 | 6 | 6 | 5 | - | 2 | 1 | - | 2 2/8 to 2 5/8 | 37.37 | 3 | Pork, poultry, fish, rice, millet, salted cabbage, vegetables. | Teeth often extensively carious. Alveolar abscess frequent. Little attrition. |
| East Indians (North) | 152 | 9 | 9 | - | - | - | 1 | - | 8 | 1 | - | - | 2 2/8 to 2 6/8 | 5.92 | 17 | Wheatmeal bread, beans, milk and its products. | Teeth very fine, white and regular, in some cases much worn. Secondary dentine. |
| East Indians (South) | 71 | 10 | 5 | 2 | 3 | - | 4 | - | 10 | - | - | - | 2 1/8 to 2 4/8 | 14.84 | 7 | Rice, millet, pulse, fish, sheep, pork, wild animals, roots. | No case of alveolar abscess. Teeth less robust, often deeply worn. |
| Africans(East) | 33 | 8 | - | 1 | 3 | 4 | 3 | 6 | 4 | 1 | 2 | 2 | 2 1/8 to 2 6/8 | 24.24 | 4 | Millet, maize, wild roots, antelopes, elephants, otter. | Caries and abscess frequent and extensive. Front teeth often filed to a pointed form. |
| Caffres | 49 | 6 | 5 | - | 1 | - | 1 | - | 6 | - | 1 | - | 2 3/8 to 2 7/8 | 14.28 | 8 | Millet, maize, milk, curds, fish, (beef occasionally) | Teeth often singularly fine. Secondary dentine formed when much worn. |
| Bosjemen and Hottentots | 29 | 6 | - | 3 | - | 3 | 3 | 2 | 9 | 1 | - | - | 2 1/8 to 2 3/8 | 20.71 | 5 | Wild animals generally, roots and berries. | Teeth often destructively worn resulting in abscess. Caries extensive. |
| Africans(West) | 236 | 66 | 22 | 7 | 23 | 14 | 35 | 31 | 23 | 2 | 6 | 9 | 2 2/8 to 2 6/8 | 27.96 | 4 | Millet, wild animals, fish, dog, roots. | Caries often extensive. Frequent irregularity and supernumerary teeth Abscess frequent. A central incisor often extracted. |
| Ashantees | 92 | 11 | 6 | 2 | 3 | - | 4 | 1 | 7 | - | - | - | 2 4/8 to 2 7/8 | 11.95 | 8 | Maize, millet, bananas, yams, beef, mutton occasionally. | Teeth usually very fine. Well formed supplementary teeth of frequent occurrence. Alveolar abscess rare. |

From this table it is seen that the Maoris of New Zealand, the Esquimaux, and the coastal North Americans had the smallest incidence of caries. These people fed mainly on flesh, and the Esquimaux in addition ate large quantities of fat blubber. It is seen that the meat-eaters had on the whole better teeth than the vegetarians. The dental condition of the Esquimaux is interesting in view of their mode of life, with its lack of green vegetables, comparatively small amount of sunshine, and large consumption of fats and proteins, while their water supply, obtained from melting snow, is almost devoid of calcium salts.

Pickerill's figures (194) for Maoris are even more significant, for he found that only 0.76% of the skulls of uncivilized Maoris showed signs of dental caries, while in the modern civilized Maoris, 95% were afflicted with the disease. This he attributed to the change in diet from primitive to civilized food.

The criticism has been made with regard to Mummery's figures that the diet of many of these tribes may have altered in recent years.

At varying intervals between 1904 and 1919, S.Colyer (50) has published information he collected regarding the primitive tribes of Central Africa. He

found great variations in the incidence of caries among the several tribes, which he attributed to differences in the food.

The Plateau tribes of Northern Rhodesia (Wasenga, etc.) had very good teeth, and 96.6% were free from caries. The Nyam-nyam tribe had excellent teeth. The Barotze of the upper Zambesi were a river people, and their teeth had been getting worse in the last 60 to 70 years, coincident with a change in their diet by the addition first of sugar, and them of cassava. (This is interesting in view of the commonly believed deterioration of the teeth of people in this country in the last 70 or 80 years.)

The "Raw Kaffirs" or "Red Kaffirs" of the Transkeian territories were still uncivilized, but suffered from extensive caries similar to that of civilized man, both among children and adults, but their food was in some ways similar to a "civilized" diet, in containing much sugar, and being soft.

Tribes from the region of Lakes Mweru and Bangweolo had only 9.8% of individuals with caries. They were very primitive. The Pygmies, for instance, were said to be without the use of fire, and in the region between Tanganyika and the Lualaba never cooked

their food. "The Bantus are largely immune to caries"(218)

In Johannisberg, Shaw (1932) (219) reported that aboriginal native children in general had bad temporary teeth, but their "permanent teeth, especially among boys, are better than those of European lads of the same age." Friel (1910) (77) reported that 61.4% of African natives living in Johannisberg were afflicted with caries, and it is interesting to note that he found 78.69% of females and only 53.84% of males among the adults as having caries. He attributed the caries to "white man's food", especially hot tea and coffee. Shaw found that 74.1% of native children had caries.

In 1908, Ottofy (189) found that among the inhabitants of the Phillipine Islands, the Igorots, the most primitive and barbarous race, had only 2.05% of permanent teeth caries, and 68.14% of the children had perfect sets of teeth, while the Filipinos, who had come into contact with the white man, had 20.9% of permanent teeth carious, and only 4.8% of the children had perfect dentitions. Out of 113 Igorot children, 12 were weakly and ill, and had 23 permanent teeth and 120 deciduous teeth carious, while the remaining 101 had only 24 permanent and 20 deciduous teeth carious.

In modern civilized countries, the prevalence of dental caries is appalling. There is thought to have

been an increase in the disease within the last 60 or 70 years. It would be interesting to examine skulls prior to this period, and it was disappointing to find that out of many hundreds of such skulls examined by me in the catacombs of Paris, not one had any teeth left *in situ*. These skulls were from people who had died from 60 to 80 years ago, and judging by the sockets, most of the teeth had been removed *post mortem*.

In the present century, sufficient statistics are available to indicate how widespread the disease is. In Shropshire in 1914, 97% of children at the age of 12 and 95% at the age of 5 were afflicted with caries (51). The British Dental Association investigation (1891) (28) gave 89% of 10,500 mouths as having dental caries present. In a series of 4,000 children aged from 5 to 12 years which I had an opportunity of inspecting, none of whom so far as could be ascertained had ever had any dental treatment whatever, 94.2% showed some degree of dental caries, *in 1927.*

Statistics from other European countries show that dental caries is just as prevalent there as in this country, and the same applies to America, where in 1931 Brekhus (270) reported on an examination of 10,445 University students whose average age was 18, and found

that on the average over the three years 1928-1930 only 2.05% had perfect mouth conditions. This group was of "socially favoured individuals, economically and educationally."

In Johannisberg, Shaw (219) found that 93.35% of 600 school children from 6 - 16 years of age had caries, and the average number of carious teeth was 4.82 per child for all children. He pointed out that rickets is almost unknown in Johannisberg and in South Africa generally, yet dental caries is just as prevalent there where the actinic rays of the sun are four or five times more intense than in England.

The disease also occurs in animals, but to a very much less extent than in human beings.

Monkeys appear to be almost the only animals which exhibit caries when living in the wild state, but in captivity many other species may be affected, although the usual disease of captivity is so-called "pyorrhoea". In 1921, J.F. Colyer reported (271) 6 out of 300 skulls (2%) of Macasus rhesus as showing caries. Attrition was marked, even though the animals were all young. Fujita in 1933 reported (80) caries in M. fuscatus.

Of 851 skulls of Old World monkeys from the wild state in the British Museum, 2.8% show caries, while

of 609 New World monkeys, 2.4% showed caries(51). Monkeys in captivity showed an incidence of dental caries of about 10%;"pyorrhoea" is more common.

In captive or domestic animals, such as the horse, caries occurs, but it has been noted that the dog is remarkably immune to caries, even when attempts are made to produce it experimentally. (Mellanby (1930) (167), and others). In no species of animals has such a high incidence of dental caries as in Man been observed. In some animals where erosion occurs, caries does not supervene, and in the case of a sea lion (Otaria jubata) reported by Murie in 1870 (184), all the teeth had annular erosion patches round the necks, but the worn surfaces were "blackened, smooth and highly polished" and no caries was seen. This appearance resembles so-called "arrested decay".

A very complete account of dental caries in wild animals and those living in captivity is given in his Dental Board Lectures, 1931 by Sir Frank Colyer (47), who has made an extensive study of this subject.

As it has been indicated in the foregoing brief survey that dental caries is a widespread disease of the human race, more common in modern civilized countries

than in primitive and prehistoric peoples, it now seems desirable for me to state what is meant by dental caries. The difficulties of framing an accurate definition are manifest, and no entirely satisfactory definition appears to have been given.

Disease "may for practical purposes be roughly defined as any state of a living organism in which it fails to respond normally to the conditions of its environment; or, in other words, in which there is a failure of some of its normal activities. Causes of disease are :

1. Predisposing. Conditions which act upon the living organism so as to render it susceptible to outside agents, and

2. Exciting. Those agents themselves.

Thus, starvation or overwork may render a person susceptible to attack by a parasitic organism which would otherwise be unable to settle in his tissues and cause disease." (Green) (92).

"A disease may be defined as any influence of whatsoever nature which is capable of disturbing the nutritive balance of any portion of the body. The branch of study which deals with the causes of disease is called Etiology. The causes of disease are exciting and predisposing, extrinsic and intrinsic." (Burchard & Inglis) (39).

"Caries of the teeth is an active destruction, by outside agencies, of formed materials which are the result of cell activity (the tissues themselves being passive). The cellular activities of organs and tissues of the body may have an influence, but this is only in producing those conditions of environment which render the activities of the destructive agents efficient in their action upon tooth tissues." (Kirk)(135)

Dental caries is "the progressive molecular disintegration of a tooth." Widdowson. (259)

"Caries of a tooth is the chemical decomposition of the earthy salts of the affected part, sometimes, but not always, accompanied by disorganization of the animal framework of this portion of the organ." (Harris)(97)

"Caries of the teeth is a chemical decomposition of the earthy part of any portion of a tooth, accompanied by a partial or complete disorganization of the animal framework of the affected part. (Gorgas)(90)

"The process (of caries) is essentially one of fermentation." (McAsh)(147)

"Dental caries may be roughly defined as a process of tooth destruction, and although involving certain complex phenomena resolves itself into an acid fermentation whereby lime salts of the tooth are first dissolved and subsequently broken up by micro-organisms. It is characterized chiefly by a localized cavity, concavity or area containing decalcified tooth-structure." (Gilmour.) (87)

"Dental caries is an acid fermentation during which the inorganic portions of teeth are first dissolved and then the organic portions disintegrated by the action of micro-organisms. More shortly, decalcification of the teeth followed by dissolution." (A. Hopewell-Smith) (107)

"Dental caries is disintegration of tooth substance extending from without inwards towards the pulp, caused by external agencies; the most prevalent disease of the human race, greater with civilization." (E.B. Dowsett) (62)

"Caries is ... a progressive destruction of the tooth tissue brought about by the agency of micro-organisms" (Colyer) (81).

It seems desirable to state clearly our point of view in approaching the subject of dental caries, and

while realizing fully the risk involved in enunciating a definition which may become a target to be shot at, we may say that "dental caries is a progressive destruction of the substance of the living tooth not due to attrition, abrasion or erosin."

This definition purposely excludes the decay of dead teeth and the production of artificial caries of dental tissues in vitro, because the essential characteristic of any disease appears to be the possibility of a vital reaction on the part of the body in response to the nocuous stimulus or stimuli.

In dental caries, the vital reaction consists in the production of a translucent zone in the enamel as described by Mummery in 1926 (180), and the translucent zone of Tomes in the dentine which was recorded in 1848 (242). More recently Fish (1932) (70) has demonstrated that the translucent zone of Tomes occurs only in certain situations, while in other places the usual response is the deposition of secondary dentine at the periphery of the pulp chanber over the proximal ends of the affected dentinal tubules. Bödecker and Applebaum (1933) (24) claim to have established the occurrence of both reactions.

We cannot agree with the latter part of the state-

ment by Colyer and Sprawson (1931) (51) that "With caries produced by artificial means there is no translucency, but translucency forms no essential part of the carious process", for to us it would seem that the essential difference between caries of a living tooth, a part of the body, and decay of a "dead" tooth, is the power of irritability as evidenced by the formation of a translucent zone in the living tooth.

In 1932, Cahn (40) wrote "The pulpless tooth ... may become brittle and fracture, but active caries ceases with the death or removal of the pulp."

The production of artificial caries in vitro results in no vital reactions - translucent zone or secondary dentine deposition - and is merely a saprophagous process of disintegration.

In the days when natural teeth were used on artificial dentures, decay of such teeth occurred, and in some cases a translucent zone in the dentine was said to be present; but this was explained by the carious process having started while the teeth were yet vital in the mouths of their original owners (Miller, 1903) (171). There is no satisfactory evidence known to us of a translucent zone, much less of secondary dentine, being actually formed as a result of decay of a dead tooth. It is well

to appreciate the distinction between a dead tooth and a devitalized tooth. Both are pulpless; but when a tooth is devitalized, as in operative dental procedures, it has no living pulp and the dentine and part of the cementum are dead, but the outer layer of radicular cementum still contains living cells and through its attachment to the peridental membrane receives nourishment and is therefore vital and still tolerated by the tissues. There is thus an outer layer of living cementum with living cells, and an inner layer of dead cementum with dead cells. This condition has been well described and illustrated by Stewart-Ross (1933) (233) who also details the various forms of response to irritation on the part of the peridental membrane and the cementum. Such a devitalized tooth, though tolerated in situ by the body, can display none of the reactions to decay characteristic of a living tooth. Only so long as the outer layer of cementum remains alive is the tooth tolerated by the tissues.

When a tooth becomes "dead", as may happen when arsenic used to devialize the pulp penetrates and kills the dentine and cementum - and according to Stewart-Ross kills the peridental membrane also - it acts as a foreign body and is gradually extruded from its

alveolus (Wilkinson, F.C.(261), Woods, E.C.(263) Young, R.J.E.(268)).

An extracted tooth, cut off from its source of nourishment, is, of course, dead.

"True caries of the enamel" stressed by some writers, is difficult to understand, because dental caries in the widest sense may affect enamel, dentine or dementum. Hess and Abramson (1931) (103) regard only dentinal caries as true caries. These distinctions are rather artificial, and are scarcely sound from a comparative anatomical point of view. It is merely a morpholigical peculiarity that human teeth happen to have a layer of enamel covering the entire crown, and in a few cases this does not hold good. Sometimes the enamel does not quite meet the cementum and an annular area of dentine is exposed at the cervical margin. This is said to occur in 10% of cases (106) but this seems rather a high percentage.

We do not need to look further than the horse or the elephant, (Tomes, 1876)(238) to find teeth composed of dental tissues arranged in rows so that enamel, dentine and cementum are all external, in turn, and in fact the very efficiency of the horse's tooth as an organ of mastication depends on the different degrees of hardness of the three tissues resulting in unequal wear

and consequent roughness of the occluding surfaces. In the horse, dental caries is not specially liable to supervene, yet it is generally supposed and frequently observed in human teeth that when the dentine is exposed by fracture of the enamel or otherwise, caries develops. There is thus some clinical support for the idea that the enamel in human teeth protects against caries, but in order to approach the subject with an unbiassed mind it is necessary to appreciate the fact that in many other animals enamel is not necessarily the outermost dental tissue. This point has been stressed by J.A. Woods (1916) (266) and others.

Colyer (1931) (51) distinguishes clinically two kinds of caries, starting in (a) the enamel, or (b) the cement or dentine respectively.

The theories advanced as the cause of dental caries are many and varied. They are nearly all concerned with human teeth, covered by enamel, and therefore tend to lose sight of the fact that caries may equally well attack dentine or cementum. In a normal human tooth, it is true that enamel is the only tissue to which fluids of the mouth have access under normal conditions, and therefore enamel must usually first be attacked. Where dentine is exposed by developmental failure of enamel

completely to cover the crown, or at the cervical margin as mentioned above, or by injury, or where cementum is exposed by recession of the gums or in the fourth stage of tooth eruption described by Orban, (1929)(186) caries may start in either of these tissues without having first to penetrate the barrier of enamel, if caries starts externally to the tooth.

The idea that caries starts from inside the tooth is not now held by many observers, and is denied by most. It was thought by Galen (94) that caries was produced "by the internal action of acrid and corroding humours", and John Hunter in 1778 mentioned the internal attack as one type of caries, since he stated (116) that "it begins sometimes in the inside of the Tooth, although but rarely. In this case the Tooth becomes of a shining black, from the dark colour being seen through the remaining shell of the tooth, and no hole is found leading into the cavity". Fox in 1806 and Bell in 1829 supported the theory of the internal origin of caries.(204)

In 1913, von Beust stated (13) "Caries is a disease which has its origin within the tooth." There are many ingenious arguments in favour of this theory of the internal origin of caries, but experimental proof seems to be completely lacking, and in the meantime I propose

to omit any discussion of it. The condition of so-called "pink spot" - chronic perforating hyperplasia of the pulp - is actually an absorption of dental tissue by odontoclastic cells of the pulp and is not dental caries. (J.H. Mummery,(178), J.A. Woods(264 265))

It is interesting to note that Magitot (1872) (158) stated in the general conclusions of his monumental work "1. La carie dentaire est une altération purement chimique de l'émail et de l'ivoire des dents. 2. Elle procède constamment de l'extérieur à l'intérieur de l'organe; il n'éxiste aucun exemple rigoureusement constaté de <u>carie interne</u>."

Most investigators to-day regard dental caries as a disease commencing externally, and Sim Wallace wrote "dental caries is the result of a chemico-parasitic process originating outside, and independently of, the substance of the tooth." (253)

The most generally accepted theory of dental caries at the present time is the chemico-parasitic theory formulated by W.D. Miller in 1882 and modified slightly in the intervening years.

Briefly, the first step is decalcification of the enamel (or demineralization, as it is nowadays more

grandiloquently termed). This is caused by acids, chiefly lactic, which are produced by the fermentation of carbohydrates lodged round the tooth. This explains plausibly why stagnation-areas of teeth show decay, while smooth surfaces and self-cleansing areas remain usually free from caries. It also explains why there is sometimes at the very earliest stage a ring of decalcification round the area of contact between two teeth, because soluble carbohydrates are held by capillary attraction, and ferment in this position, the actual so-called "contact-point" being a small area at first unaffected. Caries also frequently develops just under the "contact-point", where there is an area of stagnation sheltered from the detergent effect of mastication and inaccessible to the toothbrush bristles. Deep fissures and pits in the enamel surface are places where stagnation occurs and carbohydrate ferments, and irregularity in the position of the teeth often results in recesses where food collects and caries is likely to occur. It is a matter of common clinical experience that areas of stagnation, however situated, are more liable to caries than other parts of the teeth. It is similarly observed that where partial dentures are worn constantly and not kept clean, a ring of decalcification

and later caries occurs round the neck of a tooth encircled by the edge of the denture or a clasp. Nasmyth's membrane, if present, offers little or no protection to the enamel, as it is easilt penetrated by the acids.

The acids produced by the fermentation of carbohydrates having decalcified the enamel, disintegration of the enamel substance takes place, since its organic content is so small as to be inadequate to hold together the loosened prisms. It is immaterial from this point of view whether the interprismatic substance only or the actual enamel prisms themselves are dissolved by the acid. When the enamel is penetrated, the dentine is exposed to attack, but at this stage there is frequently a rapid spread in all directions along the dentine-enamel junction, especially if this is not well formed. In this way, a large area of enamel may be undermined and on sudden stress in mastication may give way, revealing a large cavity, and giving rise to the popular fallacy that the decay and cavity-formation occurred almost instantaneously. The acids continue their attack on the dentine, and bacteria which have found a lodgement in the cavity cause proteolytic decomposition of its organic substance. Such bacteria may be found deep in the dentinal

tubules. Usually the organic matrix of the dentine remains to preserve its form, and in some cases where rapid and complete dissolution of the enamel has occurred, the brown and softened dentine may remain and preserve the shape of the tooth-crown.

The actual details of the process, such as the mechanism of lactic acid production from carbohydrates by fermentative processes, may be found in text-books dealing with the chemistry of dental caries.

The essential point to note in this theory is that it covers the clinical features usually observed in living and dead teeth, as well as the phenomena of experimental caries. It regards the process as non-vital, a mere chemical dissolution of tooth substance brought about firstly by acids alone, then by invading saprophytic bacteria. Modifications of this theory, such as the initiation of decalcification by a bacterial plaque adhering to the tooth surface, or by acid-producing organisms, rather than by purely physical agencies, do not alter its essential features.

Finally the pulp is reached, and after a varying period of inflammation, characterized by intense pain, dies.

This theory was not entirely new, the acid theory of decay having been suggested by Robertson many years previously (204), while Bond, (1852) (25) regarded the process as purely chemical, and Harris (1839)(96) believed that caries was "caused by external agents - by the action of some corroding menstruum upon their external bony surfaces." Bacteria were also suggested.

In many cases of dental caries this is just what appears to happen. In the human subject, there is usually very little resistance to the disease, and, once started, it almost invariably proceeds to complete destruction of the tooth, unless interfered with by operation. In human subjects of to-day, an attack of dental caries usually overwhelms such feeble defences as the human tooth possesses, and penetrates enamel and dentine, reaches the pulp and kills it. In a few exceptional cases the occurrence of arrested caries may be observed. This has been regarded as a curious but unimportant phenomenon, but it seems likely that by a study of this and allied processes real knowledge of the cause of dental caries may be attained.

The chemico-parasitic theory does not account for arrested caries, which may be due to some reactive

process on the part of the tooth, nor does it explain why some people who have dirty mouths with every facility for carbohydrate stagnation do not develop caries. It is possible that erosion cavities, like arrested decay, may be evidence of a vital reaction.

It appears that in Man to-day, dental caries as a disease is rampant, and in most cases the individual has practically no resistance. In dogs, on the other hand, the disease is rare, and is difficult to produce experimentally. From Fish's dye experiments(70), the most obvious difference between the teeth of dogs and those of man is that dogs' teeth are more permeable, and those of young dogs more so than those of old ones. This suggests that permeability of the teeth may be a factor in the comparative immunity of dogs to caries, if the body fluids have any protective or defensive action against the cause or causes of dental caries.

The almost complete immunity of animals to dental caries, and the almost universal susceptibility of civilized human beings, makes it not unreasonable to suppose that dental caries may be, like syphilis, a disease which affects the human race almost exclusively.

Certain species of animals are susceptible to certain diseases, while others are not, e.g. Tuberculosis affects cows but not goats, and it may be that dental caries is essentially a human disease.

The chemico-parasitic theory was formulated when enamel was regarded as an inert, impermeable, unchanging, non-vital structure, yet it can be applied almost equally well to enamel as conceived to-day. It is now generally agreed that enamel has an appreciable organic content. This was perhaps first noted by John Hunter in 1771 (115). In parentheses, we may remark on the extraordinarily accurate observations of this worker in the eighteenth century not only on a question which was not settled until 1929, but on every other subject that he touched. Yet, as Trotter says,(274) "it is impossible, however, not to be struck by a certain disproportion between the amount of genius and energy that was expended, and the harvest of substantial knowledge that was gathered."

