are possible; but attempts to continue such enormous water traffics lead to heart failure. This rate would correspond to an output of 4,200 c.c. of urine hourly from a man weighing 70 kg., or 100 liters in twenty-four hours. *In giving intravenous injections of glucose there are then two things to avoid: too great dehydration on the one hand, and heart failure from imposing too much mechanical work on the other. These can both be avoided by knowing the number of grams of glucose which enter the body hourly, and what volume of water is moved by such a rate of sugar injection.*

CLINICAL APPLICATION

It is hardly necessary to emphasize the therapeutic possibilities which these experiments suggest. With a knowledge of the proper concentration of the solution to be used, and the rate appropriate for this particular solution, we may (1) abstract any quantity of water from the body which we deem safe or desirable, or we may (2) pass through the body a great flushing stream to wash out poisons. How far the principles worked out for normal individuals will apply in disease we have only begun to learn. In this connection it may be well to recall that the literature already contains observations on the use of sugars in therapeutics.

Sugars have been given by mouth or bowel for purposes of nutrition or to obtain other desired metabolic or chemical effects in a wide range of clinical conditions. But as pointed out, such methods of administration do not introduce sugar into the blood faster than an approximate rate of 1.8 gm. per kilogram and hour at most, which is not sufficient to permit much glucose to escape utilization. Hence, after such administrations, unburned sugar does not accumulate in the tissues except to a limited extent, and they are not diuretic except in cases with decreased rates of sugar utilization (diabetes). Intravenous injections may exceed this rate, and so cause, in addition to the ordinary chemical effects of glucose, new physicochemical effects due to the presence of unburned sugar molecules in the tissues, and expressed outwardly as diuresis. Now glucose and levulose have long been found useful when given in cases of diabetic coma, especially when given intravenously. In such cases either mode of administration produces great diuresis, but especially levulose by vein (for levulose the normal intravenous tolerance limit being only 0.15 gm. per kilogram and hour). It has not yet been recognized that the value of this treatment is due to the diuresis, but we have on other occasions given the reasons which make us believe that this is the case. In 1907, Fleig¹ made roughly timed intravenous sugar injections, was struck by the remarkable diuresis, and realized possible clinical applications, without making any, however. Kausch,² in 1911, recommended and attempted intravenous nutrition with glucose in hysterical vomiting hyperemesis gravidarum, surgical conditions and acute gastro-intestinal disease, but laid no emphasis on the diuretic action. Fischer³ recommended intravenous glucose injections in conditions of edema, nephritis and intoxication, but especially oligurias and anurias of various sorts on the basis of his observations concerning the power of sugars to dehydrate colloid gels and on their parallel diuretic effects in rabbits and men, thus bringing the

behavior of sugars in these respects into relationship with that of diuretic salt solutions and recognizing the physicochemical principles which underlie the beneficial effects of intravenous injections of sugar. In 1914, Henriques⁴ reported the use of from 250 to 300 c.c. of 30 per cent. glucose solution in the hour to about fifty clinical cases of heart disease, intoxication, and severe infections, finding special indications in oligurias of nephritis and heart disease. He did not discuss the principles involved. This year Turrettini⁵ has given details of three cases of acute nephritis and three of mercuric chlorid poisoning, in all of which the urinary output was favorably affected. Experiments have been in progress in this laboratory for some time to determine the limits of application of these principles and the present technic of administration to different grades of experimental poisoning with mercuric chlorid, arsenic and diphtheria toxin. The system is being tested in the clinic as opportunities offer. Its chief application would appear to be in acute intoxications of various sorts, acute oligurias and anurias, intoxication of pregnancy, and in cardiac and other edemas. Further work is in progress on other diuretics besides sugars and on other sugars besides glucose, and also with simultaneous injections of sugars and salts, such as acetates and bicarbonates.

SIGNIFICANCE AND PREVENTION OF AMEBIC INFECTIONS IN THE MOUTHS OF CHILDREN *

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From time to time during more than half a century, a protozoan genus called *Ameba* by some, and *Endameba* or *Entameba* by others, has been reported as occurring chiefly about the gum margins in the oral cavity of human beings.

Few details of the morphology, comparative incidence and pathogenicity of the members of this genus have been given. Until last year, most, if not all of the species, were thought to be nonpathogenic. Indeed, of so little worth were they considered that no mention of their presence is made in the general histologic review of parasitic amebas of man by several authors, who used the term "parasitic amebas" as synonymous with "intestinal amebas."