The organic content of human enamel was estimated by Sprawson & Bury (1929) (51) as 0.15%; and by Rosebury (1930) (206) as 0.3o - 0.54% in the form of keratin. On this slender percentage, Bödecker (1929) (22) has elaborated a theory of dental caries based

on the permeability of human dentine and enamel, but C.N. Johnston (126) regarded such theories as too fantastic for serious consideration.

The chemico-parasitic theory, although it explains most of the phenomena observed in dental caries, would appear to be no longer tenable as a complete explanation. It is a matter of common clinical experience that some people are more liable to have decayed teeth than others; and furthermore that some people whose mouths are filthy have very good caries-free teeth, while others whose mouths are kept scrupulously clean frequently have many teeth affected with dental caries. It thus appears that some people are immune to dental caries while others are susceptible. To explain this immunity to caries, many theories have been formulated.

Heredity.

In private dental practice where records are available for many years, it is commonly observed that some families have good teeth, others have bad, and this can be followed from one generation to another. It thus appears that the tendency or predisposition to dental caries, and immunity to it, must be inherited. In cases where brothers and sisters differ in this respect, an

enquiry will usually elicit the fact that the parents or grandparents differed also, and the incidence in the children can usually be easily explained by the theory of Mendelian segregation.

The influence of heredity in connection with dental caries was recognised by G.V. Black (17) and others, while S.P. Mummery (183) considered it the chief factor. It is of course not the acquired characteristic of dental caries which is inherited but the predisposition to the disease, just as the predisposition to tuberculosis or cancer may be inherited.

This factor is not at present of any practical importance in the control of dental caries, as one cannot be "careful in choosing one's grandparents", but in a eugenically-minded nation of the future it might be taken into consideration.

In our civilized communities, natural selection (55) (55A) is in abeyance. Everything is done to preserve the weak and unhealthy who under natural conditions would die out. Savages with decayed teeth are less fitted to survive in the struggle for existence and simply perish, (Kerr, 1920(132)) so that among savages sound teeth are the rule. Any variation towards defective teeth is checked by the ruthless

law of survival of the fittest, and so only the physically fit are left to produce offspring.

In modern civilized countries, defective teeth are restored by fillings or replaced by dentures, and their owners are thus free to reproduce children with their own inherited tendency towards the disease. In war, the fittest members of the race are killed, whilst the unfit remain at home to breed a race with inherited tendencies, by variation, to unfitness. Thus, the physical standard of the race tends to decline after a war, the condition of the teeth included. Similarly, mental defectives are allowed to breed still more defective offspring, while the weak and sickly are preserved from death by the advances of modern medical science, so that they also may reproduce their own defective kind.

At the present time, dental caries is so widespread in all modern civilized communities that almost every individual must inherit some tendency towards it, and certain adjuvant factors are present under civilized conditions which determine the individual's surrender to the exciting cause or causes. In those few individuals who even under conditions most favourable to the production of dental caries yet remain free from the disease, we may suppose that there is little or no

inherited predisposition, and their own resistance is sufficient to determine their immunity.

It may be noted that supporters of the Sim Wallace theory consider that diet and dietetic habits are alone responsible for caries, and deny that heredity has any influence. They maintain that what was formerly considered to be a hereditary disposition is in fact only a similarity of environment of the members of the same family, class, or even race. This, however, would not explain why in children living in an institution, such as a school, under precisely the same environmental conditions, some develop more caries than others.

On the other hand, hereditary predisposition may be due to the physical factors supposed to influence the incidence of dental caries, such as deep fissures, on molar-occlusal surfaces, overcrowding or irregularity of the teeth, hypoplasia and so forth; or the chemical factors such as salivary composition, unstable forms of calcium salts in the teeth themselves, etc. Certain familial features, such as the prognathism of the Royal House of Habsburg, are undoubtedly hereditary, and these characteristics may extend to the most minute physical forms, such as enlarged tubercles of Carabelli, or abnormally deep fissures in the enamel.

The exact mechanism of heredity is not definitely known (Crewe)(54) but there is as much reason to assume, from clinical observation, a hereditary predisposition in dental caries as there is in tuberculosis or cancer.

## D I E T

That diet exercises a profound effect upon the teeth is generally held by a large number of observers, who view the subject from two aspects, chemical and physical.

Chemical. It is obvious that, for satisfactory growth to occur, an adequate supply of suitable nourishment must be present. As calcification of the human teeth commences at about the seventeenth week of intrauterine life for the deciduous teeth and at the twenty-sixth week for the first permanent molars, the nutrition of the expectant mother must be included as a factor in the growth of the teeth. Calcium and Phosphorus are the two inorganic substances to which most attention has been directed. As a large bulk of the tooth substance consists of calcium phosphate, sufficient of these elements must be present for growth requirements up till the time when the third permanent molars are fully erupted, usually at from sixteen to twenty-one years of age, if perfectly formed teeth are to be produced.

Calcium and Phosphorus. It was formerly widely held that dental caries was due to calcium deficiency, and it was at one time thought that the appalling dental condition of Glasgow children was due to the soft water

supplied to the city. Röse (205) has reported investigations on this subject with regard to the inhabitants of Dalarne and Gothland. The Gothlanders had the worst teeth in Sweden, yet their drinking water had plenty of lime in it, though little permanent hardness. He attributed their bad teeth to their consumption of soft, sour, black bread containing sugar. In Dalarne, the people with soft water had teeth three times as bad as the Siljansee people, whose water was hard. His conclusion, however, was that the nature of the bread eaten in these districts had more to do with caries than the lime in the w ater. In other districts his results were more definite. Thus, in rural parts of Baden and Thuringia where the water was soft, he found 95% of the children with caries, while in places where the water was hard only 81% were affected. In two other villages, Gunthersthal had soft water and 34.6% of the teeth were carious, while at Uffhausen, with hard water, only 20.7% were carious.

At Stilli, in Aargan, 328 metres above sea level, with very hard water, P. Walter (255) found 18% of the teeth carious, while at Engelburg in Obwalden, 1019 metres above sea level, with very soft water, the

percentage was 36.

The obvious criticism of these observations is that other factors besides hard or soft drinking water may have been responsible for the differing incidence of dental caries, such as diet, sunshine, climate, heredity, race, etc. (c.f. black bread in Gothland and Dalarne). Unless all the other conditions of life of the two communities under comparison except the amount of calcium in the drinking water are the same, it is scarcely justifiable to draw the definite conclusion that this factor is responsible for the differences in the incidence of dental caries, though it may be permissable as supporting evidence.

A more direct comparison was recently (1931) published by Franci(75) in an investigation of children in the commune of Siena at two different periods. In 1913 the water had 32.03 degrees of hardness and 90% of the children had caries with an average of five carious teeth per child. A new water supply was installed with only 3.7 degrees of hardness, and 17 years later, in 1930, the prevalence of dental caries among the school children was exactly the same, viz: 90% of the children had caries with an average of five carious teeth per child. The comparison in these results is

more direct than in Röse's, but the dietetic, hygienic, prophylactic and other conditions of the people may have improved enough to counteract the possible tendency to caries to which the soft water might be expected to conduce, if Röse's theory were correct. No particulars are given of these conditions, but the published results certainly indicate that the state of hardness or softness of the water has no effect on the occurrence of caries.

It may be noted that although Röse has always been cited as supporting the theory that soft water is more conducive to caries than hard water, his published observations seem to indicate some doubt, since, for example, he invokes black bread as a possible factor.

In this country, Cook(53) investigated "the effects of drinking water upon the causation of dental caries in school children". He found that:

"Table A. The harder the water, the better the teeth (total, temporary and permanent hardness),

Table B. The harder the water, the lower the infantile mortality,

Table C. The lower the infantile mortality, the better the teeth,

Table D. The lower the general death rate, the harder the water,

The lower the general death rate, the better the teeth,

Table E. The harder the water, the better the teeth." (See Table A.)

He concluded that there was an association between excessive softness of the water and an increased amount of dental caries, and obversely, but this observation was made only for urban districts. He stressed the fact that it was merely an association of conditions and "must not be regarded as necessarily indicating cause and effect."

The Medical Research Council Report of the Committee for the Investigation of Dental Diseases published in 1925(163) on the dental inspection of 4,000 children in public elementary schools gives results showing the incidence of dental caries in "Town" and "Country" schools in districts with respectively hard and soft water supplies. Without going into actual figures, let it suffice to state that it was found that in the "Town" schools, the hard water group had a very slightly higher percentage of caries than the soft water group, while in the

"Country" schools the soft water group had a more definitely higher incidence of caries.

It is thus seen that opinions are divided regarding the possible effect of hard and soft drinking waters on the incidence of caries. It may be noted that water used in cooking may lose its temporary hardness on boiling, e.g. in making tea, and unless definite information as to the amount of water actually drunk or imbibed with its total contained hardness is given, statistics and conclusions drawn from these observations are liable to error.

On the whole, the available evidence does not seem to prove that hardness or softness of the water has any effect on dental caries. It must be left an open question.

It is interesting to note that R. Hutchison stated (120) "The fear that the use of soft water may lead to the development of rickets is quite groundless. When one remembers that even a hard water only contains about .0002 g. of lime in every 100 cc., and that an infant requires about .32 g. of lime daily, it will be evident that as a source of calcium for the bones water may be practically

disregarded." He also stated, however,(119) that "Hard waters also must be regarded as important dietetic sources of calcium."

It is difficult to arrive at the truth in view of the conflicting nature of the evidence available. The tendency nowadays is to regard the hardness or softness of drinking water as of minor importance in the aetiology of dental caries compared with other factors to which attention has recently been directed.

It has been shown that by feeding increased amounts of calcium to individuals suffering from hypocalcaemia, the normal calcium balance may be restored, while feeding an excess of calcium to normal individuals will not cause a hypercalcaemia, as the surplus calcium is simply excreted by the usual channels.

The mere feeding of excessive calcium to a normal individual will therefore not have any effect, but where calcium deficiency is suspected or proved, then calcium therapy may be of value. It would therefore appear better to be on the safe side and rather have too much than too little calcium in the diet.

In my own experience, the empirical administration of calcium was found definitely beneficial in a few cases of which careful records were kept. The most striking of these was a young male aged 16 years who was 6 feet 0½ inches in height and still growing rapidly. He attended every three months for dental inspection and treatment, and on three successive quarterly visits had from 11 to 14 cavities in the teeth. He was instructed to take calcium lactate 10 gr. t.i.d. At his next two quarterly inspections he exhibited only two cavities. At his third, he had 11 cavities, and enquiry elicited the fact that he had discontinued the calcium for over two months. On recommencing the treatment he remained caries-free for six months, and is now a firm believer in calcium therapy. It appears to me probable that on account of his rapid growth this patient actually was suffering from a deficiency of calcium, and that the administration of extra calcium did cause the improvement in his dental condition.

The other cases observed, to the number of eleven, were similar, but less striking, in their results.

The difficulty with such cases in private practice is to get the patients to adhere rigidly to the régime over a sufficiently long period of time. These results indicate that calcium therapy may affect the incidence of caries in erupted teeth, as distinct from its effect on growing teeth which must have calcium for anabolic processes.

The mode of action, if any, of such calcium therapy on an erupted tooth may be either internal, through the pulp, dentinal lymph circulation, and enamel circulation if such exists; or external, by the fluids of the mouth which bathe the tooth. However the action occurs during a period of rapid growth, it is similar to what is believed to occur in pregnancy.

It is commonly supposed that in pregnancy there is an excessive drain on the calcium resources of the mother in order to meet the needs of the growing foetus. The popular saying "for every child a tooth"

exemplifies the belief that caries is specially liable to occur in expectant mothers, the theory being that calcium is somehow absorbed from the teeth (and bones) of the mother in order to supply the foetus with the full amount required, if for any reason there is a shortage of calcium in the mother's diet.

Hess, Lewis and Roman, however, considered (1932)(104) that there was little to be gained by feeding calcium to the mother as at birth they found only 15 - 20% of the calcium content of fully developed teeth, "and the deciduous molars are less calcified than is commonly supposed."

It is true that many women produce children without suffering any dental deterioration, but these are probably exceptional, since under modern civilized conditions what should naturally be a purely physiological process so often becomes pathological that most expectant mothers show some dental signs. The least of these is a curious translucency of the tooth, supposed to be due to decalcification, though in decalcification due to dental caries the first

sign is a white opacity of the enamel.

The proverbial recognition of this association of dental caries with pregnancy must have some widespread foundation on clinical observation, and in my own experience many cases of it have been noted. It has, however, been shown by Fish (1933)(73) that the calcium content of dentine is remarkably stable, and unaffected by deprivation of calcium in other parts of the body under experimental conditions in animals. This lends some support to the view that the dental caries of pregnancy is due to altered composition of the oral secretions, rather than to an internal derangement of nutrition. Sewell, in 1863 (217), suggested that the apparent increase of the incidence of caries in pregnancy might be due to a more acid condition of the mouth irritating exposed dentine and thus calling the patient's attention to cavities which might otherwise have been overlooked till a later date. Coles (1874)(46) considered increased salivation, of an acid nature, as conducive to caries, aided by morning sickness, or the "gratification of the morbid taste that sometimes

occurs in pregnant women for acid drinks and sour and unripe fruits."

The conclusions of C.D.M. Day (1933)(56) were to the effect that dental caries is more liable to occur during pregnancy, but among many observers the modern trend is to regard the association of caries with pregnancy as a myth.

However this may be, the exhibition of calcium to pregnant women appears empirically reasonable, but the practical objection has been raised that it may cause hyper-ossification of the foetal bones with consequent increased difficulty in parturition. It has been thought that restriction of calcium intake during pregnancy results in smaller foetal bones, and no doubt this, and generalized starvation, would do so, but it is doubtful if an excess calcium intake will cause super-ossification, since, as mentioned above, it has been found that surplus calcium is simply excreted. It might, however, be better to restrict calcium therapy to cases that definitely show signs of a deficiency. In pregnancy, as in some pathological conditions, the assimilation of

calcium is thought, with some reason, to be the difficulty. Assimilation is essential apart from mere administration, and in this connection Phosphorus and various accessory food factors such as Vitamin D are believed to be of importance. Badanes (1929)[7] believed that the balance or imbalance of calcium and phosphorus were more important than the actual total amounts ingested.

While it is thought that dental caries may result from any excessive drain of calcium, it is also sometimes an indication of general bodily debility. An increased incidence of dental caries is frequently observed during or after an illness. A certain experienced dental surgeon, on finding any of his patients with an unusual number of cavities, used to ask if they had been ill, and nearly always found that they had. The teeth may thus be regarded as an index of bodily well-being, a delicate barometer of health. "General diseases ... with resulting vitiated secretions, must be considered as concomitant causes of decay." (Eames, 1899)[63].

In old people who have been free from decay for many years, the sudden appearance of numerous cavities in the teeth is often the precursor of death itself.(157)

Broderick, in 1933,(31) considered that dental caries was a sign of a particular diathesis which he called "sympathetic", associated with a "predominance of the sympathetic portion of the involuntary nervous system, occurring in persons who have a relatively great expenditure of energy, the slenders, those with a tendency to over-activity of the thyroid and adrenals, with a tendency to the formation of too much blood-acid ... the individuals who tend to be introverts." He has for long stressed the antithesis of dental caries and pyorrhoea, both of which he considers due to an upsetting of the normal acid-base balance of the body fluids, caries on the acid side and pyorrhoea on the alkaline side(30), usually with corresponding changes in the saliva.

The systemic aspects of acidosis and alkalosis have been discussed by Graham and Morris (1933)(91). Broderick's theory has not as yet got much experimental or practical proof to support it, but his arguments are plausible.

The amounts of phosphorus and calcium ingested are important and may have some bearing on the relative proportions of each which are actually assimilated. Badanes (1929)(7) found that vitamins and irradiation affect the amounts of these elements absorbed. Downs (1932)(60) found that increased vitamin D accentuated the effects of low phosphorus intake, but apparently aided the tissues to prevent the changes due to deficient calcium; but only when variations between the relative amounts of phosphorus and calcium became quite marked were histological changes noted.

Pattison (1926)(192) recorded that a high calcium content of the saliva could be produced by a diet having a large fat-soluble vitamin content, and conversely a diet with little fat-soluble vitamin and much cereal (especially oatmeal) could produce a

low calcium content, even if the amount of calcium ingested in this diet were the same or greater. He also found that tuberculous children had a lower salivary calcium content than normal.

Lebourg (1928)(146) found that the frequency of dental caries among tubercular patients was only slightly higher than among normal individuals observed - 23.4% - 29% as compared with 20%. As the factors predisposing to tuberculous disease also predisposed to dental caries, he thought that concomitance did not necessarily indicate causality, and emphasised the importance of adequate dental attention for tuberculous patients.

Horton, Marrack and Price (1929)(110) thought that low salivary calcium content and dental caries were associated; but found that there was a higher salivary calcium content in patients with early caries than in those with advanced caries, therefore the reduction of calcium was not the primary change, but secondary to dental caries. They found no relation between the serum calcium and salivary calcium in 47 adults examined.

Karshan, Krasnow and Krejci (1931)(129) studied blood and saliva in relation to immunity and susceptibility to dental caries. Using accurate methods, they found differences much slighter than those obtained by other workers, and considered them so small as perhaps to lack significance. On the whole, they found the phosphorus content of the saliva in immunes slightly higher than in susceptibles. This agreed with Hawkins (1931)(101), (1932)(272) who found low calcium, high phosphorus serum content in immunes, and considered that saliva, blood and urine were all symptomatically related. Youngberg, however, (1932)(269) considered there was "no reason to believe that the phosphorus content of saliva plays any rôle in dental caries."

The study of calcium and phosphorus metabolism has been mostly concerned with rickets, and since dental defects and rickets were thought to be due to the same causes, findings in relation to metabolism in rickets were applied to dental conditions. Though the two diseases are now thought

to be not quite analogous (Hess and Abramson, 1931 (103)), the information acquired in the study of rickets is of value in connection with dental caries. Rickets is rare in South Africa, but dental caries is just as prevalent there as in this country (Shaw, 1932 (219)).

It has been advanced by Kay and Guyatt (1933) (130) that "a chronic defect of phosphorus uptake from the diet is the most important single factor in the production of experimental rickets in rats ... vitamin D being of importance in the rat or human dietary only in so far as its presence enables the organism to increase the net intestinal absorption of phosphorus from the food."

Masaki, from work in Japan, (1931)(161) concluded development and calcification of the teeth depends less on vitamin D than on the calcium : phosphorus ratio.

Klein and McCollum (1932)(138) found that low phosphorus diets favoured the susceptibility to dental caries, although not the controlling factor.

Klein, McCollum, Buckley and Howe (1932)(140) found that swine fed on a low-calcium, high-phosphorus diet developed teeth poorly calcified but nevertheless free from caries; while Klein (1932)(136) stated that a high calcium, low phosphorus ratio results in caries. Kramer and Howland (1932)(142) found that diets low in calcium and high in phosphorus produced low blood phosphorus, and further that low blood phosphorus favours susceptibility to dental caries, although not the controlling influence. Recently, Canadian workers, have shown that deprivation of phosphorus in the diet of rats results almost invariably in the production of dental caries, and these results have been repeated in this country.

The effects of strontium administration on the histological structure of the teeth of rats were investigated by Klein, Becker and McCollum (1930) who found (137) that it caused a "proliferation of dentinoid" which they compared with the reports of Stoelzner (1908) and Lehnardt (1910) that feeding strontium salts resulted in the production of an

excessive amount of osteoid tissue in bones. Gies (1930)(83) found that strontium administered as the chloride passed into and accumulated in the solid parts, and was present in the pulp, in young dogs, but could not get definite results with fully-erupted teeth.

The experimental production of so-called "beryllium rickets" by Kay and Guyatt (loc. cit.)(130) was thought to be due to the quantitative precipitation of free phosphate in the intestine and its loss by the faeces, whereby an artificial phosphorus deficiency resulted. They found that in "beryllium rickets", there was a very low plasma inorganic phosphate content - ("in beryllium rickets values so low as 0.4 mgm. phosphorus per 100 cc. plasma have been found.") - and also a marked diminution in the phosphoric ester content of the red blood cells.

The relation of phosphorus to calcium in the blood has been summarised by Brookfield (1933)(32) and it was shown that the balance is a delicate one. It was thought by Kay and Guyatt that "the most important effect of vitamin D in physiological

quantities is to increase directly the intestinal absorption of phosphate (which normally implies increased uptake of calcium also) from the diet".

It is important also to distinguish between diffusible and non-diffusible calcium. For an exhaustive review of "Calcium and Phosphorus Metabolism", reference may be made to the Galstonian Lecture for 1930 on that subject by Donald Hunter, where further references are given(114). He stated, among other things, that in any calcium drain, as in lactation or growth of the foetus, bone was called on as the only large reservoir of calcium and phosphorus, and gave the serum calcium content in pregnancy as 9.1 mg. per 100 cc. as against the normal of 10.2 mg. per 100 cc.

Vitamins, the accessory food substances, have been intensively studied in recent years, and it seems to be established that they have some effect on the occurrence of dental caries.

Vitamin A may have some influence, since according to Boyle (1933)(26) lack of it leads to "defectively calcified" dentine, and hypoplastic

teeth; while Smith and Lantz (1932)(225) found that vitamin A deficiency in rats caused "dull white incisors" subject to excessive abrasion, with a higher Calcium : Phosphorus ratio in the ash. Three drops of cod-liver oil daily prevented the occurrence of these signs. Harris (1933)(98) considered that vitamin A was not a general anti-infective agent as generally supposed, while Korenchevsky (1908)(141) thought that the chief benefit of vitamin A administration was its power of inducing a normal state of appetite, so that this may be the means by which the effects recorded by other observers are produced.

Vitamin B ($B_1$ or $B_2$) does not so far appear to have been implicated as a causal agent of dental caries, though Kellogg and Eddy (1932)(131) have suggested it in a preliminary report.

The possible influence of vitamin C deficiency on dental caries, stressed by Appermann (1932)(5) in connection with calcium metabolism, has been investigated by numerous observers. Robb et al.

(1921)[203] found that in scurvy the odontoblasts were affected, and osteodentine was formed instead of normal dentine. Rosebury and Karshan (1931)[207] found that a Vitamin C-free diet resulted in changes in the pulps and dentine of molars only, like latent scurvy, in guinea pigs, and Eddy (1931)[65] found that vitamin C deficiency affected the odontoblasts. Downs (1932)[61] found "the presence of haemorrhage and osteoid formations in the dental pulp the most dependable criterion of vitamin C deficiency; and Indiana canned tomato-juice the most dependable curative agent." The changes in the odontoblastic layer were found to occur many days before any other symptoms of scurvy were manifest; but it would hardly be practicable to extract and examine a patient's teeth to ascertain if he were suffering from latent scurvy.

Hess and Abramson (1931)[103] considered that scurvy was not an important causal factor of dental caries, which is present in marked degree where fruit and vegetables are in greatest abundance, and least prevalent in countries such as Alaska and

Greenland, where antiscorbutic foods are scarce.

Howe thought that lack of vitamin C had no effect on monkeys, in fact monkeys with plenty of orange juice got more caries than monkeys kept on a vitamin C deficient diet.

Eddy (1931)(65) found, like Robb et al., that vitamin C deficiency affected the odontoblasts, in guinea-pigs.

Harris (1933)(98) found the same thing, and stated definitely that "it is quite likely that deficiency of vitamin C - no less than of vitamin D ... - may often be a contributing cause of dental caries."

The fact that the Eskimo, in spite of deficiency of vitamin C in the diet, has good teeth, may be due to the very large fat content of the diet preventing a high calcium : phosphorus ratio in the blood; or else there may be a relatively large amount of fat-soluble vitamins, although whale- and seal-blubber is not so rich in these substances as cod-liver-oil.

Vitamin D has been extensively investigated of recent years with a view to determining its relation to dental caries, and the contributions to the literature on the subject by M. Mellanby alone amount

to hundreds of pages of the Medical Research Council's Reports.

It has been shown by M. Mellanby that the administration of a diet deficient in vitamin D to growing dogs is followed later by the appearance of teeth which she describes as "hypoplastic" or "defectively calcified". Her definition of "hypoplastic"([166]) covers a wide range of departures from "perfect teeth". It has been found that children who were fed on a diet rich in additional vitamin D (or rather, fat-soluble vitamins) showed an increase of dental caries of only one third of that found in a similar group of children under the same conditions, who received no additional fat-soluble vitamins.

In short, the theory is that (a) deficiency of vitamin D in the diet of growing puppies and even of growing children is followed by poorly calcified or hypoplastic teeth; (b) hypoplastic teeth in children fed on a vitamin D deficient diet show a higher incidence of dental caries; therefore, deficiency of vitamin D in the diet is the cause of dental caries, which can be prevented by the addition of sufficient

vitamin D to the diet. (Dogs, even with "hypoplastic teeth", did not get caries.)

Many workers have confirmed the fact that a vitamin D deficient diet for growing puppies, guinea-pigs, etc., results in rickets and dental hypoplasia. Kronfeld and Barker (1932)(43) found that experimental avitaminosis in white rats could be "greatly influenced by the administration of a vitamin D preparation".

It would be difficult, if not impossible, to review briefly the vast amount of literature which has accumulated on the relation of vitamin D to dental caries. For some ten years the results of, and conclusions from, experiments by Mrs. Mellanby were accepted almost without question, but recently a more critical attitude has been manifest.

The argument of post hoc ergo propter hoc is easy but frequently erroneous. The evidence that deficiency of vitamin D causes dental caries appears to be insufficient to justify such a conclusion, and the fact that children deprived of an adequate supply of vitamin D did actually seem more susceptible to caries may have been due simply to the lowering

of the general powers of resistance of the body to disease through deprivation of an essential food constituent, just as starvation of any kind may predispose to disease. The very recently published work of Harris (1933)[99] would indicate that deficiency of vitamin D in the modern dietary of this country is much more widespread than is generally supposed, since he states that 80 - 90% of all elementary school children in London have rickets in some degree; hence the administration of extra fat-soluble vitamins in experiments carried out on institutional children may be merely bringing the dietary from a subnormal vitamin D diet to an adequate level. Harris even goes so far as to advocate the routine administration to all children of 1,500 international units of synthetic vitamin D (such as ostelin, radiostol, viosterol or calciferol) as a prophylactic measure.

In any case, the chief changes observed in teeth following vitamin D deprivation are in the dentine, which is found to contain many interglobular spaces.

"It was shown in Part I (of "Diet and the Teeth; an Experimental Study") that dogs fed on diets containing a liberal amount of vitamin D have white, shiny, smooth, and regularly arranged teeth, suggestive of those natives living in their natural habitats, whereas dogs fed on diets relatively deficient in vitamin D have rough and discoloured teeth. The white, shiny tooth when examined microscopically is found to have thick enamel and dentine, the former with little or no pigmentation and the latter free from interglobular spaces. In a rough, discoloured tooth on the other hand, the calcified area is comparatively thin; the enamel is pigmented and many interglobular spaces are found in the dentine ... The structure in the first case is obviously perfect or normal for dogs and the latter imperfect or abnormal. Corresponding appearances are seen in human teeth."(167)

No quantitative comparative estimations of the calcium content of the two types of dentine and enamel appear to have been made. It is implied that pigmented enamel is more liable to

caries than normal enamel, though no evidence seems to be available in support of this. Obviously the structure of the dentine is of little importance at the commencement of the carious process, since in the human subject it is the enamel that must first be attacked and penetrated, if caries starts outside the tooth. Moreover, Mackay (1929)(152) found less decay in teeth with defectively-formed "mottled" enamel than in normal teeth, and therefore concluded that "mottled enamel, by reason of its defective structure, is not thereby rendered more liable to decay than is normal enamel."

Masaki (1931)(161) working in Japan found the percentage of mottled teeth showing caries comparatively small; while Montelius, McIntosh and Ma (1933)(174) found that the amount of organic matter, and the calcium and phosphorus contents of pigmented or mottled enamel "do not show any consistent deviation from normal values."

Black and Mackay had previously (1916) recorded (18) that dental caries was less frequent in mottled teeth, but the results tended to be worse when it did occur.

The most recent report on mottled enamel (Ainsworth, 1933)( 4 ) in a small number of cases at Maldon, Essex, stated that "There was relatively little caries; 7.9 per cent. of the permanent teeth were carious, as compared with an average in all districts examined of 13.1 per cent.; and 12.9 per cent. of deciduous teeth were carious against 43.3 per cent. in all districts."

These reports seem to indicate quite definitely that pigmented and even defective enamel is less liable to caries, in contradiction to Mellanby's assumption.

The criticism has been made that the teeth which show most marked hypoplasia in cases which have been subjected to a diet deficient in vitamin D are the lower incisors, yet these teeth are the least susceptible to caries. Moreover, people with marked natural hypoplasia, wherein the upper incisors have gross macroscopical lesions, with only a thin covering of pitted and pigmented enamel, are frequently completely immune to caries. Clinical experience therefore does not support the view

that hypoplasia of the teeth *per se* is conducive to caries. In the experiments of M. Mellanby, any amount of structural imperfections, so-called hypoplasia, were produced in dogs' teeth, yet caries followed only twice. (Kesel, 1932(133))

Further elaborations of the vitamin D theory were that "cereals antagonize the action of vitamin D and tend to produce badly formed teeth." (168), and that oatmeal is the worst offender, and actually contains a toxamine substance - named "anticalciferol" - inimical to tooth-calcification. Yet for hundreds of years the Scottish nation in general subsisted on oatmeal; historians tell us that the soldiers of the fourteenth century and other periods each had a bag of oatmeal as food when on active service; and old-time University students brought a sack of oatmeal on which to live during term, "Meal Monday" being a half-term holiday to enable them to replenish the supply. The teeth of these people are believed to have been excellent (c.f. teeth from the leper colony at Bruce's well, *vide supra*) and it is only within the last 70 of 80 years that dental caries has been so rampant. Even quite recently in remote parts

of the Highlands, as in the Island of Lewis and in North-East Scotland, (Gies, 1931[84]; McCulloch, 1932[149]) inhabitants who lived on porridge had good teeth. This was explained by the vitamin D theorists as due to other substances in the diet such as fresh herrings and fresh milk being able in virtue of their vitamin D content to neutralize and even overcome the baleful effect of the oatmeal. Harris,[99] however, doubts if cow's milk contains sufficient vitamin D.

In the recent (1931) experiments of Hoppert, Weber and Canniff [108], it was found that rats fed on an adequate diet (consisting of yellow corn 60, whole milk powder 30, linseed meal 6, alfalfa meal 3, malt 1) which was considered normal over a period of three years, developed dental caries. When the ration of yellow corn was replaced by oatmeal, caries failed to develop, therefore oatmeal prevented the onset of dental caries. (Their conclusion was that dental caries is not due primarily to vitamin or mineral deficiency, but to the physical factor; vide infra).

Hitherto the experimental evidence offered by Mellanby and her co-workers was accepted without question, but in 1932 McCulloch (149) reviewed the most recent investigation at that time (169) and showed that not only were the conclusions drawn from the experiments unjustified, but the experiments themselves were fallacious.

He pointed out that in dietetic work "the first essential in testing the effects of any food material is to have dietaries which are strictly comparable, and adequate in all respects except the one under review." In much of Mellanby's work he did not find any of these postulates filled. He also stated that "the dietary was woefully deficient in every particular" for puppies, as "the essentially carnivorous dog is forced in this experimental work to obtain 50% of its energy requirements from cereals which contain the essential salts of calcification in a ratio very different from that required in the ideal dietary", and considered the Mellanby dietary as "an ideal high-phosphorus, low-calcium rachitogenic one without any necessity to postulate a harmful substance in the cereals".

After discussing the Mellanby-Pattison human dietaries, he wrote "It is now clear not a single component of the diets remained constant. They are not comparable. The widest variations are seen in the milk and calcium intake." And yet the findings of these experiments were used to support the cereal-toxamine theory. "Such variations are not permissable in first-class research, and I maintain that up to the present no evidence whatever has been produced that cereals are actively harmful."

Finally he stated regarding cereals "It is this substitution of a poor food for richer foods which has caused Mrs. Mellanby to postulate actively harmful effects from their use. Because the Hausas in times of famine use water instead of sour milk in their porridge, and they die of all sorts of nutritional diseases, I should not dare to postulate an actively harmful principle in water. The Hausa has substituted something valueless for something of value, not something harmful for something beneficient."

Although McCulloch's criticisms appeared well over

a year ago, no satisfactory reply appears to have been published by Mrs Mellanby or her co-workers.

The production of vitamin D by the action of ultra-violet radiation on the skin or the ergosterol contained in it would lead us to suppose that children living in a sunny country should have better teeth than British children, if vitamin D really is as potent in relation to dental caries as its advocates claim. In England, according to Shaw (1932)[219], the total annual insolation is less than half that at Johannisberg, where the actinic rays of the sun are more powerful than in England. Therefore he considered that the actinic rays of the sun were ten times as effective in Johannisberg as in England (actually four or five times) and dental caries ought to be very rare. Yet he found just as great an incidence of dental caries in 600 school children in Johannisberg as in England, though in South Africa rickets is almost unknown. Moreover, he stated that native children wore no clothes at all till twelve years of age, and so got all the possible actinic rays of the sun, with their vitamin D-producing

effects, yet 74.1% have caries.

That the claims of vitamin D to be a preventive agent against dental caries are overstated has long been my opinion. Experimental evidence, however well presented, may be convincing to those unacquainted with the pitfalls of such methods, but when an experienced research worker, like McCulloch, exposes the fallacies of one set of experiments, doubt is thrown on all the others. Even if the methods were unimpeachable, it does not necessarily, or even probably, follow that effects produced in rats and puppies must result in human individuals.

In my experience, one patient seems to negative the whole vitamin D theory. This patient, a boy aged 14, in 1933, whose father is a scientist, has been brought up for the last ten years on cod-liver oil in abundance, milk, orange juice, and all those things which ought to produce good teeth, including more recently synthetic vitamin D. While his temporary teeth were moderately good, his permanent dentition shows rampant caries of the rapid type. Although it

is easy to say that this single case is an exceptional one, it is difficult to explain satisfactorily to the intelligent parent why the vitamin D therapy and other measures have failed so completely.

Briefly, vitamin D is an essential constituent of the diet (McCollum, 1931(148)) particularly during the period of growth, and a hypovitaminosis D may be followed by rickets and dental caries. Deficiency of vitamin D must therefore be regarded as a predisposing cause of dental caries, but more than this can hardly be said of it.

Vitamin E does not so far appear to have been cited as a cause of dental caries. It has been observed that at and after the period of puberty there is frequently an increased tendency to dental caries, but this is no doubt accounted for by the extra strain on the body of the more rapid growth which usually occurs about this time.

It would perhaps be too sweeping a statement to say that "vitamin deficiency per se has no place in the aetiology of dental caries." (Fraser, 1933(76)), but

there seems little doubt that the importance of vitamins has been greatly exaggerated in the last ten years or so.

It is thus seen that during the period of growth, sufficient amounts of all the essential food constituents must be present in the diet of the growing child, and deficiency of any one of them, but particularly calcium and vitamin D, may lead to a tendency for dental caries to develop either then or later. It has been estimated that public schoolboys may require 10,000 calories per day, as much as Canadian lumbermen doing heavy work, (E.P. Cathcart[42]) so unless a generous diet is allowed, deficiency is more likely to occur than is generally supposed.

Deprivation of any essential food constituent may predispose to dental caries, just as starvation of any kind may predispose to disease.

It is probable that with a "good mixed diet" sufficient of all the tooth-building substances is provided for the normal healthy subject during normal growth and health. It is during the period of

rapid growth and in pregnancy that special attention may need to be given to the actual and relative amounts of the various substances present in the diet.

Under modern civilised conditions, people who live mainly on canned foods may suffer from vitamin deficiency, but this affects adults less than children. Butter, on account of its vitamin content is thus better for the growing child than ordinary margarine (Cathcart, 1921[42]). Special margarine, fortified by the addition of the missing vitamins, has been recently put on the market.

It should be noted that once the tooth has erupted, the enamel is completely formed by the ameloblastic layer, which disappears after eruption, and no further deposition of new enamel can occur. The only influences thereafter are the oral fluids which bathe the enamel, and the problematical circulation of dental lymph in the enamel from the inside. This last has not been yet generally accepted, as the brilliant speculations of Bödecker (1929)[22] already referred to are not supported by experimental proof.

If calcium therapy does really have any effect in diminishing the incidence of dental caries, as seems likely from the cases mentioned above which I have personally observed, the effect of the calcium can scarcely be postulated as exerted from the inside of the tooth in the present state of our knowledge, and it has therefore been suggested that the effect of intensive calcium therapy is to modify the oral secretions. This is dealt with in the consideration of saliva. G.V. Black stated that "the causes of immunity and susceptibility to dental caries would necessarily be found in conditions of the general system influencing qualities of the mixed oral fluids of the mouth by which the teeth are surrounded."

In 1932, Price (197) claimed that "dental caries can be largely prevented or controlled" by diet, which he discussed in detail. He concluded "the available data strongly suggest that dental caries is primarily an expression of nutritional disturbance in which the teeth become susceptible to attack by the products of acid-forming bacteria."

Attention has been directed to the influence on the teeth of milk in the diet of growing children. It had long been believed that breast-fed children have the best teeth (Kingston Barton, and others) and obviously the normal mother's own milk would naturally be expected to be the best food for the offspring. Comparing human milk with cows' milk for the feeding of human babies, Sprawson (1932) (230) wrote "Cows' milk is after all only a substitute, and it is indeed Dei gratia that it should so act at all". Wheatley's figures, however, published in 1912 (257) showed very little difference in the incidence of caries between breast-fed and bottle-fed children.

The danger of infection by tubercle bacilli through the use of cows' milk for infants and young children is so great in this country that for many years it has been customary to sterilize milk intended for the bottle by boiling or pasteurization. It is to this last practice that recent investigations have been directed, and the results of experiments have tended to prove that pasteurization and, still more, boiling deprive the milk of some important constituent or constituents and thus result in the loss of some

nutritive power with the consequence that teeth erupting subsequently are more susceptible to caries.

It has been shown by Sprawson (1932)(230) that of two groups of children living under similar institutional conditions, those in the group fed on boiled or pasteurized milk had more dental caries than those fed on raw cows' milk. By raw cows' milk he means fresh cows' milk which has never been heated above body temperature.

The numbers of children in his experiments were small, and his deductions are perhaps more definite than the amount of his experimental work would justify, but he believes that superior powers of resistance to dental caries can be found in the second permanent molars even of children whose raw milk diet was only started at the age of 10, as compared with those who had been fed throughout on boiled or pasteurized milk.

"Even when not started till nearly ten, the incidence of caries in second permanent molars in children on raw milk as compared with those on boiled milk and cod-liver oil was as 11 to 77 a few years later"

Sprawson points out that from the age of 9 months or whenever breast-feeding is discontinued, children are usually given boiled or pasteurized milk up to the age of over two years when the last deciduous teeth erupt, and he maintains that therefore the deciduous teeth are afforded no protection whatever. He believes that "if a child is started on a daily ration of raw milk at any time before the eruption of the first permanent molars, it does not get caries in teeth which erupt subsequently; but this daily ration has to be kept up ( under civilized conditions) till the child is certainly not less than about 14 years old."

It should be noted that Sprawson only advocates the use of clean raw milk, "certified", from double intradermally-tested cows, and it would appear that such milk should be sufficiently pure and free from organisms to render pasteurization unnecessary.

Discussing these results, Frazer (1933)(76) stated that "there is no evidence to show that milk must necessarily be "raw" to produce the effects described by Sprawson." He suggested that, as "daily administration of raw milk appears to prevent the occurrence of dental caries", this action may be due

to the fat contained in the milk causing increased phosphorus absorption and diminished calcium absorption, and thus preventing the possibility of a high calcium-phosphorus ratio in the blood, which is associated with caries.

In 1928, Orr (187) reported the effects of the addition of milk to the ordinary diet of school children and found that it resulted in a 20% increase in height and weight over children not receiving extra milk. These results were over a short period. He also concluded that separated milk was almost as good. From this, it was suggested (144) that the salts in the milk were more important than the fats, as the fats were not present in the separated milk.

The report by Stirling and Blackwood (1933) (234) (which N.C.Wright stated to be a review of evidence, not a summary of opinions) maintained that milk pasteurized by the low-temperature holder process (145-150°F for 30 minutes in a closed container, with subsequent cooling) undergoes no diminution in nutritive value that cannot easily be remedied by the simple addition of orange or lemon juice and cod-liver oil, and further claimed that pathogenic organisms present are killed. "The exact effect of heat on vitamin D is

not known with certainity, but since fresh cows' milk has an antirachitic potency only $\frac{1}{500}$ of that of cod-liver oil, it is clearly dangerous to rely on milk for the proper development of the bones and teeth of the growing child." (This agrees with L.J.Harris,loc.cit.) "There are therefore strong grounds for the belief that infants can satisfy all their requirements on diets of adequate amounts of pasteurized milk provided that extra vitamin D and, of course, vitamin C are added to the diet."

The heat-labile ingredients of cows' milk are the soluble ferments and the enzymes, casein and lecithin, and the vitamins, of which the chief is vitamin D.

Blackwood and Wright (1933)(19)also criticize Sprawson's work, and his claims for raw milk, which they consider exaggerated.

The evidence does not appear sufficiently conclusive to justify the assumption that raw milk is a specific means of preventing the occurrence of caries, and it is doubtful if it can be regarded as a physiological food up to the age of fourteen years.

It seems more likely that the beneficial results which were observed to follow its administration in a small number of cases were due to its value as a food rather than to any specific effect. The undoubted value of milk as a food would in itself make milk a valuable component of the dietary of growing children, whether raw, or boiled and fortified by the addition of extra vitamins.

It has been recorded elsewhere (1933)[29] that milk was responsible for immunity to caries, and, further "that the immunity was soon lost when the consumption of milk was stopped." Also "the relative immunity to caries which milk gives to the children does not appear to be permanent. It is as though the actual structure of the teeth is not affected by the increase in the mineral reserves of the body, but some factor---possibly the saliva---perhaps the lymph, which changes its character when the supply of milk is reduced."[29]

Savage (1933)(212) considered that pasteurization improved the nutritive properties of cows' milk for humans, as the chemical changes due to heating made it more like human milk, and the reduction of vitamin C was unimportant, as there was not enough vitamin C in any case, and extra vitamin C had to be added even to raw cows' milk. He further stated that there were "no human experiments which demonstrated that pasteurized milk is less nutritive to young children than raw milk", and maintained that the 6% reduction of Calcium does not affect even older children whose calcium need is greater, provided sufficient calcium is present in their diet otherwise. He quoted the Lanarkshire experiment of Leighton and McKinley in support of his conclusions.

In addition to the danger of infection by tubercle bacilli in cows' milk, Maclean (1933) (155) has recently suggested that raw milk might be one of the causes of dental caries. "The only two organisms which appeared in the dentine in early caries were Streptococcus mutans and B.acidophilus odontolyticus. It was rare to find either of these organisms in nature, but both occurred in faeces and were certainly found in town milk and in bovine faeces. Neither organism could survive very long in human saliva, so that in

order that they might be planted in the site of a future carious cavity there must be a constant supply. What better vehicle could there be than raw milk?"

Milk in any form is good for the growing child. This has long been recognized clinically. While it would appear probable that raw "certified" milk may be free from risk, ordinary milk should be boiled or pasteurized to render it safe for small children. Only the well-to-do can afford "certified" milk costing sixpence a pint, and for the great bulk of the growing population even separated milk is better than no milk.

Physical.

The form in which the food is presented for eating has an important bearing on the incidence of dental caries. It has been seen that dental caries occurs most frequently in highly civilized peoples, whereas savages and primitive men were almost free from the disease. The rich in ancient Egypt were more subject to caries than the poorer classes. At the present day, Sim Wallace (251) concludes that

"dental caries is not a defieiency disease; rather is it a luxury disease", and examinations of multitudes of school children tend to show that "the better the school, the worse the teeth". The views of Sim Wallace have received more general recognition than any other theory of the causation of dental caries, and the dental policy of the school medical services for most of Britain, which was approved of by the Council of the Society of Medical Officers of Health, is based on his teaching.

He thinks that caries is due to the lodgement round about the teeth of easily fermentable carbohydrates, especially sugars, of which he says "sugar is so highly refined that we might almost say it is not a food, but a pure chemical product produced in quantity for the destruction of the teeth." Wheatley's figures (1912)(257) strongly support this.

Investigations have shown that there is more caries in children eating sweets in quantity, and the less sweets eaten, the less the number of carious teeth. The consumption of sugar in this country has increased greatly during the last 60 or 80 years

and even in the last 20 or 30 years, and so has the incidence of caries.

To prevent this stagnation and the consequent decay, food of a tough, coarse, fibrous nature should as far as possible always be chosen, and "in the event of non-fibrous food being eaten, of a physical consistency making it liable to lodge about the crevices of the teeth, this should be followed by foods of a detergent nature." Raw celery is recommended at the end of a meal or, still better, an apple. The rôle of the saliva is less that of a chemical digestive juice than of a physical agent to flush out the crevices of the teeth, and aid in deglutition.

In addition to coarse, fibrous food, cleanliness of the teeth should be achieved by regular, correct use of the toothbrush, preferably after each meal and last thing at night, and dental floss silk should be used to clean the spaces between teeth that are closely placed together.

There is no doubt that the work of Sim Wallace is one of the most important contributions to the practical side of preventive dentistry, and his writings on the subject are well worthy of perusal.

In spite of the widespread acceptance of the Sim Wallace theory and its undoubted success when applied practically, it does nothing to explain the immunity to caries shown by some individuals who have dirty mouths, eat sweets, and in fact ought to have rampant caries, but who are nevertheless free from the disease. However, he shows clearly the best means to adopt for preventing the onset of caries in people who are susceptible to the disease, just as those liable to tuberculosis or exposed to infection by the pneumococcus may take precautions to avert disease by avoiding chills,etc.

As mentioned previously, Hoppert, Weber and Canniff (108) showed that the dental caries which occurred in rats fed on an adequate stock diet could be prevented by giving oatmeal instead of yellow corn meal, while the addition to the stock

diet of cod-liver oil, orange juice, calcium carbonate or tricalcium phosphate were of no avail in preventing the onset of caries. They concluded that dental caries was not primarily due to vitamin or mineral deficiency.

They then passed the yellow ground corn through a 60-mesh sieve and gave only the portion that passed through the sieve, in the stock diet, and found that caries failed to develop in six months. They therefore concluded that the size of the food particles affected the incidence of caries and that a chemically adequate diet is no guarantee against caries.

Klein and McCollum (1933)(139) repeated these experiments with a Steenbock diet for two groups of rats. One group had coarse particles, the other fine particles passed through a 60-mesh sieve. The second group had no caries, the first group developed a dental caries incidence of 64% for a period similar to, or lesser than, the first group.