Thus Craig says:

There is no experimental evidence connecting *Ent. buccalis* (or the mouth ameba) with disease. It is very doubtful if it has anything to do with caries of the teeth, although it is most frequently encountered in the cavities of carious teeth. However, it may be frequently demonstrated in material scraped from the roots of perfectly normal teeth, so that, as far as the evidence goes, we must regard *Ent. buccalis* as only a secondary invader. The amebae known as *Ent. gingivalis*, Gross, 1849, and *Ent. dentalis*, Grassi, 1879, are probably identical with *Ent. buccalis* and do not merit separate description.

4. Henriques, E.: Presse méd., 1914, xxii, 121.

5. Turrettini: Rev. méd. de la Suisse romande, 1915, xxxv, 204.

* From the Bureau of Laboratories, Department of Health, New York City.

* Read before the Section on Preventive Medicine and Public Health at the Sixty-Sixth Annual Session of the American Medical Association, San Francisco, June, 1915.

1. Fleig: Compt. rend. Soc. de biol., 1907, lxiii, 190.

2. Kausch: Deutsch. med. Wchnschr., 1911, xxxvii, 8.

3. Fischer, M. H.: Edema and Nephritis, New York, John Wiley & Sons, 1915.

In 1914, Smith and Barrett, followed by Bass and Johns and several other investigators, asserted that mouth amebas occurred chiefly in cases of pyorrhea alveolaris or Riggs' disease, and that they were the probable cause of this common septic infection of the jaws. Other observers have not agreed with this assertion because sufficient evidence has not been given by its authors to make us sure of the types of amebas found and of the extent, if any, of their pathogenic action. So several hypotheses may be formulated as to the part thought to be played by amebas in mouth infections.

HYPOTHESES IN REGARD TO ACTION OF AMEBAS IN HUMAN MOUTHS

1. Amebas are accidental invaders; they produce no pathogenic or other reaction in the mouth (Provazek Hartman and others).
2. Amebas in the mouth have a beneficent action as scavengers (Chiavaro and others).
3. Amebas may be sometimes pathogenic, producing or helping to produce certain cases of pyorrhea alveolaris, certain abscesses and certain other mouth infections (Leidy, Gross, Flexner, Smith and Barrett and others).
4. Amebas are the specific cause of pyorrhea alveolaris (Bass and Johns and others).
5. Amebas may be pathogenic only in connection with certain other micro-organisms.

The comparative worth of these hypotheses may be discussed as follows:

In favor of the first hypothesis, that is, that amebas are accidental, nonpathogenic invaders, are the facts that amebas have been reported so frequently in apparently normal mouths and that when found in diseased conditions, they are always accompanied by other micro-organisms of pathogenic types, which might be sufficient to produce the disease. Against it is the fact that they are found so much more frequently and in so much larger numbers in the lesions.

As for the second hypothesis, that amebas are beneficent scavengers, there is no question but that amebas are scavengers both of other micro-organisms and of tissue host cells—at least, of leukocytes, red blood cells and broken down epithelial cells. But they probably take up as many harmless as well as harmful germs and leave enough of the latter to exert their full power in harming the tissues of the host. Then, too, since we know that certain amebas will live on brain, liver and kidney cells of human beings, there is no *a priori* reason to suppose that the mouth amebas will not help in destroying the fixed tissue cells in the mouth, especially after a certain amount of injury has been done them by other micro-organisms. The evidence, then, is against this second hypothesis as it stands.

As regards the third hypothesis, that amebas are sometimes pathogenic, we can only say that the large number of amebas in most cases of pyorrhea and in some abscesses of the jaw, and their infrequent presence in absolutely healthy mouths make this a tenable hypothesis.

We may state, concerning the fourth hypothesis, that is, that amebas are the specific cause of pyorrhea alveolaris, that whether or not they are the specific cause of this condition can only be determined after further study. Some facts brought forward in this paper bring new evidence on this point.

In regard to the last hypothesis, that is, that amebas may be pathogenic only in connection with certain other micro-organisms, we must remember that if amebas absorb and digest fixed tissues as well as leukocytes, blood cells and other wandering cells, they may help decidedly in keeping up a lesion bacteria have started. This fifth hypothesis is also tenable.

ORIGINAL WORK

In order to help determine which, if any, of these hypotheses is correct, we have been trying to find out, first, how many children are infected and at what age the amebas begin to appear in their mouths, as well as to study the condition of the mouths showing the amebas. Second, what types of amebas, if more than one, are present. Third, the relation, if any, of these amebas to the diseased condition of the children's mouths, and fourth, the necessity and worth of preventive measures.

We have begun our study by examining the mouths of the schoolchildren in one of the poorer districts of New York City. We have already started the examination of a similar group in one of the more well-to-do districts. At the same time, the Bureau of Laboratories of the New York City Health Department has begun a more general study of focal mouth infections, particularly of pyorrhea alveolaris.

Soon after our observations on adults were begun, we found that all patients presented to us by the several dentists who are collaborating with us already had pus pockets formed in the jaw alveoli—that is, they were well-established cases—and when we began to inquire for beginning cases, we learned that the diagnosis of pyorrhea alveolaris is made by most dentists only after pus pockets can be demonstrated, so this added interest to our decision to hunt for beginnings in schoolchildren.

So far 1,678 children (those in only one session of the school) between the ages of 5 and 15 years have had a preliminary examination, and from 995 representative ones stained smears have been made and examined for amebas. The 1,678 children have been divided roughly into those having healthy gums and those with receding or spongy and bleeding gums. The healthy looking gums are further divided into those with one or more decayed teeth, and those with apparently sound teeth. Needless to say, we found very few of the latter, that is, of those with apparently healthy gums and sound teeth, and about one half of all showed unhealthy gums.

From many cases, two smears were taken. The smears were made as follows: In the bad cases, the teeth and gums were cleaned with a cotton swab dipped in 50 per cent. alcohol; then with the flat end of a hard wood sterile toothpick, material was scraped from the margin of the gums (there were no pus pockets in these children, so we did not need a scaler) and stroked lightly over a clean glass slide, three or four streaks being made. The smear was then fixed while still moist by dropping methyl alcohol on it, and it was ready to send to the laboratory. This was called the superficial smear. A second toothpick was used to get material from beneath the margin of the gums, from which another smear was made and fixed. This was called the deep smear. In the cases with clean teeth and tight healthy looking gums, the first smear was made before the cleaning with alcohol. All of these smears were stained with Giemsa's stain and

examined for amebas. Other stains have also been used in the more minute study of types of amebas. In regard to the identification of amebas by stained films, we would say that they are more certainly and easily identified by this method than by warm living preparations, though, of course, both methods should be used in studying all of the characteristics of these micro-organisms. In hunting for amebas in these smears, we have found that most of them seem to be between the superficial bacterial layer and the deep leukocytic layer, so one may find them only in large numbers in one small part of a smear, especially in a smear taken in a routine way.

Stained by Giemsa, the delicately but definitely reticulated cytoplasm of the ameba stains a clear blue, while the small nucleus shows as a delicate rounded or irregular mass of red granules and threads, with often a central light blue staining karyosome which sometimes contains a vacuole-like body. This nucleus no doubt corresponds to that most frequently described for *Endameba histolytica-tetragena*. There has been found no marked variation from this type of nucleus.

Amebas are so notoriously variable in their entire morphology, according to their stage of development, and to the conditions of growth, that no definite opin-

found in healthy mouths. Whether or not one or more varieties are taken from adult pyorrhea patients and are simply waiting their opportunity to exert a pathogenic action, we cannot yet say. But the evidence seems to point toward the establishment of either the first or the fifth hypothesis already given.

These results, however, as they stand, are very striking, especially when we must add that most of the cases of spongy and bleeding gums show more amebas in the films than the other cases. If we could only rule out other micro-organisms, the evidence for the pathogenic action of the amebas would be stronger.

We have, in these schoolchildren carriers of amebas, a marked opportunity to test the prophylactic effect of ipecac or its emetin alkaloid on mouth amebas.

We have divided the cases showing amebas into three groups, one to be watched without treatment, and others to be treated in different ways, one with emetin and the other by ordinary cleanliness. We have a tooth wash made up containing a dilution of emetin, and have the washing of the teeth done morning and afternoon in the school clinic under the supervision of the school nurse.

The first examinations, one week after the beginning of this treatment, gave marked results, in that every child but one, receiving emetin, showed no amebas, while many of the others showed them. In the later examinations, however, amebas were found in a few of the emetin-treated which were negative before, while a few more of the controls showed none.

The following points have been brought out so far by these examinations:

Amebas are demonstrated irregularly in all mouths once showing them, most constantly and in largest numbers in mouths showing gingivitis, least so in healthy mouths.