The explanation was that the coarser particles lodged in crevices in the teeth and gave rise to fermentation, while the finely pulverized diet was easily washed away by the saliva. These experiments, which are to be amplified, tend to support the Sim Wallace theory that keeping the teeth free from stagnating débris by choice of suitable food prevents the occurrence of caries.

The idea that giving oatmeal instead of yellow ground corn prevented the onset of caries is rather paradoxical in view of the harmful effects of oatmeal with its contained "anticalciferal" so much stressed by Mrs. Mellanby.

There is much clinical evidence that of all the fermentable carbohydrates, sugar is the worst for the teeth. Statistics show that children who eat sweets have more caries than those who do not, and the more sweets eaten, the more caries present. Thus "the better the school, the worse the teeth", because the luxury of sweet-eating is more prevalent. Coolie labourers imported from

India to work on Natal plantations were reported by Ricard (1926)[202] as poorly fed, and therefore they sucked sugar-cane. After two years on the plantations, caries was universal, and the teeth as bad as could be.

Wheatley (1920) attributes the improvement in the condition of the teeth of children in 1919-1920 as due to war food, with less sugar, (the price having risen from 2½d. to 1/2½d. per pound), no sweets, and plainer food during the war.[258]

A factor of some importance was noted by Breeze in 1913, and he reported[27] the observation that the mouths of some children were self-cleansing, while others remained dirty after a meal, with food clinging about the teeth. This self-cleansing action may be due to differences in the quantity and viscosity of the saliva. It has been noted that fever patients are liable to an accumulation of sordes on the teeth, due to a reduction in the flow of saliva with consequent dry mouth; it is therefore important to pay special attention to cleaning the teeth of such patients.

It was considered by some (e.g.Read, 1912 [201]) that brown, whole-meal bread was better for the teeth than white bread, but Sim Wallace strongly refuted this, and maintained that it was the cleansing of the teeth at the end of a meal by detersive food or artificial means that determined freedom from caries.

Schnack (1932, loc.cit.) instances the highly fermentable carbohydrate Hawaiian foodstuff "poi", which is cohesive in the mouth and thus never clings to the teeth. Hawaiian natives have little dental caries.

It thus seems indubitable that sticky food of a fermentable nature clinging about the teeth in areas of stagnation is an important predisposing cause in the aetiology of dental caries, by the production of acids tending to decalcify the enamel of teeth which are not "immune" to caries, and laying them open to attack by the exciting cause, whatever it may be.

Sugars, especially glucose, seem to have a particularly deleterious effect. The eating of sweets has long been regarded as conducive to caries. It appears that sugar in solution is held against the teeth by capillary attraction, especially round the contact point in posterior teeth. It would be interesting if investigations dould be made regarding the site of "sugar-caries". Obviously, after sweets are eaten, the teeth should be cleaned, and the mouth rinsed. Indiscriminate sweet-eating "between meals" should be discouraged.

Agnew and Agnew attributed the higher incidence of caries in Chinese children of the Tibetan borderland chiefly to sweets, while the aboriginal children with no sweets had better teeth (1931)(3).

Tooth Structure.

The hardness of the enamel was thought to affect the incidence of caries in human teeth. Both Tomes (1895)(239) and Black (1895)(16) found that the molar teeth contain more calcium salts

than the incisors, yet according to Bennett (1931)(10) and Colyer (1931)(52) the molars are 10 times more frequently affected by caries than the upper incisors, and the mandibular incisors are very rarely affected at all. Black wrote in 1899(17) that "caries is not dependent upon the quality of the teeth as to their structure or their perfect or imperfect calcification". This is still the general opinion.

Pickerill, however,(1914)(194) regarded the hardness of the teeth as important in relation to caries, and devised an instrument for comparing the degree of hardness of the enamel of different teeth. He found that the teeth of immune native races had the hardest enamel, while sclerotic, or hard, teeth were less liable to caries than malacotic, or soft, teeth. He also showed that malacotic teeth were more permeable to silver nitrate than sclerotic teeth. This seemed to indicate that malacotic teeth had more organic matter which lessened the resistance of the enamel. It should be noted that the teeth of the dog are much

more permeable than human teeth, and yet the dog is almost completely immune to caries.

Spencer-Payne (1927)(228) found no difference in the calcium content of erupted and unerupted teeth, though it has been suggested that the teeth may undergo a process of hardening or calcification in the mouth, and this has even been advanced to explain the fact that caries is more prevalent in youth, than in middle age and later life (51). Hess, Lewis and Roman (1932)(104) found that at birth the deciduous teeth contained only 15-20% of their normal calcium, so that in the first few months of life a comparatively large amount of calcium is needed to ensure an adequate supply for the developing teeth.

In practice, great differences are observed clinically in the hardness of enamel in different subjects, yet the results of large numbers of estimations show little variation in the calcium content of different teeth. The form in which the calcium salts are combined with the organic matrix of the tooth may decide the degree of resistance to caries. If the first stage of caries

is simply an acid attack on the enamel, the more pure the calcium salts were, the more liable they would be to solution by the acid; whereas combination with the organic matrix, small though it is, might increase their resistance to decalcification. According to Benedict and Kanthak, (1932)[9] the calcium:phosphorus ratio indicates that enamel is not pure $Ca_3(PO_4)_2$, but a complex salt. The rôle of Nasmyth's membrane as a defensive mechanism is not of much importance, as it is soon worn by attrition off the exposed surfaces of the teeth, yet these surfaces seldom show caries.

Sometimes hard teeth, the enamel of which is evidently well formed, are found to be prone to caries, while soft teeth remain free. In gross "hypoplasia" and in defectively-formed Hutchinsonian teeth, especially incisors, caries is frequently completely absent.

The chemical composition of the enamel thus appears to have little connection with its liability to caries, and Black after much study came to this conclusion and wrote (1895)[16] that "neither the

density nor the percentage of lime salts, nor the strength, is in any degree a factor in predisposing the teeth to caries or in hindering its inception or progress."

The commonly accepted idea that teeth change as regards hardness or softness after eruption was denied by C.N.Johnston, who pointed out that enamel varies materially from other structures of the body in that it never regenerates.(1933)(126). Beretta, in 1928(11) found that in cattle from two to five years old, and from eight to eleven years old there was practically no change in the composition of the enamel occurring with age.

The conclusion of J. Leon Williams in 1927(262) was that "We have no reason for supposing that the most meticulous attention to diet can change the structure of the enamel of erupted teeth so as to render it less immune to caries....All the structural, physical and chemical qualities of enamel are determined and fixed during the prenatal period and the first twelve years of life, and,

consequently, all sttention to good food habits should be concentrated on these periods."

The physical configuration of the tooth surface appears to be more intimately connected with caries. Pits and fissures are clinically found to be the most frequent sites of caries in molars. On smooth surfaces a defect in the enamel may be the starting-point of a cavity. Such pits and fissures, or defects and flaws in the enamel are difficult to keep clean, are not self-cleansing during mastication, and afford stagnation areas for carbohydrate fermentation. The so-called "prophylactic odontotomy" advocated by Thaddeus P. Hyatt (1933)(121) is based on the assumption that all pits and fissures in molars will ultimately decay, and clinical observation and his own statistics give strong support to the theory.

The form and arrangement of the teeth may be considered here. Teeth which have bulbous crowns sometimes have a zone of stagnation along the gum margins, since owing to their form this area,

especially on the buccal surfaces, is not scoured by mastication. Decay frequently occurs in this situation. Teeth which are irregularly arranged in the dental arch frequently show areas of stagnation. Even well arranged teeth have a stagnation area immediately gingivally to the contact point---a situation where caries is specially prone to occur, Arthur's "separation treatment" for the prevention of caries was devised to counteract this tendency.(1879)(6). There is little doubt clinically that places where stagnation occurs, as in closely-placed, teeth, are more frequently the site of caries; and conversely in the microdont, widely-spread teeth occasionally seen, and in Hutchinsonian teeth, proximal decay seldom occurs. In a patient of mine who shows congenital absence of several teeth, confirmed by radiographic examination, caries has occurred in all the fissures on the occlusal surfaces of the only four molars present in his mouth. None of the few teeth he has are in contact with any others, and he

has had no interproximal caries. Two other patients with small widely-spaced teeth not making contact with other teeth both show caries on the occlusal surfaces of the molars, but nowhere else.

The structure of the enamel is obviously of importance in human teeth, since we have seen that the enamel must usually first be attached and penetrated. J.H.Mummery (1922)(179) among others has shown that fissures in the enamel may extend right into the dentine; and von Beust, in 1923(14) and 1926(15) demonstrated a connection between cementum and dentine. Bödecker in 1927(21) and 1929(22) advanced a theory of the vascularity of enamel (which, by the way, was suggested in 1838 by John Tomes in a paper read before the Royal Society where, referring to his own work, he stated "These investigations of the structure of the different parts of the teeth furnish abundant evidence of their vascularity and consequent vitality")(241), and suggested that the enamel rods are dissolved before the rod sheaths and cement substance by the acids which are produced by bacteria adherent to the tooth

surface; and hence he advocated polishing the teeth to prevent decay.

The effect of vitamin D deficiency on the structure of growing teeth has been mentioned above. It is claimed that "defectively calcified" and "hypoplastic" teeth are more liable to decay than those which are well formed, although in actual practice it is found that macroscopically hypoplastic teeth are often free from caries. A diet deficient in vitamin D administered to puppies will always result in defective teeth, yet these teeth will remain free from decay, and in fact it is almost impossible to produce dental caries in dogs, however hypoplastic the teeth.

"The experiments on dogs with well and poorly calcified teeth have lasted for varying periods from three to eight years, and have consisted in testing:

1) The effect of soft and sticky foods.

2) Diets varying in their vitamin A and D content and cereal content.

3) The addition to the diet of large quantities of fermentable carbohydrates.

4) The presence in the mouth of Streptococcus mutans and Bacillus acidophilus.

5) The effects of artificial breaks in the enamel.

6) The diminution of the flow of saliva, the results are in most cases negative." (164)

The dog would thus seem to be indeed immune to dental caries.

Under the heading of tooth-structure may be considered the theory of R.Eckermann (1919)(64), who regarded the enamel and dentine as an osmotic membrane interposed between the blood serum internally and the saliva externally. He wrote "Granted that osmosis takes place, the absolutely inevitable consequence will be that plasma from the blood vessels of the pulp is forced up to the osmotic entrance of the tooth, contributing to the formation of the carious area." His theory does not appear to have received general acceptance, though Bunting and Rickert from a series of experiments (1917)(37) (1918)(38) concluded "that the enamel and dentin of the teeth are as a rule more or less porous, and that they will admit of a passage of salts and solvent

both from the blood to the surface and from the saliva to the interior."

The fact that, as noted elsewhere, the dog with permeable teeth is immune to caries, while man with almost impermeable teeth is susceptible, suggests that work should be done with a view to finding out if human teeth immune to caries are more permeable than teeth which are liable to decay. If so, this would be a strong argument in favour of Bödecker's theory.

Saliva.

The part played by saliva in the aetiology of dental caries has received a great deal of attention, since a fluid which constantly bathes the teeth may obviously have a profound effect on them. Its main action appears to be that of a mechanical cleansing agent. A copious flow of saliva washes out particles from between the teeth and generally cleanses the mouth.

There are clinically great variations in saliva, from the thin watery mobile saliva, usually abundant,

to the thick, ropy, viscous saliva which can be drawn out in long threads from the mouth. The latter is thought to be conducive to caries by encouraging stagnation, while the thin, watery saliva flushes and cleanses the mouth. If much saliva is secreted, it is always being swallowed, and replaced by fresh saliva, so that the environment of the teeth is constantly changing, and stagnation is less likely to occur. Sim Wallace (1929) (252) regards a copious flow of saliva caused by acids, and pungent, bitter principles as "the natural mouthwash that should be stimulated."

Pickerill (1914) (194) who has made extensive studies of saliva, found that acid foods, such as fruit, cause a greater flow of saliva and therefore a cleaner mouth, while insipid foods depress the flow of saliva.

He also estimated the reaction of saliva after stimulation by various substances taken into the mouth, in conjunction with the amount of saliva

secreted, to which he attached great importance, as he thought that "The mere analysis of a sample of saliva in a test-tube without knowing anything as to the conditions and rate of secretion is utterly valueless." Mathur (1930)[162] found great variations in the alkalinity of saliva due to the slightest disturbances during its secretion. Pickerill calculated the "alkalinity per minute in the mouth" as the rate of flow of saliva multiplied by the alkalinity index. He found as a result of investigations among native children of the Urewara, New Zealand, in 1912, that the "total alkalinity per minute was six times greater in Maori than in European children, and the ptyalin index was double." He attributed the greater freedom of Maori children from caries to this alkalinity, as an acid saliva was thought to be conducive to caries.

The increased incidence of dental caries supposed to occur in pregnancy was thus explained by older writers as due to "morning sickness"

causing acidity through gastric juice being brought into the mouth. Clinical support seemed to be lacking, and some women seemed to develop excessive caries without having suffered from morning sickness. Schnack (1932)(214) noted an imperceptible acid regurgitation from the stomach as a cause of caries, in conjunction with a diminished buffer action of the saliva.

Stern (1931)(232) concluded that the pH of normal resting saliva in children was from 6·6 to 7·0; that there was no difference between the acidity of saliva in boys and girls; that the buffer action of the saliva tended to prevent too great acidity; and that there was no apparent relation between dental caries and acidity of the mouth.

Mathur (loc.cit.) found that in healthy adults the pH of fasting saliva varied from 6·5 to 8·0, the average being 7·5, and the degree of alkalinity was determined by the rate of secretion, chiefly, "though many other factors must be considered".

Friesell and Vogt (1926)(78) found that the saliva in pregnant women had a pH of 6·61, or 0·2 lower than the average of 6·8 for normal controls.

Hawkins (1931)(101) stated that the normal reaction of saliva is alkaline, Gans (1926)(82) stated that saliva is normally slightly acid, pH from 6·4 to 7·0.

The normal pH of saliva is usually taken as 7·0.

Broderick (30)(31) considered an acid saliva associated with dental caries the antithesis of an alkaline saliva and pyorrhoea, and regarded both as a manisfestation of some systemic disorder; and Price (1932)(148) supported this theory. Jones, Larsen and Pritchard (1930)(128) in an extensive investigation of native children in Hawaii, found that a particular kind of caries was rampant, which they named "odontoclasia", in spite of foods rich in vitamin C, and abundant sunlight. They correlated these findings with experiments on puppies, by Simonton and Jones, and found certain differences between the incidence of rickets and caries by varying the diet to produce variations in the acid-base balance of the blood. They concluded that an "explanation of a number of clinical phenomena of

obscure etiology was offered from the standpoint that dental disease is a systemic disorder directly related to the composition of the blood." Later (1930) Jones stated [127] that the Hawaiian researches merely suggested a "promising approach to the problem of dental disease" as proof "must come through chemical analyses of blood and body fluids in apparently normal individuals with rampant decay, and in those with uncomplicated alveolar atrophy of systemic origin" and she pointed out that Broderick presented "no direct experimental evidence or clinical data to support his view."

While the evidence is not conclusive, there is a general impression that an acid saliva is associated in some way with the occurence of dental caries. However that may be, in the worst case of dental caries which I have ever seen, wherein a girl of twelve had <u>all</u> her teeth carious, and showed ten cavities, all of the rapid caries type, in her lower six anterior teeth----a most unusual occurrence---an estimation of the salivary reaction revealed a pH of 7·4. (The normal is usually taken as 7·0). In this case, as in other similar ones, there was

an excessive flow of watery saliva, which should have tended to keep the mouth clean. This may have been due to the pain or irritation of the carious process. The patient did not complain of pain.

Ptyalin, according to Pickerill, is an important agent in the saliva for protecting the teeth against caries, as it converts sticky starches into soluble substances, which are easily washed away by the saliva.

Pickerill and Champtaloup (1914)(196) investigated the immunity of the Maori of the Urewera, and considered it not due to the absence of organisms which are usually regarded as causal factors. Later, Pickerill (1924)(195) stated that it was the superior power of resistance of the Maori children, due to harder enamel and more alkaline saliva index, that resulted in their teeth being better than those of European children, as the attacking forces---- micro-organisms and carbohydrates----were the same

in both groups investigated. He added that "This finding places dental caries in line with nearly all other diseases of bacterial origin", and "our hope must lie in building up the resistance of the fixed tissues (enamel) and of the body fluids (saliva)."

Potassium sulphocyanate in saliva was at one time regarded as inhibitory to dental caries, and was even administered as a preventive. Pickerill concluded that the amount present in saliva bore no relation to the incidence of caries. The amount normally present is so small (about 0·01%) as to have no appreciable effect, and Gies (1914)(86) has concluded it is probably an excretory product, or partly formed by bacteria in the mouth; Bunting (1914)(35) agreed with this. Waugh (1910)(256) found that "plaque formation" was inhibited by potassium sulphocyanate in test-tube experiments.

More recently, lysozyme in saliva was suggested (Schulz, 1932[215] and others) as responsible for a supposed bactericidal action of saliva, and hence as a retarding agent in dental caries. Reference to the literature on the subject enabled me to show[156] that its concentration in saliva is not sufficiently great to account for the powers claimed for it.

Mucin in saliva if present in excess in alkaline solutions, in which it is soluble, forming a ropy, viscid saliva, may render the saliva less effective as a cleansing agent, and hence encourage stagnation. It is precipitated in an acid saliva, which therefore is more mobile. Mucin and mucinate readily undergo bacterial hydrolysis and provide pabulum for bacteria, while a "Mucin plaque" holds and nourishes the organisms. (Inouye, 1930)[123]. Kirk (1910)[134] also attributed an important rôle to oral mucus in the saliva.

The calcium content of saliva was regarded as important, since it was thought that a highly concentrated solution of calcium in the saliva would not only not tend to decalcify the teeth, but might even permeate the enamel and calcify it still forther. Ehrensberger (1931)(66) favoured the "remineralization" theory and advocated correcting the saliva by a diet of vegetables and fruits, with no cereals. He thought that it was only possible to remineralize the enamel if the organic matrix was still intact.

It was even supposed that the enamel could undergo a process of hardening after eruption, if bathed in alkaline saliva highly charged with calcium in solution, while Benedict and Kanthak (1932)(9) stated that enamel would dissolve in the saliva if it were not for the protective action exerted by the calcium and phosphorus ions.

The technical difficulties of estimating accurately the small amounts of calcium present in saliva (from 6 to 12 m.g. per 100 c.c.) have

resulted in conflicting results by different investigators. It has been found that the calcium content of saliva varies in different individuals normally, and even in the same individual at different times, and, as mentioned above, under varying rates and conditions of secretion. (Spencer-Payne,1924)(227).

The influence of diet upon the inorganic constituents of human saliva was investigated by Clark and Shell (1927)(44) but they could not demonstrate any relationship between the amount of a given element ingested and retained, and the amount of it appearing in the blood or saliva, or between the concentration of inorganic constituents of the blood and the amount appearing in the saliva. They found that saliva contains from 3 to 5 times as much phosphorus as blood plasma, which they thought provided an excellent buffer system for saliva.

On the other hand, Forbes and Gurley (1932)(74) found that a high-cereal, or a high-grain diet tended to increase the salivary acid-neutralizing power, while other diets, such as a high-meat or high-egg diet had the reverse effect.

Fetterley and Maughan (1931)(68) found that there was apparently "no difference in salivary calcium in those who have always had healthy teeth and in those having many repairs."

The glucose content of saliva was investigated by Entin and Schmidt (1927)(67) who found no relation between the quantities of glucose and other reducing substances in the saliva, and dental caries. The results of Simon (1926)(221) were similarly inconclusive. The presence of glucose, or indeed any fermentable carbohydrate of that nature in the saliva was denied by Prinz in 1918(119), "even in a diabetic patient with a known sugar content of 7 per cent in the urine", but his method of estimation may not have been sufficiently delicate.

The supposed antiseptic power of saliva was thought to inhibit the growth of organisms in the mouth which might be causative of dental caries. In 1903 Miller (171) published results of his experiments from which he concluded that saliva was not really antiseptic, and later he amplified his results (172) (173) and stated that "Neither the oral fluids as a whole, nor their separate constituents (mucus, potassium sulphocyanate, etc.) have the power to prohibit or even perceptibly retard the growth of bacteria" and "There are neither simple chemical antiseptic compounds present in sufficient concentration to exert a bactericidal action, nor have I been able to find the more subtle protective bodies (analogous to the alexins of the blood) in the saliva. In other words, the protective bodies of the normal blood serum (alexins, complements, cytase) do not under normal conditions pass into the saliva in sufficient quantity to be detected by ordinary means."

This confirmed the conclusions of Hugenschmidt in 1896(112).

In 1931, Sugg and Neill(275) claimed to have found detectable amounts of diphtheria antitoxin in human saliva, and stated that its concentration was directly related to the concentration in the blood. They admitted, however, that "exception to this usual ratio of saliva to serum antitoxin appeared to be characteristic of certain individual persons"; and the number of their subjects was small. This was not convincing in view of the fact that Dobbs (1926)(59) had found no constant relationship between any constituents of blood and saliva examined, which was confirmed for calcium by Horton, Marrack and Price (1929)(110), and Fetterly and Maughan in 1931(68).

Amylase in saliva was investigated by Prinz in 1918(199) and later by Adams and Myers (1933)(2) who concluded it might "be an etiological factor

in dental caries, provided acid fermentation of carbohydrates is a cause of tooth decay."

In short, the results of different workers on saliva are inconclusive,and sometimes even conflicting.

Saliva, if mobile and copious, washes out the mouth and removes food particles from the teeth, and hence tends to prevent the occurrence of caries. Acid saliva is probably conducive to caries, as is viscid and scanty saliva. A high calcium content would be expected to counteract a tendency towards decalcification of the teeth by acids produced locally by fermentation.

Bacteria.

That bacteria play a part in the aetiology of dental caries is the basis of most modern theories, though the rôles assigned to the organisms are varied and their types legion.

Miller was the chief originator of a bacterial cause, with his chemico-parasitic theory, and he further studied the bacteriology of the mouth (1890)[170] Goadby in 1903 also published a work [88], which has become a classic.

Innumerable types of organism flourish in the mouth; many of them are normal harmless inhabitants of the bucc̸al cavity, while others are potentially harmful.

Out of a mass of literature, much of it conflicting in character, the two chief contributions to the study of the bacteriology of dental caries were the discoveries of Bacillus acidophilus odontolyticus (Lactobacillus acidophilus odontolyticus) and Streptococcus mutans.

Previously, Leber and Rottenstein (1878)[145] had regarded Leptothrix buccalis as the causative organism of caries, following the action of acids on the teeth, but apart from that, the results of other workers were too vague to be of much significance.

In 1917, Howe and Hatch [111] found the Bacillus acidophilus of Moro in dental caries in almost all cases examined, and concluded that "The Moro-Tissier group of micro-organisms is the constant and predominant flora of dental caries."

In 1922, McIntosh, James and Lazarus-Barlow [180] confirmed these results and amplified them. They stated that the examination of selected carious teeth showed the constant presence of the same definite type of bacillus to which they proposed to give the name Bacillus acidophilus odontolyticus. Later, [181] they described two types, I and II, and found that these could produce, and thrive in, a high degree of acidity of the medium from the fermentation of carbohydrates "the average final pH value of nine strains was 2·75, which is sufficient to decalcify teeth."

Clarke, in 1924(45), found another organism constantly present in dental carious lesions, which he named Streptococcus mutans, as it was normally a coccus on neutral media, but tended to assume a bacillary form if the reaction became slightly acid. It was killed if the reaction became more acid than pH5·6, and hence was distinct from B.acidophilus.

The chief interest of investigators has been centered on these two bacteria. Aspergillus niger, regarded by Hartzell (1924)(100) as a cause of "black decay", a type of enamel caries, and other organisms described from time to time, were only of ephemeral interest.

Schlirf (1926) considered aciduric bacilli, particularly Goadby's B.necrodentalis as the chief decalcifying agents, though streptococci derived from the mouth, and belonging to its ordinary flora, were also involved, as well as "anaerobic fusiform bacilli." (213)

It is possible that on account of their pleomorphic forms, the two organisms, Bacidophilus, and S.mutans may have been mistaken for separate entities by some of the observers who described them. Thus, Okumara and Nikai (1927)(185) described two rod-shaped bacilli as constantly present in dental caries, and named them Bacillus necrodentalis and Bacillus N, and considered them the primary co-factors in caries formation.

Morishita (1929)(176) recorded that high-acid-tolerating organisms were almost always found in carious mouths, but were rare in the saliva of non-carious mouths.

Later workers to some extent confirmed the results of McIntosh, James and Lazarus-Barlow. Bunting, Nickerson, Hard and Crowley (1928)(36) found B. acidophilus directly related to dental caries in 1335 children, and cessation of caries coincident with the disappearance of B.acidophilus from the mouth. They even considered the presence or absence of B.acidophilus as constituting a more

accurate means of diagnosing the activity of dental caries than any clinical examination,(!) Metaphen inhibited B.acidophilus and therefore according to them was a specific for dental caries.

Hadley and Bunting (1932)[95] likewise found a direct relationship between B.acidophilus and dental caries, and also that the intestinal tract duplicated the mouth findings. Jay, Crowley and Bunting (1932)[124] found a skin-reactive substance in B.acidophilus filtrate, and observed a modified Arthus reaction in one case. In two cases they found negative skin reactions associated with B.acidophilus agglutinins in the blood serum after the use of a polyvalent B.acidophilus vaccine. Later,[125] they claimed to have demonstrated B.acidophilus agglutinins in the blood sera of caries-free individuals in higher dilutions than in the sera of caries-susceptibles, and that the B.acidophilus counts in the saliva were very high in susceptibles and negative or very low in immunes.

Maclean (1927)(154) critized the work of Bunting and his co-workers, who, he said, were "evidently unable to distinguish between Bacillus acidophilus and Streptococcus mutans". He himself showed that they are in fact separate and distinct organisms and worked out a means of differentiating them. He was inclined to believe that S.mutans was more likely to be the causal organism, as he got sterile cultures in a large number of cases when working with B.acidophilus, and he could not definitely confirm the results of McIntosh, James and Lazarus-Barlow. On the other hand, his work tended to confirm Clarke's results with S.mutans.

As a result of extensive investigations, Thomson and Thomson (1930)(236) apparently concluded that S.mutans was more likely to be causative of dental caries than any other organism so far implicated.

This is interesting in view of the fact that S.mutans has been reported as the causative organism in a case of infective endocarditis

(Abercrombie and Scott, 1928)[ / ]. The importance of dental and oral sepsis as a cause of systemic and localised disorders was stressed by W.Hunter in 1900[117], and especially in 1911[118]. While S.mutans has frequently been found in carious cavities, its importance would be greatly enhanced if it were proved to be capable of initiating the lesion in dental caries. In view of Rosenow's experiments (vide infra) the chain of evidence in favour of S.mutans from the buccal cavity passing via carious cavities, dental pulps, and the blood stream to sites of elective localization would be much strengthened.

The available evidence does not seem sufficient to prove either B.acidophilus or S.mutans to be the specific cause of dental caries. There are, however, strong grounds for supposing that some form of organism is responsible, and it is hoped that the experiments which follow will be sufficient to support this hypothesis.

## Various Theories.

### Food.

The continual ingestion of some particular food substance was thought to influence the incidence of dental caries. Thus, grape-fruit eaten every morning, or lemons frequently sucked, were supposed to decalcify the enamel and lead to caries. In the "grape cure" wherein large quantities of grapes are consumed (as is practised at Merano, in Italy) it has been observed that caries is frequently rampant.

### Occupation.

Flour-millers and bakers are noted for their bad teeth. The explanation has been suggested that fine dust in the air they breathes consists of particles of fermentable carbohydrates, and this dust settles in the crevices of the teeth, especially if dental hygiene is neglected. Workers in chemical factories may have the teeth affected by acid fumes, and those engaged in the confectionery trade are liable to the deleterious influence already noticed as associated with sugar.

Sex.

It has been noted that females have a slightly higher incidence of caries than males of the same age, in school children. The difference is slight, and may be explained by the fact that the teeth of girls usually erupt a little earlier than those of boys, and at a given age have therefore been exposed longer to whatever causes of caries there may be.

Race.

Certain races living under modern conditions are less susceptible to dental caries than others; thus Jews are supposed to be remarkably immune; and Hills (1928)(105) found that although up to the age of twelve years, the incidance in both races was the same, after that age, the incidence of dental caries in English school children increased, while in the Jewish children it decreased.

The apparent immunity of other races living under primitive conditions is more probably due to other factors, such as environment and diet, though racial immunity may be of some importance.

Electrolysis.

The electrolytic theory was in vogue some 40 years ago. It was discussed by W.D.Miller (1890) but rejected. In 1894 Palmer(190), and, later Patrick(191) supported it. The various substances composing the tooth, and occurring in the buccal cavity, were supposed to act like an electric cell, the saliva being the electrolyte, and calcium was thereby extracted from the teeth.

Internal Gland Secretions.

Waller (1913)(254) considered internal secretions as the first links in the chain of dental caries. Kirk (1913)(135) also investigated this possibility, and Gies (1918) experimented by removing various glands in albino rats. He found that there were no effects on the dentition after thymectomy, thyroparathyroidectomy or castration, but deficient calcification of incisors followed simple parathyroidectomy. He also found that dental calcification was decreased by oral administration of lymphatic, salivary and thyroid gland substance,

increased by oral administration of testicle, and unaffected by corpus luteum, parathyroid, pineal, pituitary, spleen, suprarenal and thymus.(83)

Parathormone (the active principle of the parathyroid gland) appears to play an important rôle in calcium metabolism, as in parathyroid overdosage hypercalcaemia may be produced, while after parathyroidectomy the serum calcium content may be reduced.

The evidence on this and kindred effects is concisely reviewed by Donald Hunter (loc.cit.).

It is thus clear that "hormones" indirectly are of importance in considering the incidence of dental caries.

Blood groups. The results of an examination of the incidence of dental caries in relation to blood groups in 700 male students of from 18 to 22 years of age published by Suk (1933)(235) showed that "good teeth" occurred in 26·8%

of O group, 18·9% of A group, 16·2% of B group and 21·8% of A.B. group.

He therefore suggested that a tendency towards dental caries and particular blood groups, may be constitutional characters and not particularly related to race. He pointed out, however, that the O group, like good teeth, is more common in primitive races than in civilized people.

Salt.

S. Colyer (loc.cit.) mentioned that of three groups of natives from the region of Lakes Mweru and Bangweolo, the group which had least caries lived in the salt-producing area for the surrounding country.

In 1918, Bloom stated that erosion was not observed in races which do not add salt to the diet, and suggested that excess of common salt resulted in the production of hydrochloric acid; this was supported by Eckermann (loc.cit.) He also pointed out that patients with erosion like much salt in

their food. The suggestion is, of course, that excessive salt in the diet may also predispose to dental caries. Not much work has been done on this subject, but it would seem to offer a fruitful line of investigation. It is a matter of common observation that some individuals take large quantities of additional salt with their food, while others take the food as it is presented to them. Exact records could be made to ascertain if the incidence of dental caries varies appreciably in the two types.

Civilization.

The effects of civilization have been referred to in connection with Egyptian mummies, Mummery's researches, and elsewhere. Seiler, in 1931,(216) published the results of investigations into the condition of the teeth of the adult inhabitants in the Valley of Conches in Valais, from which he concluded that the opening-up of the district had greatly diminished the powers of resistance of the

people to dental disease. Formerly, rye-bread was consumed, eaten stale and hard. Now, white flour, sugar, jam and macaroni were largely consumed. He considered that the advent of "civilization" to this isolated valley had resulted in dental deterioration.

It has been almost universally observed that primitive people living in their natural habitat have sound teeth, but as soon as they come in contact with civilization their teeth become carious. The chief factor would appear to be the change in diet, from hard, coarse food, to soft, cooked food. According to the commonly-accepted theory, stagnation of civilized food, with consequent acid-production might be regarded as the <u>determining</u> cause which lays the tooth open to the action of the actual specific cause of dental caries, whatever it may be, and precipitates an attack of the disease.

Age.

Dental caries is a disease of youth. I think almost all writers are agreed on this point. The chief exception is in the case of old people who develop a sudden attack of caries which is in many cases a sign of impending dissolution.

Many explanations have been attempted. One is that as cavity after cavity is filled, the places where decay is liable to occur are replaced by inert filling materials and so are no longer possible sites of caries. Another is that the teeth harden as the years pass, but this is denied by some, and experimental evidence does not seem to support it. Still another is that the individual acquires immunity to the disease, and evidence in support of this will be suggested in the experiments to follow.

Exanthemata.

Exanthematous fevers occurring during tooth-formation may, and usually do, affect the developing enamel, leaving furrows or pits, which in later life are more likely to decay if not kept clean, owing to carbohydrate stagnation.

The effect of ill-health on increasing the incidence of caries has been noted above.

Various other theories have been advanced to account for dental caries, most of them now only of historical interest. The disease was even supposed by Trillot and Fouassier (1914)(243) to be contagious, and of course atrophy through disuse in modern peoples has been postulated as an explanation of its ravages.

The discovery that the inhabitants of Tristan da Cunha had teeth almost completely free from caries was surprizing in view of the fact that they ate only soft foods and never

cleaned their teeth. The staple diet was found to be potatoes, fish, milk and eggs. Flour and other groceries were only available at long intervals when obtained from visiting ships. Cereals were not grown on the island.

The supporters of almost every prevalent theory derived much encouragement from this discovery, reported by Marshall in 1926[159], and more recently discussed in a series of letters in The Times in 1932, and by Gane[81]. Heredity, lack of sugar, absence of cereals, and presence of vitamins, were all in turn held responsible for the good dental condition of the islanders. So much of a contradictory nature was published that it seemed as if none of these factors could be held to have much importance.

The obvious feature of their existence was that these people lived the "simple life". They had to work for their daily food, collecting eggs, catching fish, and so forth. They did not tend to overeat, but their diet appeared to be adequate. They had no luxuries. The Pitcairn Islanders were found to have bad teeth.

My own observations regarding the incidence of dental caries among 4,000 school children attending public elementary schools may be mentioned here. The district was a rural area where no facilities for dental treatment had ever existed prior to the inauguration of dental centres by the county council. Even the simple extraction of an aching tooth was of the rarest occurrence. It was thus virgin ground.

The children were mostly aged from five to eight years, though a few ranged up to twelve, and could be divided into three groups.

Group A lived in a village of about 2,000 inhabitants where umemployment had been rife for the previous nine years, owing to the closing of the one and only works on which the whole community depended. The entire population appeared to subsist mainly on "the dole". Poverty was the dominating feature of their lives.

Their diet was naturally of the plainest, and my colleague Dr. Tomb [237] stated "They live chiefly on bread, dripping, margarine, potato, and cereals such as porridge, rice, etc. The population is poor, and the supply of sweets, candies, etc., is limited. They bake and eat large quantities of pastry. In fact, one might say the diet is chiefly carbohydrate, owing to the fact that this is the cheapest form of food". As the village is beside a tidal estuary, shell-fish are obtained and largely consumed at certain times, but this appeared to be the only luxury they enjoyed. These children seemed to be on the whole healthy, but not well nourished.

Group B lived in a large mining village of 14,000 inhabitants, surrounded by open country, and apparently as prosperous as post-war conditions would permit. The diet was more generous than that of Group A. Meat, fish and vegetables were consumed. There were several confectioners' shops

where the usual cheap varieties of sweets could be obtained. Fruit appeared to be plentiful. There were no obvious signs of the extreme poverty manifested in Group A. Tinned foods appeared to be eaten to a considerable extent. These children were on the whole well-nourished, but seemed to suffer more from minor ailments than those of the other two groups.

Group C lived in the surrounding country districts. They were mostly the children of farmers and farm labourers. Their conditions of life seemed to be better than in Groups A or B. They had healthy surroundings, fresh air, while butter, eggs, and raw milk were abundant. Sweets and candies were limited, as in Group A, and likewise large quantities of pastry were consumed, as well as home-made white bread. For the most part, these children had the air of being healthy and well-nourished, and many of them were robust.

At the preliminary dental inspections, those children with more than four carious teeth were recorded as +, and those with less than four as —. Only 5·8% were caries-free.

Without going into tedious statistical details, suffice to say that the children in Group A had the best teeth, those in Group C the worst, and the ones in Group B were intermediate.

This result was somewhat surprizing, and I made further enquiries, but the essential facts remained the same. The determining factor in Group A appeared to be poverty. This agrees to some extent with other observers, who found that poorer children frequently have better teeth, caries is a luxury disease, and "the better the school, the worse the teeth".

It was rather astonishing to find that the children in Group C had the worst teeth. These children had most nourishment of a wholesome kind---farm produce---with most "vitamins" in the food, and most raw milk. From the laboratory experiments on rats and dogs, and the institutional experiments on human children, one would have been justified in predicting the best teeth in Group C. In both Groups A and C, the supply of confectionery was small, but we find the consumption of large quantities of pastry, which tends to cling to the teeth.

In Group A, carbohydrates formed the bulk of the diet, yet carbohydrates have been regarded as associated with dental caries. Poverty prevented the use of such detergent foods as the apple to conclude each meal, as advocated by Sim Wallace.

In Group C, apples were obtainable from orchards, and other fruits of the earth could be enjoyed in due season. For this reason, further, the children in Group C might be expected to have had better teeth.

None of the children in any of the groups were in the habit of cleaning the teeth, prior to the institution of the dental service.

No explanation based on the results of the conventionally-approved experiments appears to account for the superiority of the dental condition of Group A over that of Group C, unless shell-fish have an extraordinary effect in diminishing the incidence of dental caries.

If an explanation must be given, I would suggest that (i) In Group C, too much food was

available, the children tended to over-eat habitually, and at the conclusion of each meal the salivary flow was depressed so that surplus food tended to cling in sticky masses about the teeth, where it could ferment and initiate the carious process by tending to decalcify the enamel and expose the tooth to the causal agency of dental caries.

(ii) In Group A, food was scarce, and the children were still hungry at the end of each meal; there was still a flow of saliva adequate to flush out all the food débris from between the teeth, leaving the mouth clean; all the available food was utilized for nutrition and there was no surplus to lodge about the teeth. The total amount of food, however, must in most cases have been sufficient to prevent deficiency diseases, and possibly in the fewer individuals who exhibited caries, this may have been secondary to malnutrition.

It is a trite aphorism that more people are ill through over-eating, than through under-eating; and an old saying that one should rise from the table with a feeling of appetite still unsatisfied.

It is unlikely that parents who are economically capable of overfeeding their children would dare to take the risk of starving the growing child in order to conform to this concept. Yet, if there is any truth in my suggested explanation, over-eating would seem to render the teeth liable to dental caries just as starvation or malnutrition does and this would help to account for the fact that children of the well-to-do have on the whole worse teeth than those of the poorer classes, and "the better the school, the worse the teeth", though for different reasons.

In the case of malnutrition, a definite deficiency may result in imperfect tooth-formation, predisposing to caries.

In over-feeding, surplus food clinging round the teeth may result in carbohydrate stagnation with acid formation, and attack on the enamel, predisposing to caries.

Although enamel decalcification precedes caries, usually, in the human individual, it is not per se caries, since dentine exposed by attrition or otherwise does not always become carious; while some animals (Edentates) do not develop enamel at all, and yet are free from caries.

In some animals, and primitive races of man, the interstitial wear between molars may remove all the enamel so that dentine touches dentine at the contact points, and yet caries does not supervene. (Woods).

Another puzzling observation I have made refers to certain teeth in middle-aged individuals. These teeth have remained perfectly sound for 30 or 40 years while exposed in the mouth to whatever may be the cause or causes of dental caries, and then for no

apparent reason they suddenly develop cavities. This occurs often in individuals who appear to be quite healthy, as distinct from the cases mentioned above where sudden rampant caries may precede death. It is probable that some derangement so slight as to pass unobserved may determine the onset of caries. Similarly, an individual who has always seemed healthy may suddenly develop pneumonia.

From the foregoing review of the present state of our knowledge regarding dental caries, it is manifest that the actual specific cause is unknown. All the factors proved to have a positive effect in increasing the incidence of dental caries can be regarded as only predisposing causes.

Exposure to cold, malnutrition, and fatigue may all predispose to tuberculosis, but the actual exciting cause of the disease is the tubercle bacillus. In the same way, carbohydrate stagnation, starvation in its broadest sense---including insufficient calcium or vitamin D in the diet---and acidosis may all be regarded as predisposing causes of dental caries, but the actual exciting cause has not yet been discovered.

It is a not unreasonable hypothesis to assume that dental caries, like most other diseases, may be caused by some contagium vivum, either a microscopic organism or a filter-passing virus. It is stated quite definitely in the authoritative Dental Board book(57) that "The immediate cause of dental disease is certainly a microbe"; and Badcock(276) wrote that dental caries is distinctly a microbic and not a deficiency disease, though vitamin deficiency may be one of its most important predisposing causes.

Even if the primary lesion in the human subject is acid decalcification of the enamel (whether due to bacteria or mere chemical fermentation of sugars), the continuation of the disease is probably microbic, and the phenomena of arrested decay, and translucant zones in enamel and dentine, indicate some vital reaction in the tooth.

The investigations of the bacteriology of dental caries have resulted in a mass of literature, but no definite conclusion has been reached. Attention has always been focussed on the microscopically visible organisms present in the mouth and dental tissues, and consideration of the possibility of a filter-passing virus being the causative agent appears to have been overlooked. If this hypothesis were correct, it would not be unlikely that laboratory tests might demonstrate some positive evidence of its truth, and with a view to investigating this possibility, the experiments described in the following pages were devised.

# Preliminary Report

# on

# IMMUNOLOGICAL REACTIONS IN DENTAL CARIES.

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## C O N T E N T S



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## INTRODUCTION

As none of the research on the aetiology of dental caries has so far done more than indicate predisposing causes and accompanying phenomena, it was felt that a fresh line of investigation should be followed.

Theo von Beust (1912) [12] wrote, "This vascularity of the enamel renders it possible, even probable, that the immunity to caries observed in some teeth lies in the tooth itself, and is the result of the formation of antibodies".

E.W. Fish (1926) [69] wrote, "Since calcium deficiency is not the predisposing cause of caries, and since there is such a vigorous circulation of lymph in the dentine and (?) enamel, I think it worth while investigating the possible existence of an active immunity to caries in the blood stream."

These are the only references to the subject of this paper, so far discovered, which were published before the work described in this preliminary report was begun.

## PRELIMINARY CONSIDERATIONS

Lethal tendencies in conditions entailing germ cell products, e.g. pregnancy in mammals and ovulation in many orders of insects and pests, are well known. In the animal kingdom immune reactions are demonstrable by laboratory tests. In pregnancy we have an instance of a reaction - antagonism - between the serum of the host and an antigen. There is reason then to suspect the possibility of a similar antagonism between serum and tooth substance originating in blastodermic or blastothelial cells. It is probable, however, that two main factors would render the demonstration of this principle a difficult matter in the laboratory test. Firstly, the protein content of tooth substance likely to act as an antigen is in small proportion to the inorganic material. Secondly, the intensity of the reaction would tend to decrease with the age of the host, especially on complete eruption of the tooth. On the other hand, antagonism between serum and foreign substance, possibly in the form of bacterial body end products, present in carious dentine should appear in intensity proportional to the capacity of immunity centres to engender immune bodies against a continual supply of foreign substance.

The microscopic pathology of the carious lesion is beautifully described and illustrated by E.W. Fish (1932) (70) in his recent excellent monograph, where he brings together sufficient evidence of a process of reaction on the part of the tooth to experimentally-produced or naturally-occurring carious lesions. In this reaction the carious lesion becomes isolated from the dental pulp by an impermeable calcium barrier which prevents interchange of fluids between the lesion and the pulp, so that in the complete reaction toxic fluids would not be absorbed into the body. Permeability is adjudged by Fish from staining experiments covering permeation in any one tooth of a duration of usually twenty-four to forty-eight hours only, and it is conceivable that in natural conditions in which the lesion persists over a period of many months permeation of fluids actually does take place slowly through the calcium barrier in sufficient quantities to generate antibodies to counteract the toxicity of the lesion.

Tomes (1848) (242), who first described the translucent zone in dentine, considered that it was often an imperfect barrier. The process once commenced would be easily excited to further action by constantly repeated doses of toxic matter from the lesion. Fish (71), however, states definitely that "The translucent zone

is always a complete barrier to the passage of fluids between the lesion and the pulp (not sometimes imperfect as suggested by Tomes)".

On the other hand, it is conceivable that, if the calcium barrier actually is completely impermeable, toxic matter from the carious lesion enters the body through the pulp before the dental calcium barrier is laid down, and may even be the exciting cause of its deposition; and that repeated doses of toxic matter from successive carious lesions gradually stimulate the reactive forces of the body.

It was the object of the present research to prove by laboratory methods that such a process actually takes place.

The phenomenon of arrested caries may be capable of explanation by the suggestion that the toxic matter excites the deposition of the calcium barrier, and that in those individuals who possess a high degree of immunity, inherited or acquired, the reactive powers of the body are such as to produce a vigorous reaction to the carious lesion with production of a complete and impermeable calcium barrier. The suggestion occurs that this barrier may be an actual precipitate resulting from the reaction between transuding serum (or "dental lymph") and the infection, as in the precipitin test to be described

later, and this seems the more probable as arrested caries occurs most frequently on the occlusal aspect of molar crowns in the same positions as the translucent zone of Tomes - at the peripheral ends of the dentinal tubules, farthest removed from the influence of the odontoblasts. In the dead-tract reactions, the calific barrier is deposited apparently by the still living odontoblasts as secondary dentine, and appears to be a vital reaction on the part of those cells. Arrested caries seldom occurs at these sites.

Some support is given to this suggestion by what is known of calcareous depositions in normal and pathological states in shell-fish. J.H. Orton (1927) (188) in a study of shell depositions in oysters remarks on "the microscopic characters of a calcareous deposit on an oyster shell opposite a suppuration in the body. The calcareous deposit was exceedingly thin and covered numerous small cells or platelets up to 10$\mu$ in diameter containing branching rod-like structures similar to those found in platelets in brown spots (excretion on the shell covered with conchyolin only)".