With ordinary teeth cleansing methods, the number of mouths showing amebas is reduced one half. With emetin in the tooth wash, the number showing amebas is greatly reduced, only about 10 per cent. showing them. The second set of controls—those doing their cleaning *at home* by ordinary methods—continue to show amebas in about 75 per cent. of the cases.

The question as to the amount of emetin to be used has not yet been settled. We began with a 1:200 solution, then reduced it to a 1:400 strength. Probably a much smaller amount would be sufficient to keep down the development of the amebas.

We hope to carry on these investigations for several years and, if necessary, to extend the number of children included in the groups. Whether or not we can demonstrate fully the efficiency of the use of an amebicide in preventing many cases of pyorrhea alveolaris remains to be seen. We hope by combining these practical investigations with the laboratory studies that we may be able to help demonstrate the relationship of these amebas in septic mouth infections.

ABSTRACT OF DISCUSSION

DR. WOODS HUTCHINSON, New York: It is an interesting and important subject, especially in view of the effect of the organisms involved in later life. My impression is that we shall find that the diseases of the gums in later life bear the same relation to the infections of childhood that the diseases of so-called middle age do to the infectious diseases of younger life. We elderly individuals, who ought to be Oslerized probably, are beginning to be terribly worried about diseases that attack us over 50. By taking proper steps we may very early remove the cause of our worries.

NUMBER AND PERCENTAGE OF AMEBAS FOUND IN EACH GROUP OF CHILDREN

Condition of Mouth	Between 5 and 16 Years		Between 5 and 7 Years	
	Total	Amebas Present	Total	Amebas Present
1. Healthy gums	(+ 38) (— 92)	= 29 per cent.	(+ 3) (— 31)	= 9 per cent.
2. Healthy gums, decayed teeth	(+ 109) (— 183)	= 37 per cent.	(+ 32) (— 105)	= 23 per cent.
3. Tartar and receding gums	(+ 182) (— 96)	= 65 per cent.	(+ 19) (— 17)	= 53 per cent.
4. Spongy, bleeding gums	(+ 268) (— 28)	= 91 per cent.	(+ 34) (— 5)	= 70 per cent.

ion should be expressed as to the species of the forms found in these mouths until much further study has been made. We may say, however, that there seem to be at least two morphologic types of amebas present: one small, with more or less rounded, dense nucleus, with a few or no inclusions or vacuoles, found in the majority of healthy mouths, and the other large, with more irregular nucleus with many large vacuoles and containing many red blood cells, leukocytes and other dense masses formed by undigested material. The latter type is found in the majority of unhealthy mouths. Its appearance agrees with that of the type most frequently seen in the well-developed cases of pyorrhea alveolaris. We have seen no cysts. We have seen evidence of budding and of more equal division.

We have found that amebas begin to appear at a very early age, 35 per cent. of children between 5 and 7 years of age being infected. The numbers of those infected with amebas increase in frequency with age. Between 5 and 15 years, 60 per cent. show these protozoa on their gum margins. In apparently normal mouths, only 29 per cent. show amebas. Taking the percentages by groups as in the accompanying table, the results are more significant.

Finding the amebas so often in apparently healthy mouths and in such young children does not agree with the statement of Bass and Barrett that they are not

The ameba in its relations with the pathogenic organisms may prove to be the keynote of the diseases and loss of teeth at an immature and undesirable time.

DR. C. C. BASS, New Orleans: I do not know of any other series of cases as large as that reported by Dr. Williams. Most examinations that have been reported and come to my attention have been in older people, practically all of whom were infected. The study, then, of Dr. Williams and her associates is especially valuable because it takes up the subject of the disease at a stage at which it could be prevented, perhaps, whenever we know enough about it, or cured before too great damage is done. As to the pathogenicity of the amebas that we find in the mouth and in pyorrhea lesions, the evidence at hand leaves the question somewhat open. Many good authorities are confident that this ameba is not the cause of pyorrhea and does not play a part at all. Others are willing to grant that it may play a part. Still others, including ourselves, feel certain that the chief specific cause of alveolodental pyorrhea is *Endameba buccalis*. One of the things that we have observed we believe as certainly as any other thing is that these endamebas feed on certain nucleated cells that are to be found only in inflamed tissue. If we make sections of the diseased tissue we find these same cells distributed not only on the surface, but actually in the inflamed tissue. Now for endamebas to get these cells, they must crawl about or burrow about in the inflamed tissue searching for them. We have been able to demonstrate and find the endamebas in the tissue and determine to what extent they burrow. We find that they burrow for a distance many times their diameter, but possibly they do not burrow as deeply as the endamebas in amebic ulcers of the intestine do. Not only do we find them in the tissue, but we find them also on the surface of the closed ulcerated tissue in the bottom of the pyorrhea pocket. In burrowing back and forth from the pyorrhea pocket and then into the tissue, many different species of bacteria are transported or transplanted and retransplanted by the amebas. Whether endamebas