Calcareous deposits, therefore, consist generally of two kinds; that form laid down in response to the general functions of the body, and that in response to

stimulus from the site of a pathological process. In the case of normal functioning, the calcium layer is intimately connected with a layer of "special leucocytes functioning as calcoblasts" in Lima Scabra (Carpenter, quoted by Orton); odontoblasts beneath the dentine of the tooth; and ameloblasts depositing the enamel. The deposition of calcium resulting from infection is a familiar feature. The pearl is a typical example of deposit round a disintegrating cestode larval focus of irritation. Cestode larvae are characterized by the calcareous "corpuscles" present within the body during life. Counter-irritant calcium deposit within the bladder accompanies Bilharziosis. As a final example, the group of streptococcal infections might be mentioned, especially in view of the possible implication of Streptococcus mutans (Clarke, 1924) (45) in the causation or continuance of dental caries. The aetiological relationship of streptococci to the formation of gallstones was demonstrated by Rosenow (1916) (208) in experimental cholecystitis produced by intravenous injection of streptococci from cholecystitis in man.

The conclusion of Rosenow (1923) (210) "that primary urinary calculi are often due to streptococci which have elective affinity for the urinary tract"

seems justified from his experiments in which dogs whose teeth had been infected by streptococci from catheterized urine, tonsils and teeth of human patients suffering from nephrolithiasis developed typical calculi or lesions of the medulla. "The experimentally produced calculi were similar in physical properties and chemical composition to those found in nephrolithiasis in man".

At the conclusion of another paper, Rosenow (1922)[209] writes, "there can be no doubt of the importance of the specific localizing power of bacteria and of foci of infection in the causation of many diseases". In view of the possibility of dental caries being due to an organism, this selective affinity should be borne in mind.

No special attention has been given in the present work to the identity of organisms present in the carious lesion. There is a mass of literature on the subject, mostly disappointing in character, and, with the exceptions of Lactobacillus acidophilus odontolyticus and Streptococcus mutans no recent or definite evidence is offered associating specific organisms with the lesion. This remarkable failure is probably due to the lack of suitable means of isolating pathogens from among the bacterial flora.

Four patients from among those selected for tests by immunological methods also submitted voluntarily to the withdrawal of venous blood from the arm for the purpose of pathogen-selection culture of the organisms from their own carious lesions, by the method of Solis-Cohen (1926) [226]. The results of these cultures are given below:

RESULTS OF PATHOGEN-SELECTIVE CULTURES FROM CARIOUS LESIONS

| Case[x] | Colonies by Direct Plating | | | Colonies by Pathogen-Selection | | | Organisms |
|---|---|---|---|---|---|---|---|
| | Small | Medium | Large | Small | Medium | Large | |
| MA | - | - | - | - | - | - | |
| ED | - | - | - | - | - | - | |
| LO | - | 3 | - | - | 300 | - | Streptococci |
| RE | 60 | - | - | 360 | - | - | Streptococci |
| | - | 60 | - | - | 30 | - | Gram+diplococci |
| | - | - | 2 | - | - | 2 | Staphylococci |

[x] See Case List, Section I (f)

These results were encouraging, and gave great impetus to the investigations which followed, since the method adopted is considered as "the most dependable criterion of the actual immunity of the animal". (Black, Fowler and Pierce (1920) quoted by M. Solis-Cohen (1926))

The sequence in which the experiments were done was largely determined by the opportunities of obtaining material. In general the scheme of investigation included experiments under two sections.

Failure to isolate a causative organism by ordinary bacteriological methods led to a search for a precipitin test. The phenomena associated with pathogen-selection culture methods applied to dental caries, as above, were sufficiently convincing in the first few attempts to support the suggestion that blood or blood serum developed immunity against organisms found within the active lesion. The formation of a "Calcium barrier" beneath a cavity and the laying-down of secondary dentine are sufficient reasons to suspect a reactive process. When considered in the light of a bacterial infection such a reaction could only be one generated by immune processes and it appeared reasonable further to suspect that evidence of such a process could be obtained from serum. The "gen" obtained from the lesion consisted of a saline emulsion

of the bulk of the carious dentine together with detritus from enamel sources and, of course, bacterial bodies, live or dead, whole or disintegrated. When filtered or centrifuged, such a "gen" would contain bacterial end products, together with soluble substances from the dentine. The interpretation of precipitin phenomena between such a precipitinogen and serum would need to be guarded, and the following points are borne in mind:

(i) Previous infections, particularly streptococcal, giving reactions of the group type.

(ii) Reactions against saprophytes finding a pabulum within the lesion.

(iii) Antagonism of body fluids to affected or unaffected dentine.

(iv) Precipitability of tissue products by serum from heterologous sources.

# SECTION I

## Precipitin reaction between Serum and Saline Emulsions of Carious Material.

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The test materials consisted of one unit volume of

i. Serum or dilutions of serum in saline.

ii. Precipitinogen obtained in saline emulsion or suspension from carious dentine.

(a) Method of Dilution

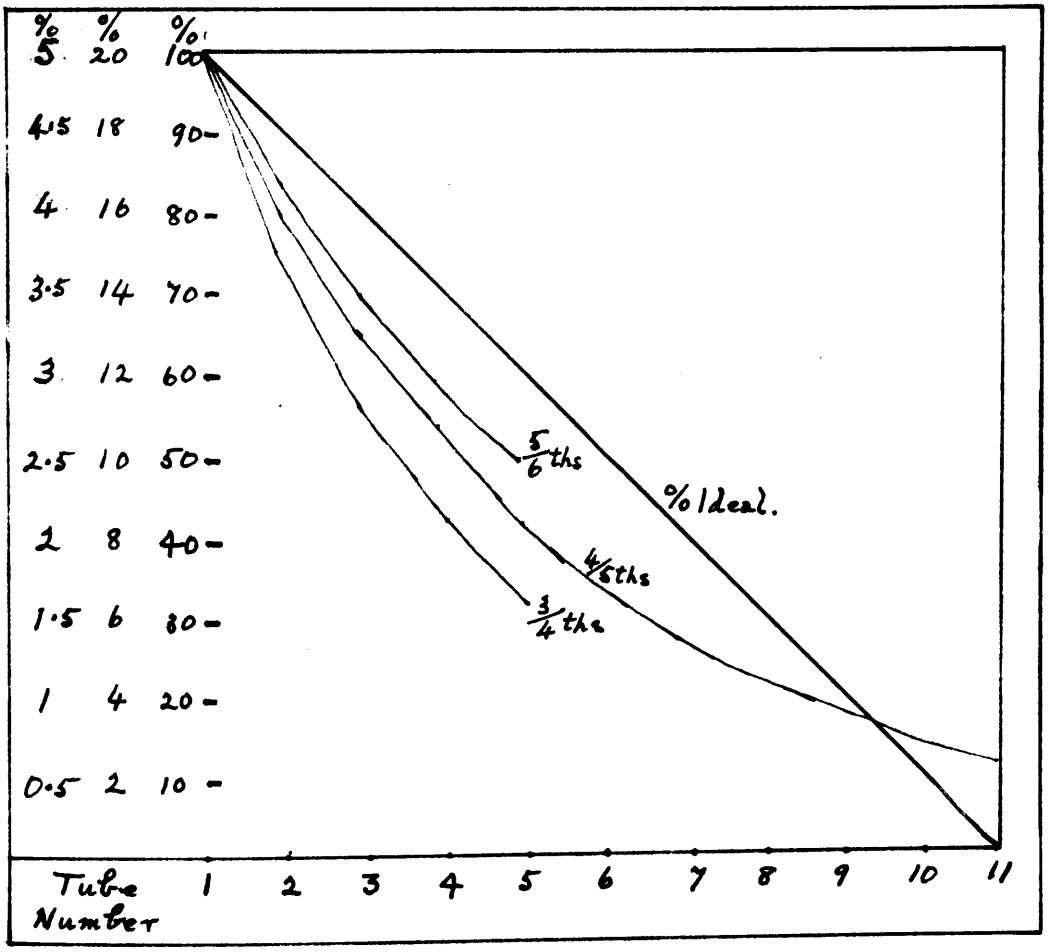
By the method used in putting up the test, that component requiring dilution was first made up in the strongest dilution required and one unit of this strength was placed in tube number one. Four units were mixed with one unit of saline in tube number two, and after this dilution was made, four units of the mixture were carried to tube number three, and mixed with one unit of saline. This process was repeated till a series of dilutions by four-fifths was obtained between two suitable points. Experiments given below determine the critical zone for the test.

It may be of interest to observe the graph of

dilutions which aided the choice of a simple dilution method. The ideal solution by descending percentages of even distance is impractable by simple method without extravagance in the use of material, and is extremely difficult to obtain with small units, such as the amount of carious emulsion available from one of the patient's own teeth. The method used here was devised in order to overcome this difficulty, and so far as I know has not been recorded in the literature.

## Graph of Dilutions

# GRAPH OF DILUTIONS.



The graph indicates the advantages of a simple dilution method using amounts of diluent decreasing by four fifths.

a. Titration of "gen" from carious dentine gave a reactive titre in the region of a 10% dilution.

b. Titration of "serum" indicated three average end points:

i. Non-immunes, 3rd tube = 3.5%

ii. Moderate immunes, 7th tube = 1.3%

iii. Total immunes, 17th tube = 0.15%

The amount of serum required for the test when using a unit volume of four drops (of diluted serum) is one drop, an amount easily available from a finger puncture.

The "gen" required for a single test, 20 tubes of unit volumes of four drops is usually available from a single tooth by the method used.

b. Method of Obtaining Serum for the Test Reactions.

Venous blood was obtained by the usual aseptic method from those cases in which comparative pathogen-selection culture tests were made. These tests have been referred to already in the text.

Ordinarily, blood was obtained from the finger as follows:-

i. The finger was cleaned with soap and water, then rinsed with alcohol and allowed to dry.

ii. A glass slide was sterilized in alcohol and broken smartly on sterile filter paper to produce sterile glass spicules.

iii. Blood was 'centrifuged' into the prepared finger by swinging the extended arm vigorously with a circular motion from the shoulder, the finger being then wrapped quickly from below upwards with several turns of a length of fine rubber tubing.

iv. A puncture made with a sterile glass spicule behind the nail yielded sufficient blood to half-fill a Widal tube. Further quantities were obtained by loosening the rubber and repeating the process iii above, no further puncturing being necessary. The amount of

blood obtained depended chiefly on the speed with which centrifuged blood was trapped in the finger by the rubber wrap.

v. Serum was separated from the clot in the ordinary way and stored in the ice chest till required.

c. Method of Obtaining Carious "gen" for the Test Reaction.

Teeth were obtained fresh from the Extraction Room of the Dental Hospital and those showing carious cavities without obvious exposure of the pulp were selected. The tooth was scrubbed with a brush under flowing hot water and dried on filter paper. As much carious material as possible was extracted from the cavity and weighed. Physiological saline solution was added to the carious material in the proportion of 20 cc. saline to 1 g. of material. Glass beads were poured into the tube and by means of a glass rod the material was crushed when possible and in every case mixed with the beads to allow of an even distribution of any extracted substances. Extraction was done by several methods, but that finally adopted was by leaving the tube in the ice

chest at under 0°c. for 20 or 24 hours.

After extraction the fluid was removed, centrifuged at 3,000 r.p.m. for 10 minutes and in the first few tests was passed through a Jenkin's filter block capable of holding back the enterococcus. The resulting clear fluid was diluted as required for test. A dilution of 10% in saline was eventually used as a satisfactory reactive "gen".

In cases where the patient's own carious material was used, the selected tooth (or teeth) was isolated with rubber-dam, cleaned, and sterilized with alcohol. The carious dentine was then excavated as thoroughly as possible and received in an aseptic container. Extraction of the "gen" was then proceeded with as before. The carious material thus obtained from an individual was usually necessarily small in amount.

Modifications of the above method for obtaining "gen" from normal dentine are given in the "gen" list which follows ((g), N.D.).

d. Examination for Results of Test.

On mixing the test materials the tubes were quickly shaken and then kept at room temperature for 15 minutes. At the end of this time any reaction seen was recorded as an immediate reaction. As compared with serum and antigen

control tubes, the first evidence of reaction was cloudiness. The strength and character of the formed precipitate was indicated by symbols. A further reading was made after two hours during which time the tubes were incubated at 37°c. The symbols of the reaction were adjusted when necessary. In some of the tests - but not all - phenol to 0.4% was added to the test materials at this stage in the procedure to guard against bacterial contamination. Such preserved tests are indicated by an asterisk. After a complete 24 hours incubation at 37°c. the symbols were again adjusted, if necessary, to record the final character of the precipitate. The end-point of the reaction was determined as the tube containing the least quantity of precipitate, this being finally decided by observing precipitate by lens x 10 at the bottom of the tube after centrifuging at 3,000 r.p.m. for 5 minutes. At this speed any extraneous material seen by lens x 10 in uncentrifuged control tubes had apparently disappeared after centrifuging.

At the final examination of the test, after 24 hours, and before centrifuging, the first impression is that of an increased translucent opacity towards the left of the series when viewed against a strong opalite

lamp. In strong reactions this opacity is accompanied by a precipitate which in some cases is "tan" coloured, visible to the unaided eye. On examination with the lens x 10 the opacity resolves itself into innumerable minute particles suspended evenly throughout the medium. In appearance these minute particles resemble breadcrumbs in the tubes towards the right, the particles become fewer and of a more spiky snow-crystal character, and are gradually less distinguishable from the extraneous suspensions commonly seen by hand lens in fluids not effectively filtered. These extraneous suspensions apparently consisted of extremely minute particles so highly refractile that they appeared larger than they really were. As has been previously stated, they disappeared entirely after centrifuging, and could not be detected as a sediment even with the lens x 10. With experience, no confusion need arise between these extraneous suspensions and the precipitate, but to the inexperienced centrifugalization will be a help in determining the end point of the reaction.

e. Symbols Used.

In the results recorded below, symbols to indicate reactions are used with the same meaning throughout. Control tubes were put up consisting of equal units of "gen" and saline, or serum dilution and saline. Where

one or both of these tubes showed a tendency to cloud or precipitate, which in every case was of a very fine nature, the "amount" of cloud or precipitate was deducted from the reading of every tube in that test series. Latterly, however, controls have been neglected, as it has been shown that precipitation decreases in amount from left to right in the series whenever "gen" is diluted by 4/5ths from 10% strength (and serum remains constant), or serum is diluted by 4/5ths from 5% strength with a constant strength of "gen".

Symbols used

|| An immediate reaction, i.e. occurring within 15 minutes at room temperature.

— Having a brownish tint (tan-coloured).

++ Precipitate visible to the eye.

\+ Precipitate visible with lens x 10.

⊕ Precipitate very weak.

? Precipitate very doubtful.

– no precipitate.

□ Test not done owing to lack of material.

f. Case List

| Case | Sex | Age | Dental State | Remarks |
|---|---|---|---|---|
| MA | ♂ | 16 | 2 cavities | Arrested caries present for many years and not progressing. |
| RE | ♂ | 19 | Many cavities | Progressive caries |
| LO | ♂ | 30 | 1 small cavity | ? Progressive - robust health. |
| ED | ♂ | 25 | Few cavities | ? Arrested caries, not progressing. |
| LE | ♂ | 37 | No cavities | Had caries in youth. Now has upper denture. |
| WO | ♂ | 21 | No cavities | Perfect teeth |
| BE | ♂ | 34 | Some cavities | Not progressive - ? arrested |
| JO | ♂ | 19 | No cavities | Perfect teeth |
| GA | ♂ | 16 | 2 cavities | Progressing slowly |
| FE | ♂ | 20 | 4 cavities | " " |
| GR | ♂ | 20 | Over 60 fillings | Regular dental attention. Considered non-immune. |
| CL | ♂ | 23 | Many small fillings | Regular dental attention |
| HO | ♂ | 20 | 6 teeth with arrested caries | Arrested |
| CO | ♂ | 21 | 12 fillings, 2 crowns | Formerly very susceptible to caries. Considers himself liable to caries. |

The cases about which some knowledge of the dental state could be obtained are referred to by initials as shown above. In addition to these sera from unknown sources were tested. The latter, referred to by numbers, were mainly sera left over from the Wassermann test and were kindly supplied by Professor J.H. Dible.

g. "Gen" List

1. Saline emulsion of subject's own carious material, stood upon ice overnight, filtered through Jenkin's filter block. 2 cc. saline per tooth.

2. Saline emulsion of carious material from other patients prepared exactly as "Gen" No. 1.

3. Saline emulsion of patient's own carious material centrifuged at 3,000 r.p.m. for 10 minutes (not passed through Jenkin's block) prepared exactly as "Gen" No. 1.

4. Saline emulsion of carious material from other patients centrifuged at 3,000 r.p.m. for 10 minutes (not passed through Jenkin's block ) prepared exactly as "Gen" No. 1.

5. Carious material from several teeth from different patients weighed and extracted in ice chest overnight, with 20 cc. of saline per 1 g. carious material. Material mixed and supported among glass beads. Fluid centrifuged at 3,000 r.p.m. for 10 minutes.

Where the "gen" was produced from more than one tooth, the number of teeth used is indicated in brackets in the tables of experiments given below, e.g. 5(7). Modifications of the "gen" were attempted in investigating the possibility of improving the visibility of the reaction. These modifications are referred to separately.

"Gen" List (continued)

N.D. Teeth were selected which from external inspection appeared to be normal (free from caries). The roots were removed and the crowns freed from enamel by grinding. The remaining portions of the crowns were split and freed from all traces of pulp and discoloured dentine by further grinding. The apparently normal dentine was then washed rapidly in hot water, dried on filter paper and crushed to powder by pestle and mortar. The remainder of the procedure was the same as for carious "gen" given under (c) above, and (5) in this list, except that at first the quantity of saline used for extraction was 5. cc. per 1. g. of material, or four times stronger than "gen" No. 5. above.

Later 20 cc. per 1 g. was used to compare with the carious "gen".

The "gens" in this list were employed in 10% dilution for the experiments.

h. Experiment No. 1.

Determining the presence of precipitin in serum against a possible "gen" obtained from carious dentine.

Gen = No. 5 (7) diluted to 50% with saline.

Serum dilution = 100, 75, 50 and 25%.

| Serum | Tube No. 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| MA | ++ | ++ | ++ | ++ |
| JO | ++ | ++ | + | + |
| LE | □ | ++ | ++ | + |
| FE | □ | □ | + | + |
| GR | + | + | + | + |

The reaction is proved possible and varies in intensity directly with the degree of arrestation in the cases observed by less precise methods.

Experiment No. 2.

First titration of "gen" against diluted serum.

Gen = 4 (7) in dilutions 100, 75, 50 and 25%

Serum dilutions = 5%

| Serum | Tube No. 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| MA | ++ | ++ | ++ | ++ |
| BE | ++ | ++ | ++ | ++ |
| BE | □ | □ | □ | ++ |
| WO | ++ | ++ | ++ | + |
| LE | ++ | ++ | ++ | ++ |
| WO | □ | □ | □ | + |
| ED | ++ | ++ | ++ | ++ |
| LO | + | + | + | - |
| LO | □ | □ | □ | - |

The relative intensity of the reaction is not appreciably diminished in a dilution of "gen" of 25% strength except to exclude one case LO whose dental history shows no natural test of his liability to caries. It may be noted that case LO is a regular 3 monthly blood donor and a few days prior to this test

had given over a pint of blood. This may have some bearing on the results found in his case.

Experiment No. 3.

Second titration of "gen" against diluted serum.

Gen = 5 (7) in dilutions of 50% by 4/5ths down to 20 tubes.

Serum dilution = 1%.

| Serum | Tube No. 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JO | ± | ± | ± | ± | ± | ± | ± | ± | + | + | + | + | + | + | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ |
| MA | ± | ± | ± | ± | ± | + | + | + | + | + | + | + | + | + | + | + | + | ⊕ | ⊕ | ⊕ |

↑

"Gen" in a dilution of 10% is more than sufficient to indicate the reaction (10% dilution occurs between the 7th and 8th tube in this series). To the right of this mark there is indication that the amount of precipitable "gen" decreases.

Experiment No. 4.

Combining observations from previous experiments to elucidate a simple routine test.

i. An immediate reaction is produced with a serum unit of 5% strength.

ii. Tan coloured precipitate occurs in some cases with serum of 1% strength when used with "gen" diluted to at least 10%.

iii. In experiment No. 3 the precipitate persists in very minute doses of "gen" (Tube No. 20 = a dilution of "gen" of approximately 0.8%) suggesting firstly that precipitate will occur in the presence of sufficient serum from any strength of "gen", and secondly, titration of serum against adequate "gen" is necessary.

Titration of serum against an adequate "gen".

"Gen" = 1,2,3,4 or 5 as shown below diluted after preparation to 10% strength. Where the "gen" was obtained from a case in the Case List such is indicated by initials.

Serum = Tube No. 1 contains one unit of 5%

Tube No. 2. contains one unit of 4/5ths the strength of that in Tube No. 1.

and so on to Tube No. 20.

| Serum | "Gen" 10% | Tube No. 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | Ages in days where older than 24 hrs. Undil-uted Serum | Undil-uted Gen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MA | MA.1 | ++ | ++ | + | + | + | + | + | + | + | + | + | + | ⊕ | ⊕ | ⊕ | - | - | - | - | - | | |
| MA | MA.1 | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | | |
| MA | RE.2 | ++ | ++ | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | | |
| MA | ED.2 | ++ | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | 3 | |
| MA | LO.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 | |
| MA | 3 | ++ | | | | | | | | | | | | | | | | | | | | 5 | |
| MA | 4(2) | ++ | ++ | ++ | + | + | + | + | + | + | + | + | + | + | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - | | |
| MA | 5(7) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | ⊕ | - | - | - | - | - | 4 | |
| RE | RE.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| RE | MA.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| LO | LO.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| LO | ED.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| LO | 4 | + | - | - | - | - | - | - | - | - | - | | | | | | | | | | | 40 | |
| LO | 4 | + | - | - | - | - | - | - | - | - | - | | | | | | | | | | | 40 | |
| LO | 4 | ⊕ | - | - | - | - | - | - | - | | | | | | | | | | | | | 40 | |
| ED | ED.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 40 | |
| ED | LO.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| ED | ED.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| ED | 4 | + | ⊕ | ⊕ | ⊕ | - | - | - | - | - | - | | | | | | | | | | | 40 | |
| ED | 4 | - | - | - | - | - | - | - | - | - | - | | | | | | | | | | | 40 | |
| ED | 4 | + | + | + | ⊕ | ⊕ | - | - | - | | | | | | | | | | | | | | |
| LE | ED.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| LE | LO.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LE | ED.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| LE | 4 | + | + | + | + | + | + | + | ⊕ | ⊕ | ⊕ | | | | | | | | | | | 5 |
| LE | 4 | + | + | + | + | + | + | + | - | - | - | | | | | | | | | | | 5 |
| LE | 4 | + | + | + | ⊕ | - | - | - | - | | | | | | | | | | | | | |
| LE | 4 | ++ | + | + | ⊕ | - | - | - | - | | | | | | | | | | | | | |
| LE | 4 | + | + | - | - | - | - | - | - | | | | | | | | | | | | | |
| LE | 4(2) | + | + | + | + | + | ⊕ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| LE | 5(7) | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | 4 |
| WO | 1 | ++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | ⊕ | ⊕ | - | - | - | |
| WO | 4 | + | + | + | - | - | - | - | - | - | - | | | | | | | | | | | 30 |
| WO | 4 | - | - | - | - | - | - | - | - | - | - | | | | | | | | | | | 30 |
| WO | 4 | + | + | ⊕ | - | - | - | - | - | | | | | | | | | | | | | 30 |
| BE | BE.1 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | |
| BE | 4 | + | + | + | + | + | + | ⊕ | - | - | - | - | - | - | - | - | - | - | - | - | - | 10 |
| BE | 4 | + | + | + | + | ⊕ | - | - | - | | | | | | | | | | | | | 10 |
| JO | 4 | + | + | + | + | + | + | + | - | - | - | | | | | | | | | | | |
| JO | 4 | + | + | + | + | + | ⊕ | - | - | - | - | | | | | | | | | | | |
| JO | 4 | ++ | ++ | ++ | + | + | + | + | + | | | | | | | | | | | | | 2 |
| JO | 5(7) | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | |
| GA | 4 | ++ | + | ⊕ | ⊕ | - | - | - | - | | | | | | | | | | | | | |
| GA | 4 | + | + | - | - | - | - | - | - | | | | | | | | | | | | | |
| GA | 4(2) | + | ⊕ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| GA | 5(7) | + | + | ⊕ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 4 |
| FE | 4 | + | + | + | + | + | + | + | + | + | ⊕ | | | | | | | | | | | |
| FE | 4 | + | + | + | + | + | + | + | + | ⊕ | ⊕ | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FE | 4(2) | ++ | + | + | + | + | + | + | ⊖ | ⊖ | - | - | - | - | - | - | - | - | - | - | 7 | |
| FE | 5(7) | + | + | + | + | + | + | + | + | + | + | ⊖ | - | - | - | - | - | - | - | - | 11 | |
| GR | 4(2) | + | ⊖ | ⊖ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| GR | 5(7) | + | + | ⊖ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 4 | |
| MA | 5(7) | ++ | ++ | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 17 | 14 |
| * LE | 5(7) | ++ | ++ | ++ | + | + | ⊖ | ⊖ | ⊖ | ⊖ | ⊖ | | | | | | | | | | | |
| * GR | 5(12) | + | + | + | ⊖ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| JO | 5(12) | ++ | ++ | ++ | ++ | ++ | + | + | ⊖ | ⊖ | ⊖ | ⊖ | - | - | - | - | - | - | - | - | | |
| * LE | 5(12) | + | + | ⊖ | ⊖ | ⊖ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | |
| * HO | 5(7) | ++ | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | |

Observations upon the results of the Series of Tests in Experiment No. 4.

i. The immediate reaction given in the first few tubes in a strongly positive series occurs only with freshly prepared "gen" and serum no more than three days old. This reaction is visible to the eye and the formed precipitate is tan coloured, usually.

ii. The dental state and history of the cases investigated agree to some extent with the types of reaction obtained.

iii. "Gens" vary a little in their precipitability against fresh serum but not outside the range of three types of reaction viz.,

A. Non-immunes precipitating not further than the third tube of the series, i.e. in serum dilution to 3.5%.

B. Cases moderately immune precipitating to about the seventh tube in the series, i.e. in serum dilution to about 1.3%.

C. Totally immunes precipitating beyond the seventh tube, usually between the twelfth and seventeenth tube, i.e. in serum dilutions to between 0.4 and 0.15%.

iv. Confusing results occur with serum ten or more days old. Sera were stored on ice with no special precautions against contamination.

v. The table shows results built up from three observations viz., after 15 minutes, 2 hours and 24 hours. In general the reaction continued by increasing the intensity of precipitate already formed and by precipitate occurring in tubes further to the right at each succeeding inspection. One possible irregularity of precipitation occurred in tests of serum MA, "Gens" 4(2) and 5(7). That was that the first tube of the series showed much less precipitate than that in the second tube at the 2 hours reading, thus depressing the importance of the immediate reaction frequently observed with this and other serum.

## Miscellaneous Experiments

### Experiment No. 5.

Precipitation as occurring under the conditions of Experiment No. 4 yields an indefinite end point especially where extraneous particles may be seen in the control tubes. These extraneous particles tend to disappear from view after centrifugalization at 3,000 r.p.m. for 5 minutes.

| | End Point by hand lens x 10 24 hours. | End point by visible deposit after centrifugalization at 24 hours | End point by visible deposit after centrifugalization at 30 hours. |
|---|---|---|---|
| Tube No. | 3 | □ | 4 |
| Tube No. | 3 | □ | 4 |
| Tube No. | 8 | □ | 9 |
| Tube No. | 11 | □ | 12 |
| Tube No. | 15 | □ | 15 |
| Tube No. | 11 | □ | 12 |
| Tube No. | 3 | 3 | □ |
| Tube No. | 3 | 3 | □ |
| Tube No. | 8 | 8 | □ |

Centrifugalization is likely to aid the inexperienced in determining the end point of the reaction at 24 hours. That the reaction continues after this period necessitates a time limit for the final examination, viz., 24 hours if comparable results are to be recorded.

Experiment No. 6.

Means to fortify the "gen" with a view to producing a clearer "end point" reaction and probably increased visibility throughout the series giving precipitate was sought by the addition (one experiment only) of cholesterol in quantity and manner used to fortify antigens in the Wassermann test.

End point without addition of cholesterol - Tube No. 15.

End point with addition of cholesterol - Tube No. 8.

Cholesterol apparently inhibits the reaction.

Experiment No. 7.

Thirteen carious teeth were placed in absolute alcohol 1 cc. per tooth, and extracted at room temperatures. Minute samples of the extract were withdrawn and tested against precipitating and nonprecipitating sera used in Experiment No. 4.

"Gen" = Dilutions in saline as shown in the table were constant throughout each series. In Test marked (x) the extract was diluted 4/5ths in a series of 10 tubes commencing with 5% (serum being constant at 5%)

Serum (except test marked (x)) in a series of dilutions by 4/5ths commencing with 5%.

| Serum | End point tubes in Experiment No. 3 with fresh serum | Duration of extraction in days | "Gen" dilution % | Tube No. 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ED | - | 1 | 1.25 | - | - | - | - | - | - | - | - | - | - |
| MA | 17 | 3 | 1.25 | - | - | - | - | - | - | - | - | - | - |
| MA | 17 | 3 | 1.25 | - | - | - | - | - | - | - | - | - | - |
| ED | - | 8 | 2.5 | - | - | - | - | - | - | - | - | - | - |
| LE | 10 | 8 | 2.5 | - | - | - | - | - | - | - | - | - | - |
| LO | - | 8 | 2.5 | - | - | - | - | - | - | - | - | - | - |
| MA | 17 | 8 | 2.5 | - | - | - | - | - | - | - | - | - | - |
| MA | 17 | 8 | (x) | - | - | - | - | - | - | - | - | - | - |
| MA | 17 | 14 | 2.5 | - | - | - | - | - | - | - | - | - | - |
| MA | 17 | 14 | 2.5 | - | - | - | - | - | - | - | - | - | - |
| BE | 12 | 28 | 2.5 | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - | - | - |
| BE | 12 | 28 | 2.5 | ⊕ | ⊕ | ⊕ | ⊕ | | | | | | |

The feeble reaction shown with serum of case BE might indicate that extraction of longer than 28 days is required. The hygroscopic nature of alcohol may, however, be responsible for a watery extract from the preserved principle accumulating in the alcoholic "gen" after a period of 28 days.

## Experiment No.8

Comparison of Precipitin reactions between serum dilutions and Gens from normal and carious dentine, the normal "gen" being four times stronger than the carious gen.(See "gen" list).

| Serum | Normal Gen | % Serum 5 | 3.75 | 2.5 | 1.25 | Serum 5 | 3.75 | 2.5 | 1.25 | Carious Gen |
|---|---|---|---|---|---|---|---|---|---|---|
| GA | ND/4 | + | ⊕ | - | - | + | ⊖ | - | - | 5/16 |
| * GA | ND.4 | - | - | - | ⊕ | + | + | + | ⊕ | " |
| CL | " | + | - | - | - | + | ⊕ | ⊕ | - | " |
| * CL | " | □ | □ | - | - | + | + | + | - | " |
| A 1460 | " | + | + | - | - | ++ | + | + | + | " |
| 12648 | " | + | - | - | - | + | ⊕ | ⊕ | ⊕ | " |
| * " | " | ⊕ | - | - | - | ++ | + | ⊕ | ⊕ | " |
| 12615 | " | + | + | - | - | + | + | - | - | " |
| 12613 | " | ⊕ | - | - | - | ++ | ++ | + | + | " |
| 32724 | " | + | - | - | - | ⊕ | - | - | - | " |
| 32718 | " | + | ⊕ | - | - | ++ | + | ⊕ | - | " |
| 12621 | " | ⊖ | - | - | - | + | - | - | - | " |
| * " | " | - | - | - | - | - | - | - | - | " |
| 12634 | " | ⊕ | - | - | - | ⊖ | ⊖ | - | - | " |
| 12647 | " | + | + | ⊕ | - | ++ | + | + | + | " |
| 12630 | " | - | - | - | - | ++ | + | - | - | " |
| * " | " | ⊕ | - | - | - | ++ | + | ⊖ | - | " |
| 32731 | " | + | - | - | - | + | + | + | - | " |
| * " | " | + | ⊕ | - | - | ++ | ⊕ | ⊕ | - | " |
| 12652 | " | ⊕ | - | - | - | + | - | - | - | " |
| 12632 | " | ⊖ | - | - | - | ⊖ | - | - | ⊕ | " |

| | Serum | Normal Gen | Percentage of Serum 5 | 3.75 | 2.5 | 1.25 | Percentage of Serum 5 | 3.75 | 2.5 | 1.25 | Carcinous Gen |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12600 | ND.4 | + | - | - | - | ++ | + | ⊕ | - | 5/16 |
| | 12631 | " | + | + | ⊕ | - | ++ | + | + | ⊕ | " |
| | 12640 | " | ⊕ | - | - | - | + | ⊕ | ⊕ | ⊕ | " |
| | 12646 | " | - | - | - | - | ⊕ | ⊕ | - | - | " |
| * | " | " | - | - | - | - | + | ⊕ | - | - | " |
| | 12618 | " | + | + | ⊕ | - | ++ | ++ | + | ⊕ | " |
| | 12635 | " | ⊕ | - | - | - | ++ | + | + | ⊕ | " |
| | 12645 | " | ⊕ | ⊕ | ⊕ | - | + | ⊕ | - | - | " |
| * | " | " | ⊕ | - | - | - | ++ | ++ | ⊕ | - | " |
| * | CO | " | - | - | - | - | ++ | ++ | ⊕ | - | " |
| * | LE | ND.3 | + | + | ⊕ | ⊕ | ++ | ++ | + | ⊕ | 5/7 |

These results indicate that the precipitating principle is absent from, or present in much weaker concentration in, normal "gen" four times stronger than the carious "gen" against which comparison is made.

Under Section II, complement fixation is compared with precipitates from the same sera. These precipitates are almost absent in tests with normal "gens" of the same strength as those of carious origin.

## j. DISCUSSION

1. A reaction such as that given by serums of the order MA, WO, BE, JO and HO - wherein precipitate is visible to the unaided eye in at least the first tube of the series (Experiment No. 4) before the expiry of 2 hours - is obtained with a mixture of serum containing precipitin and a saline-solution-soluble substance derived from carious dentine acting as precipitinogen. The tendency of the precipitate to be of a brownish hue (tan coloured) is perhaps an indication of the origin of this tint in the affected dentine of the carious cavity. The brownish colour sometimes seen clinically during cavity preparation in the 'hard and shiny' surface of dentine left when the softened carious material is removed may be due to an actual precipitate caused by the interaction of the body fluids with toxic material from the carious lesion, similar in character to the tan coloured precipitate occurring in these tests.

2. The formation of the precipitate in this reaction occurs in a manner similar to that observed in other bacterial affections of the animal body. Firstly, cloudiness appears in the mixtures, later the cloudiness resolves itself into formed

precipitate, flocculent in character, and where this precipitate is produced in great quantity, deposit occurs.

3. The reaction, whether observed as cloudiness, flocculent precipitate or deposited precipitate, occurs in greatest intensity to the left of the series of tubes in the test, moderating towards the right of the series with successive dilutions of serum.

4. Bacterial contamination of isolated tubes in certain series has been observed. In no case has the interpretation of the result of the test been affected. The appearance of the contaminated tube was different from that of the positive precipitin test. Microscopical examinations of the deposits obtained by centrifuging positive tubes of the tests done on serum BE revealed - by Gram staining - a few organisms only. The precipitate was not therefore a mass of bacterial bodies. Contamination of individual tubes in the test series did not occur in any particular order, and is preventable by the addition of phenol to 0.4% to the mixed test materials, as recommended in "Muir and Ritchie" ([177]).

5. The determination of the end point of the reaction is difficult. The test materials often contain floating

particles not unlike precipitate at or near the end point of the reaction. Centrifuging tubes at and around the possible end-point is described in the text, and it is sufficient to observe deposit obtained by centrifuging in recording a positive result in high serum dilution.

The reaction is thus brought within the range of the precipitin serum dilutions in the common bacterial infections. It is to be remembered that if the ocular visible precipitate alone is taken as proof of immunity, sera MA, WO, BE, JO, and HO remain in the immune group, such precipitate having been produced by a combination between "gen" and "in". It is obviously impossible for a "gen" finally diluted as in the test to 0.25% strength to provide the amount of precipitate observed.

6. Three points of minor interest which arose during the investigations of which this report is a record may be mentioned here:

(i) Haemagglutinins and haemolysins against blood cells of Moss Group No. 4 (inagglutinable by sera of any of the four groups) were not demonstrated in "gen" used in these tests.

(ii) In the search - so far unsuccessful - for a substitute "gen" for the precipitin test, two sera, GR and LE, of nine tested gave a positive reaction to Urea stibamine in the Chopra test for Kala Azar. Though precipitates were given eventually with all sera tested against Urea stibamine and incubated at 37°C., and these varied in intensity proportionally to the quantity of serum or drug present, there was no relation shown to exist between these reactions and immunological reactions with the same sera.

(iii) Preliminary tests with saline or serum extractions of tooth pulp indicate that the precipitin reaction obtained when using these extracts against "gens" of carious dentine is not greater than that produced by the amount of residual pulp serum or/and the amount of serum used in proportion for the extraction. The results are too few to record here but are mentioned because the "vital reaction" "within the tooth" has been the subject of keen research by Fish in his histological studies, and the same idea was

suggested by von Beust. Results so far indicate that the reaction is in proportion to the immunity present in the circulating serum.

7. A vital theory to account for the translucent zone of Tomes has been advanced by Fish (1932)[71] and he writes "the fact remains that sometimes the fibril survives, and by its increased activity - for no other method seems possible - lays down the plugs of calcific tissue in the cut ends of the tubules which collectively form the translucent zone." The results of this present research suggest that the translucent zone is due to an actual precipitation near the peripheral ends of the exposed dentinal tubules caused by the interaction of toxic substances (precipitinogen) from the carious lesion, and serum precipitin from the body.

A B C

Photograph of precipitates resulting from reaction between serum JO in 75% strength and:-

A. Saline control.

B. Gen. ND/4.

C. Gen. 5/14.

For photographic purposes the precipitates were centrifuged out of the original test materials, washed in saline and recentrifuged, then rewashed and centrifuged in water. Finally the precipitates were taken up in a thymol gelatine preparation, which was allowed to set in such a manner as to present the appearance of precipitate forming at 24 hours in the test series described. A - shows little or no deposit, and no suspended particles. B - shows precipitate which tends to lodge on the side of the conical base of the tube. C - shows precipitate much more abundant than that produced with normal dentinal gen (Tube B.).

## SUMMARY OF SECTION I

1. A simple precipitin test is described between blood serum and a saline filtered or centrifuged emulsion of carious dentine.

2. The test in the cases described confirms the evidence available from oral inspection and previous dental history. The measure of immunity to dental caries is indicated by the power of serum to precipitate the prepared "gen".

3. The reaction, though definite and convincing, is feeble in character, and means are still required to fortify the "gen" or a substitute for the present "gen" must be obtained.

4. Sufficient evidence is obtained to show a greater precipitation from carious "gens" than from "gens" prepared from presumably normal dentine.

5. It is suggested that the precipitin reaction described in these experiments may occur in the living tooth as part of the natural defences of the body against caries, and that the formation of a translucent zone in dentine is the result of this reaction.

## Section II

## Complement Fixation

Precipitin reactions between "gen" from the affected lesion and serum are sufficiently definite in character to the experienced worker. Difficulty is found in reading the end point of the reaction which is frequently obscured by residual suspended particles visible by lens x 10. These particles apparently disappear from the tubes when centrifuged at 3,000 r.p.m. for five minutes, the particles being evidently of minute dimensions, though highly refractile. The possibility of an end point reaction being clearer with a complement fixation test prompted an investigation of this phenomenon in dental caries.

### The Complement Fixation Test

The complement fixation test consists essentially of the interaction of four unit volumes made up in normal saline solution.

1. Serum or serum dilution.
2. Complement.
3. Antigen.
4. Red Blood Corpuscles, sensitized by haemolytic serum.

Unit volumes of Nos. 1,2 and 3 are allowed to act together in suitably titrated strength for a definite time and temperature, after which one unit volume of No. 4 is added. The presence of complement not fixed in the first part of

of the test is indicated by haemolysis of the red blood corpuscles in the second. Haemolysin in serum requires to be combined with complement to perform its function.

Should, however the complement be completely fixed in the first part of the test, the red blood corpuscles remain unhaemolysed in the second.

Inactivation and dilution of test sera.

(a). The complement content of human serum varies within narrow limits, and in order to standardize results, the serum is inactivated by heating at 55°C for 30 minutes. (Complement is added in known amounts in unit volume No. 2 to replace this loss in the final test.)

(b). No actual titration of test sera takes place, but the unit volume contains an amount of serum which in the case of the non-immune series would be

i. the maximum quantity of serum which would not in itself fix an appreciable quantity of complement in the presence of antigen.

or

ii. that quantity of serum which would fix a known quantity of complement, e.g. 1 minimum haemolytic dose of complement, in the presence of antigen.

20% serum satisfies the conditions under (i) for the Wassermann test and it is probable that the condition

under (ii) could only be applied with difficulty or not at all. The principle underlying both conditions is that the complement fixed in the test series increases with the degree of immunity resident in the test serum.

Other titrations of test materials being satisfactory, suitable serum dilution is finally decided on.

Titration of complement.

The normal source of complement for fixation tests is guinea-pig serum in which 1 minimum dose (1 M.H.D.) haemolytic against a 2½% suspension of sensitized red blood corpuscles is usually contained in unit volumes of between 1 and 3 per cent serum.

The titration is carried out in a series of tubes containing decreasing amounts of complement put up in the manner eventually used in the final test, substituting 1 unit of saline for the serum unit of the test and another unit of saline for the antigen unit of the test. In this titration, one unit of the dilution of serum providing the least quantity of serum capable of haemolysing the red blood corpuscles completely, i.e. without deposit, equals 1 M.H.D. A unit twice this strength of serum equals 2 M.H.D. and so on.

For the purpose of a particular test it is to be remembered that reagents finally used may fix complement independently. In the case of serum there may be a

proportion of complement fixed by the serum independent of that fixed in the presence of immune body. Such a serum is rare and the test should be repeated with fresh serum. The M.H.D. of complement does not vary - except by time depreciation - because of these independent fixations by reagents. The sum of the calculated M.H.D. of complement fixed by all reagents independently is deducted from the total complement in the final test. The amount of complement fixed by serum is negligible and it is frequently convenient to reduce the strength of antigen so as not to affect the final reading.

Antigen:

The antigen used in these tests was No. 5 of the series described under Section I, g. The number of teeth used for its production is indicated by a second figure bracketed after the antigen number, e.g. 5(7), 5(6), 5(16). This antigen has been tested at intervals and does not of itself bind complement in appreciable amounts.

R.B.C.s.

The corpuscles used were those of the sheep collected in 1.5% Sodium Citrate Saline and washed three times in normal saline. A 5% suspension was used in equal proportions with a 1 - 60 dilution of Burroughs Wellcome

Haemolytic Serum for Sheep Corpuscles (glycerinated).

Complement fixation of reagents

This test consists, as in the main test, of four unit volumes made up in normal saline solution. The four unit volumes are retained merely to provide a comparable reading with that of the final test.

1. Saline.
2. Complement of known strength,
3. Reagent for test, as used in the final test.
4. R.B.Cs. sensitized by haemolytic serum.

It will be seen that it is possible to substitute any substance for Unit No. 3 above in testing its complement fixation powers. The procedure is the same as in the main complement fixation test described above.

Experiment No. 9.

Complement fixation of Serum in presence of Gen 5/7 and Gen ND/3.

| | Carious Gen 5/7 | | | | Normal Gen ND/3 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sera | 4 | 3 | 2 | 1 | 4 | 3 | 2 | 1 | = Complement doses |
| GR 7.7% | + | + | + | + | - | + | + | + | |
| JO 16% | + | + | + | + | + | + | + | + | |
| MA 2.5% | + | + | + | + | + | + | + | + | |
| LE 20% | + | + | + | + | + | + | + | + | |
| CL 20% | - | - | - | - | - | - | - | - | |

These tests were done with unheated serum and consequently Complement fixed by serum alone must be taken into account, in addition to complement normally present in the serum.

| | Complement fixed | Complement present | |
|---|---|---|---|
| GR 7.7% | 3 MHD | - | Old Serum |
| LE 20% | - | 1 MHD | Fresh serum |
| CL 20% | - | 1 MHD | " " |

It is therefore possible to correct the experiment result to read as follows:-

| | Carious Gen 5/7 | | | | | Normal Gen ND/3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sera | 5 | 4 | 3 | 2 | 1 | 5 | 4 | 3 | 2 | 1 | = Complement doses |
| GR 7.7% | | | | | + | | | | | - | |
| JO 16% | | + | + | + | + | | + | + | + | + | |
| MA 2.5% | | + | + | + | + | | + | + | + | + | |
| LE 20% | + | + | + | + | + | + | + | + | + | + | |
| CL 20% | - | - | - | - | ?- | - | - | - | - | ?- | |

The power of the serum to fix complement studied in comparable dilutions of 20% would therefore be:-

| | Carious Gen 5/7 | | | | | Normal Gen ND/3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sera 20% | 5 | 4 | 3 | 2 | 1 | 5 | 4 | 3 | 2 | 1 | = Complement doses |
| GR | | | + | + | + | | | - | ? | ? | |
| JO | + | + | + | + | + | + | + | + | + | + | |
| MA | + | + | + | + | + | + | + | + | + | + | |
| LE | + | + | + | + | + | + | + | + | + | + | |
| CL | - | - | - | - | ?- | - | - | - | - | ?- | |

## Experiment No. 10.

Complement fixation by Serum in presence of Gen 5/16 and Gen ND/4 (compared with precipitin tests on the same sera where possible).

| Sera | COMPLEMENT FIXATION | | | | | | | | PRECIPITATE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Carious Gen 5/16 | | | | Normal Gen ND/4 | | | | Carious Gen 5/16 | | | | Normal Gen ND/4 | | | |
| | MHD comp | | | | MHD Comp | | | | % Serum | | | | % Serum | | | |
| | 6 | 4.5 | 3 | 1.5 | 6 | 4.5 | 3 | 1.5 | 5 | 3.75 | 2.5 | 1.25 | 5 | 3.75 | 2.5 | 1.25 |
| A 1460 | + | + | + | + | + | + | + | + | ++ | + | + | + | + | + | - | - |
| 12648 | - | - | - | + | - | - | ∓ | + | + | ⊕ | ⊕ | ⊕ | + | - | - | - |
| 12615 | - | - | - | + | - | - | ∓ | + | + | + | - | - | + | + | - | - |
| 12613 | - | - | ∓ | + | - | - | ∓ | + | ++ | ++ | + | + | ⊕ | - | - | - |
| 12650 | - | - | - | + | - | - | ∓ | □ | | | | | | | | |
| 32724 | - | - | - | + | - | - | ∓ | + | ⊕ | - | - | - | + | - | - | - |
| 32718 | - | ∓ | + | + | - | ∓ | + | + | ++ | + | ⊕ | - | + | ⊕ | - | - |
| 12621 | - | - | - | + | - | - | ∓ | + | + | - | - | - | ⊕ | - | - | - |
| 12634 | - | - | - | + | - | - | ∓ | + | ⊕ | ⊕ | - | - | ⊕ | - | - | - |
| 12647 | - | - | - | + | - | - | ∓ | + | ++ | + | + | + | + | + | ⊕ | - |
| 12630 | - | - | - | + | - | - | ∓ | + | ++ | + | - | - | - | - | - | - |
| 32731 | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - | - |
| 12652 | - | - | - | + | - | - | ∓ | + | + | - | - | - | ⊕ | - | - | - |
| 12632 | - | - | ∓ | + | - | - | + | + | ⊕ | - | - | ⊕ | ⊕ | - | - | - |
| 12600 | - | - | - | + | - | - | - | + | ++ | + | ⊕ | - | + | - | - | - |
| 12631 | - | - | - | + | - | - | ∓ | + | ++ | + | + | ⊕ | + | + | ⊕ | - |
| 12640 | - | - | - | + | - | - | - | + | + | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - |
| 12646 | - | - | + | + | - | - | - | + | ⊕ | ⊕ | - | - | - | - | - | - |
| 12618 | - | - | + | + | - | - | - | + | ++ | ++ | + | ⊕ | + | + | ⊕ | - |
| 12635 | - | - | - | + | - | - | - | + | ++ | + | + | ⊕ | ⊕ | - | - | - |

Experiment No. 10 (contd.)

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12645 | - | - | - | + | - | - | - | + | + | ⊕ | - | - | ⊕ | ⊕ | ⊕ | - |
| 12653 | - | - | - | + | - | - | - | + | | | | | | | | |
| 12629 | - | - | - | + | - | - | - | + | | | | | | | | |
| CL | - | - | - | ∓ | - | - | - | - | + | ⊕ | ⊕ | - | + | - | - | - |
| GA | - | - | - | - | - | - | - | - | + | ⊕ | - | - | + | ⊕ | - | - |

Conclusions from this test series indicate that there is variation of complement fixation by human serum in contact with a gen of dentinal extract. When it is remembered that the normal gen ND/4 was four times the strength of carious gen 5/16 it can be safely assumed that the use of a carious gen of four times the strength of 5/16 would result in a higher fixation of complement particularly as precipitate from interaction of serum and gen is responsible for fixation. There is an apparently more satisfactory reading for the precipitin reactions indicating a response more specific in nature towards the gen of carious origin than that from normal source despite the greater strength of the latter. It is not unusual for precipitate to be reabsorbed when gen to excess is used in the test. That this is not the case in the present series is indicated clearly by order of the readings, i.e. precipitate to greatest intensity occurring to the left of the series.

The Wassermann readings for the numbered sera in the above series are as follows:

+ + A 1460, 12650, 12632, 12600, 12618

- ± 12630

- The remaining numbered sera.

It will be seen, therefore, that there is no relation between fixation of complement in the Wassermann test and fixation of complement in the test with dentinal antigen.

## Experiment No. 11.

Complement fixation by Serum in presence of Gen 5/16 and Gen ND/6. The normal gen is reduced to a dilution equal to that of the carious gen. (Compared with precipitin tests on the same sera using Gen ND/6 reduced to a dilution equal to the carious gen).

| | COMPLEMENT FIXATION | | | | | | | | PRECIPITATE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Carious Gen 5/16 | | | | Normal Gen ND/6 | | | | Normal Gen ND/6 | | | |
| Sera | MHD Comp | | | | MHD Comp | | | | % Serum | | | |
| | 12 | 9 | 6 | 3 | 12 | 9 | 6 | 3 | 5 | 3.75 | 2.5 | 1.25 |
| 32776 | - | - | - | ⊕ | □ | □ | - | - | | | | |
| 10843 | ⊕ | + | + | + | - | - | ⊕ | + | + | - | - | - |
| C3530 | - | - | + | + | - | - | - | - | | | | |
| 12659 | - | - | - | - | - | - | - | - | - | - | - | - |
| 12670 | - | - | - | ⊕ | - | - | - | - | - | - | - | - |
| 10842 | - | - | - | - | - | - | - | - | | | | |
| 32805 | - | - | - | - | - | - | - | - | | | | |
| 12642 | - | - | - | - | - | - | - | - | | | | |
| 12674 | - | - | - | - | - | - | - | - | | | | |
| 12668 | - | - | - | ⊕ | - | - | - | - | - | - | - | - |
| 32784 | - | - | ⊕ | + | □ | - | - | - | | | | |

## Experiment No. 11 (contd.)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12675 | - | - | - | - | | | | | | | | |
| 12680 | - | - | - | - | | | | | | | | |
| 12568 | - | - | - | ⊕ | | | | | | | | |
| 10852 | - | - | - | - | | | | | | | | |
| 32777 | - | - | - | - | - | - | - | - | - | - | - | - |
| 12677 | - | - | - | - | - | - | - | - | ⊕ | - | - | - |
| 12678 | - | - | - | + | - | - | - | - | - | - | - | - |
| 32785 | - | + | + | + | - | - | - | + | + | + | - | - |
| 12667 | - | - | - | - | | | | | | | | |

It is again shown that there is variation of complement fixation by human serum in contact with a gen of dentinal extract. Dentinal gens of equal strength are compared and in every case showing complement fixation in the carious series there is either much less or none fixed in the normal gen series. There is a maximum limit placed to the complement fixation which is not shown in Exp. 10. The limit is shown as ⟨12 MHD and ⟩ 9 MHD.

Of the sera tested for precipitate with Gen ND/6 diluted to the strength of carious gens used in other experiments none show a strong reaction and in general the positives are weaker than those obtained with the stronger gen ND/4 used in Experiment 10.

That there is no relation between fixation of complement in the Wassermann test and fixation of complement in the test with dentinal antigens is apparent. Wassermann results for this series are as follows:

\+ + 12670, 10842, 12642, 12680.

\- The remaining sera.

## Experiment No. 12.

Complement fixation by precipitates formed from interaction between "Gen" and unheated sera.
Complement dose = 1 MHD.

Tubes were selected from precipitate reactions recorded as + +, +, ⊕, or -, centrifuged at 3,000 r.p.m. for five minutes and the supernatant fluid removed to within 1 mm. of any possible deposit.

| Origin of Ppt. "In" | "Gen" | Ppt. record for the tubes selected. | | | | | | | | | | | All tubes show some haemolysis. + indicates deposit of r.b.cs. ⊕ = little deposit | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HO | 5/7 | + | + | + | + | + | + | - | - | - | - | - | + | + | + | + | ⊕ | | | | | | |
| HO | 5/7 steamed | + | + | + | + | + | + | | | | | | - | - | + | ⊕ | - | | | | | | |
| LE | 5/7 | + | + | + | + | + | + | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | + | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ? | - | ⊕ | + |
| LE | ND/3 | + | + | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | - | + | + | ⊕ | - | - | + | ⊕ | - | - | ⊕ | | |

The necessity for inactivation of sera used to form precipitates for use in a further test of the capacity of the precipitate to fix complement is indicated in the next experiment wherein it is clearly shown that a precipitate reading of + + or + invariably fixes at least 1 MHD Complement.

## Experiment No. 13

Complement fixation by precipitate. Complement dose = 1 MHD.

Comparison of fixation of complement by precipitates obtained from carious and non-carious dentine Gens.

| In | Gen 5/16 | | | | Gen ND4 | | | |
|---|---|---|---|---|---|---|---|---|
| | Ppt Record | | Fixation | | Ppt Record | | Fixation | |
| A1460 | ++ | + | + | + | + | - | + | + |
| 12648 | + | ⊕ | + | + | + | - | + | +L |
| 12615 | + | - | + | + | + | - | + | + |
| 12613 | ++ | + | + | + | ⊕ | - | + | +L |
| 32724 | ⊕ | - | + | + | + | - | + | +L |
| 32718 | ++ | - | + | + | + | - | + | +L |
| 12621 | + | - | + | +L | ⊕ | - | + | +L |
| 12634 | ⊕ | - | + | +L | ⊕ | - | + | +L |
| 12647 | ++ | + | + | + | + | - | + | + |
| 32731 | + | - | + | + | + | - | + | + |
| 12652 | + | - | + | + | ⊕ | - | + | + |
| 12632 | ⊕ | ⊕ | + | + | ⊕ | - | + | + |
| 12600 | ++ | - | + | + | + | - | + | +L |
| 12631 | ++ | ⊕ | + | + | + | - | + | +L |
| 12640 | + | ⊕ | + | + | ⊕ | - | + | + |
| 12646 | ⊕ | - | + | + | - | - | + | +L |
| 12618 | ++ | ⊕ | + | + | + | - | + | + |

## Experiment No. 13 (contd.)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12635 | ++ | ⊖ | + | + | ⊕ | - | + | + |
| 12645 | + | - | + | + | ⊕ | - | + | +L |
| CL | + | - | + | +L | + | - | + | +L |
| GA | + | - | + | + | + | - | + | +L |

L = Presence of some haemolysis = limit of complement fixation reached.

## Analysis of Experiment No. 13

| Ppt. record | Origin Gen 5/16 Complement fixation | | | Origin Gen ND4 Complement fixation | | |
|---|---|---|---|---|---|---|
| | >1 MHD | <1 MHD | - | >1 MHD | <1 MHD | - |
| ++ | 8 | - | - | - | - | - |
| + | 12 | - | - | 12 | - | - |
| ⊕ | 10 | - | - | 7 | 1 | - |
| - | 9 | 3 | - | 10 | 12 | - |

It is apparent that the maximum fixation of complement may be above 1 MHD unless the empirical reading of the precipitin tests are greatly at fault.

## Experiment No. 14

Complement fixation by precipitate
Complement dose = 1.5 MHD

Comparison of fixation of complement by precipitates obtained from carious and non-carious gens.

| lu | Gen 5/16 | | | | | | | | Gen ND4 | | | | | | | | 1/20 Serum Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ppt record | | | | Fixation | | | | Ppt record | | | | Fixation | | | | Fixation |
| GA | + | + | + | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - | - | ⊕ | - | - | - | - | - |
| CL | + | + | + | - | - | - | - | - | ◻ | ◻ | - | - | ◻ | ◻ | - | - | ◻ |
| 12648 | ++ | + | ⊕ | ⊕ | + | + | + | + | ⊕ | - | - | - | + | + | + | - | + |
| 12621 | + | - | - | - | ⊕ | ⊕ | ⊕ | - | ⊕ | - | - | - | ⊕ | - | - | - | - |
| 12630 | ++ | + | ⊕ | - | ⊕ | - | - | - | ⊕ | - | - | - | - | - | - | - | - |
| 32731 | ++ | ⊕ | ⊕ | - | - | - | - | - | + | ⊕ | - | - | ⊕ | - | - | - | - |
| 12646 | + | ⊕ | - | - | - | - | - | - | - | - | - | - | ◻ | ◻ | ◻ | ◻ | - |
| CO | ++ | ++ | ⊕ | - | ◻ | ◻ | ◻ | ◻ | - | - | - | - | - | - | - | - | - |
| CO | + | + | + | ⊕ | ⊕ | ⊕ | - | - | - | - | - | - | - | - | - | - | - |

Serum 12648 is removed from consideration as its power to fix complement is shown. The complement fixed by precipitate is obviously less than 1.5 MHD

⊕ under fixation = some deposit of corpuscles but haemolysis nearly complete.

It has been shown that precipitates obtained from carious dentinal gens fix more than 1 MHD and less than 1.5 MHD of complement when contained in the fraction of test solutions at the bottom 1 mm. or less after centrifugalisation and removal of supernatant fluid. The calculation of complement fixed by precipitate independent of fixation by the reagents used in the production of precipitate is of interest.

Complement fixed by serum alone. Nil usually.

Complement fixed by Gen alone. An inconsiderable quantity $<0.05$ MHD.

Maximum complement fixed by serum 1 - 5 in the presence of gen = 9 MHD.

∴ Complement fixed by serum 1 - 20, - the maximum strength of serum in the precipitin test = 2.25 MHD.

The fraction of test solutions containing precipitate separated for fixation test cannot be greater than 1/8th of the bulk, i.e. 1/8th of the serum unit of a maximum strength of 1 - 20 dilution. $1_n$ proportion to the amount of complement fixed by this strength of serum - in the test on serum - 1/8 Unit accounts for 0.28 MHD. Precipitate in fractions of test solutions which has been shown to fix more than 1 but less than 1.5 MHD of complement can be assumed to fix approximately 1 MHD

independently. That it does so strongly supports a suggestion that the precipitate is bacterial in origin.

## Experiment No. 15

Complement fixation by precipitate
Complement dose = 1.25 MHD

Comparison of fixation of complement by precipitates obtained from carious and non-carious gens.

| | Gen 3/14 | | | | | | | | Gen ND/4 | | | | | | | | 1/20 Serum Control Fixation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ppt record | | | | Fixation | | | | Ppt record | | | | Fixation | | | | |
| GA | ⊕ | ⊕ | - | - | - | - | - | - | - | - | - | - | - | - | - | ⊕ | - |
| LE | ⊕ | ⊕ | ⊕ | - | - | - | - | - | ⊕ | - | - | - | - | - | - | - | ⊕ |
| JO | ++ | + | + | + | ⊕ | - | - | - | ⊕ | - | - | - | - | - | - | -- | ⊕ |
| 32777 | ⊕ | ⊕ | - | - | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - | - | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ |
| 32818 | ⊕ | - | - | - | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - | - | ⊕ | ⊕ | ⊕ | - | ⊕ |
| 12700 | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - | - | ⊕ | ⊕ | ⊕ | ⊕ | ⊕ |

The conclusion from Experiment No. 15 is that precipitate fixes 1 MHD Complement (Exp.No.13) but not 1.25 MHD Complement.

## Experiment No. 16

An evaluation of the reliability of results obtained by the methods described in the text.

i) Reagents prepared in the presence of two workers.

ii) Sera used in the test not known to one (GG.M) until the results of tests were recorded.

| Serum | MHD complement fixation Gen5/14 12 | 9 | 6 | 3 | GenND/4 12 | 9 | 6 | 3 | Precipitation % Serum Gen 5/14 5 | 3.75 | 2.5 | 1.25 | Gen ND/4 5 | 3.75 | 2.5 | 1.25 | Wassermann result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GA | - | - | - | - | - | - | - | - | ⊕ | ⊕ | - | - | - | - | - | - | |
| LE | - | - | - | - | - | - | - | - | ⊕ | ⊕ | ⊕ | - | ⊕ | - | - | - | - |
| JO | - | - | - | + | - | - | - | - | ++ | + | + | + | ⊕ | - | - | - | |
| 32777 | - | - | - | ± | - | - | - | ± | ⊕ | ⊕ | - | - | - | - | - | - | |
| 32818 | - | - | - | - | - | - | - | - | ⊕ | - | - | - | - | - | - | - | - |
| 12700 | - | - | - | - | - | - | - | - | ⊕ | ⊕ | ⊕ | ⊕ | - | - | - | - | ++ |

Serums were available in the first three cases to make a further titration of complement fixation in the presence of carious Gen 5/14.

| Serums | MHD Complement 3.2 | 2.6 | 2.05 | 1.64 | 1.31 | 1.05 |
|---|---|---|---|---|---|---|
| GA | - | - | - | - | - | ⊕ |
| LE | - | - | - | - | ⊕ | + |
| JO | - | + | + | + | + | + |

From Experiment No. 16 facts emerging from previous experiments are confirmed.

1. Though Gen 5/14 is poor in character in both tests, the results are comparable with results obtained previously using the same sera.

2. Serum JO retains its character in producing visible precipitate.

3. In two sera tested the complement fixation with dentinal gen bears no relation to that obtaining in the Wassermann test.

4. Means to fortify and standardize dentinal "gens" must be found.

## Summary of Section II

1. Complement fixation by serum in the presence of dentinal antigen bears no relation to that fixed in the Wassermann test.

2. Fixation of complement by serum in the presence of caribus dentinal antigen is greater in measurable quantities than that fixed in the presence of a gen from normal dentine of similar manufacture.

3. The extent of complement fixation bears some relation to the extent of the precipitation obtaining between the same sera and gens.

4. Precipitates fix complement to the extent of 1 MHD approximately and do so independently of that fixed by the separate test materials and that fixed by the combination.

# Proposal for future study of the Immunological Reactions in Dental Caries.

1. Determination of optimum conditions for the precipitin tests, having regard to the laws of proportion which apply to precipitin tests in other states, e.g. in horse serum precipitin.

2. Means to fortify the gen in precipitin tests to be sought or failing a satisfactory adsorbing agent to seek antigen substitutes as applied in the Wassermann test.

3. Further study of substances extractable from tooth pulp to settle once for all that the vital reaction does or does not become concentrated within the tooth substance.

4. Comparison between pulp extracts from normal and carious teeth, acting against gens from normal and carious dentine.

5. Chemical examination of the precipitate, carious and normal dentine and the calcium barrier between lesion and pulp with a view to correlating the reaction *in vitro* and *in vivo*.

6. Further correlation between the precipitating and complement fixing properties of serum in the presence of dentinal antigens and these properties as exerted by serum against filtered bacterial or other virus isolated from carious lesions.

## General Summary

1. Failure of research to discover a specific cause of dental caries led to a new line of investigation being followed, viz., immunological reactions in the human subject.

2. A definite precipitin reaction was found to occur between filtered or centrifuged emulsions of carious dentine and serum from the same or other subjects.

3. The intensity of this reaction was greatest with subjects immune to caries, as judged by clinical examination, and least with caries-susceptibles. With improved technique, such as fortified and standardized precipitinogen, this test might give a useful measure of the individual's immunity to dental caries.

4. It is suggested that this precipitate may account for the calcium barrier in the translucent layer of Tomes, and that the complete reaction in the tooth, when successful, may result in the condition known clinically as arrested caries.

5. Complement fixation tests gave a positive result, greater with antigen from carious dentine than with antigen from normal dentine.

6. The extent of complement fixation bore some relation to the precipitin reaction between the same sera and "gens", and therefore to the patients' state of immunity or susceptibility.

7. Complement fixation by serum in the presence of antigen from carious dentine had no relation to the results of the Wassermann test.

8. The occurrence of immunological reactions on the part of the body, as shown by these investigations, suggests that dental caries is a definite infective process in the nature of a bacterial infection or one due to a filter-passing virus.

9. Suggestions for further research on this subject are indicated.

This work was done in the Pathological and Bacteriological Laboratory of the University of Liverpool School of Dental Surgery.

Discussion.

Attention has been focussed on the enamel in most investigations of dental caries in the human subject. In most cases it is actually the enamel which has first to be penetrated, hence the acid-fermentation theories have attained undue importance.

The old idea that enamel was an inert inorganic substance is no longer tenable. The presence of an organic matrix has been proved. The diffusion of dye into the enamel from the pulp has been demonstrated. The vascularity of the enamel has been postulated, and a circulation in it suggested. If this were proved, it would be credible that the immunity of some teeth to caries was due to resisting powers of the tooth fluids (dental lymph) exerted from inside the tooth. The fact that the dog, with permeable teeth, is immune to caries, tends to support this hypothesis. The occurrence of

a translucent zone in enamel still further supports the theory of the permeability of enamel. The fact that dyes have not so far been found to diffuse into human enamel does not negative this view. Human enamel may retain so little permeability that long periods of time may be required; or else the dyes commonly used in such experiments may not be "subtle" (Hunter) enough to penetrate it.

The hardness of human teeth, with their small degree of permeability, may be a factor in determining their liability to caries.

The occurrence of a translucent zone in enamel is almost certainly evidence of a vital reaction on the part of the tooth against the attack of the causal factor in dental caries. It may be regarded as the first line of defence. The fact that it is so seldom observed may indicate that the defence in the human tooth is weak.

Similarly, the translucent zone in dentine may be regarded as the second line of defence. When highly developed, it is sufficient to check the carious attack, and results in "arrested caries".

The deposition of secondary dentine is a third line of defence, which may be repeated again and again so long as the pulp remains sufficiently active to respond to the stimuli.

Such reactions would seem reasonable in response to attack by some contagium vivum, as has been suggested in the summary at the conclusion of the experiments just described.

The chemical part of the chemico-parasitic theory is not excluded, as acid attack of the enamel may predispose to invasion of the tooth by the causal factor. That acid attack is not the sole factor, is proved by the observation that many teeth in dirty mouths are never affected by caries, although subjected to the influence of

acid fermentation of foodstuffs.

That none of the bacteria so far isolated are causal factors seems likely from the contradictory observations of most investigators, who conspicuously fail to agree on any one organism. Most of the bacteria hitherto described are probably normal inhabitants of the mouth, and merely follow the carious process.

The extremely minute size of the vascular channels in human enamel, if such exist, make it much more probable that an ultra-microscopic virus is the causal factor, if the disease is due to some contagium vivum. The experiments here described suggest that this is so, and to some extent account for the vital phenomena observed clinically in carious lesions in the human tooth, as far as laboratory tests can be expected to confirm processes not fully understood in vivo.

As mentioned before, so-called "caries" of dead teeth and *in vitro* experiments on extracted teeth are in my opinion merely a saprophagous process of decay, and do not constitute true caries, although they may simulate it in all respects except the production of vital reactions.

## Conclusion.

The available evidence seems to indicate that dental caries is due to some contagium vivum, possibly one of the numerous types of bacteria already described in the literature, or, more probably, a hitherto undiscovered filter-passing virus.

Predisposing causes are food deficiency in its broadest sense (especially in the growing child); heredity; ill-health (especially if resulting in vitiated oral secretions); luxurious living (especially over-eating, and eating soft, sticky foods); and local anatomical abnormalities of tooth form and position favouring carbohydrate stagnation,

which by acid-production on the enamel surface may be the determining factor in the onset of caries.

The possibility of a vital reaction on the part of the tooth has been indicated, which, if sufficiently active, may result in arrest of the carious process.

If further investigations confirm the truth of the conclusion that some contagium vivum is the causal factor, it is not too much to hope that some day dental caries may be controllable by prophylactic measures, just as are smallpox, rabies, and yellow fever.

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Abbreviations used.

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| Brit.J.Exp.Path. | British Journal of Experimental Pathology. | London |
| Brit.Med.J. | British Medical Journal. | London |
| Dent.Cosmos | Dental Cosmos. | Philadelphia |
| D.M. and O.T. | Dental Magazine and Oral Topics. | London |
| Dent. Record | Dental Record. | London |
| J.Amer.Dent Assoc. | Journal of the American Dental Association. | Chicago |
| J.Amer.Med.Assoc. | Journal of the American Medical Association. | Chicago |
| J.Anat. | Journal of Anatomy. | Cambridge |
| J.Dent.Res. | Journal of Dental Research. | Baltimore |
| J.Med.Res. | Journal of Medical Research. | Boston |
| J.Nat.Dent.Assoc. | Journal of the National Dental Association. | Chicago |
| Liv.M.-C. J. | Liverpool Medico-Chirurgical Journal. | Liverpool |
| Pac.Dent.Gaz. | Pacific Dental Gazette. | San Francisco |
| Proc.Roy.Soc. | Proceedings of the Royal Society. | London |
| Proc.Roy.Soc.Med. | Proceedings of the Royal Society of Medicine. | London |

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