are present in all cases of pyorrhea we will be obliged to wait for further studies to determine until those working on the subject have acquired such technic as will permit them to make accurate negative diagnoses. It is quite difficult to make an examination which justifies one in saying positively that the individual has not some endamebas present. We may say he has no endamebas demonstrable by our technic but a microscopic lesion may be large enough to hold many endamebas. We cannot give a mouth a casual macroscopic observation, and determine that there is no inflammation of peridental membrane around or between any of the teeth. The disease begins between the teeth, where it cannot be seen. After it is advanced, then we may see evidence of the disease labially or lingually. It is difficult if not impossible to determine that an individual has a normal mouth. The fact that some endamebas have been found and are found in what are called apparently normal mouths cannot be taken too seriously on this account. Our experience has been that in the examination of probably 500 individuals having pyorrhea, we found endamebas in all of the patients after we had acquired the technic which seems to us quite necessary.

DR. ANNA W. WILLIAMS, New York: Just such work as Drs. Bass and Johns are doing in histologic biology will no doubt be of much aid in helping us to get more evidence in regard to the relationship of this organism to disease. In regard to the use of the toothpick, I would say that we simply use a toothpick in our routine examinations of the schoolchildren. These children do not need a scaler since they do not have pus pockets. We have only found one of the older boys having small pus pockets. We use Young's dental scaler No. 22 for obtaining material from the adult pyorrhea cases that we have studied.

COMPLEMENT FIXATION IN DIAGNOSIS WITH BACTERIAL EXTRACTS PRE- PARED WITH DIGESTIVE FERMENTS

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SAN FRANCISCO

In a preliminary report¹ in THE JOURNAL a method was described by me for the preparation of a soluble vaccine against the pneumococcus, and the evidence presented of its efficacy in producing active and passive immunity. Ten cases of pneumonia treated with the extract were tabulated and reference was made to experiments with other micro-organisms. Since then, extracts have been prepared with greater accuracy by improved methods and the immunizing effects have been made more constant. At the same time, it has been found that these extracts are valuable as antigens in complement fixation for diagnostic purposes.

PREPARATION OF EXTRACTS

As the standardization of bacterial suspensions by the known methods of counting is notably uncertain, a gravimetric method was devised based on their opacity as compared by means of a delicate photometer with that of a suspension containing a known quantity of the micro-organism as determined by its weight when dried at 110 C. The germs were grown in bouillon that had been passed

Diagram of light for determining amount of micro-organisms in suspensions. See full description in text. A, A', screw adjustment for lamp; B, standard bottle; C, Lummer-Brodhun photometer; D, test bottle.

through a Berkefeld filter and then autoclaved. After growth, the culture was filtered through a Berkefeld and the germs washed several times with distilled water. The bacteria were then gently rubbed off under a fine stream of distilled water with absorbent cotton on the end of a wire and the suspension well shaken.

A portion was weighed and dried at 110 C. and the dry weight determined. Weighed portions of the suspension were diluted with measured quantities of distilled water until the opacity corresponded to that of the standard. The amount of the dried bacteria in the standard could thus be readily calculated. Conversely, it became easy to calculate the amount of the dry micro-organism in an unknown suspension by diluting to the opacity of the standard.

The apparatus used consists of a Lummer-Brodhun photometer head to which two light-proof arms are attached, in each of which a small electric lamp can be moved back and forth by means of a screw. After the light on the two sides has been equalized, the standard test bottle is placed between the photometer and the lamp on one side, and on the other side a measured quantity of the bacterial suspension to be determined in a tested bottle. This is then diluted with distilled water until the light which passes through both sides is the same. When this is the case, the suspension contains the amount of dry micro-organism represented by the standard. All of the bottles used must be first tested optically when filled with distilled water. The standard is made by using a suspension of electrosilicon in distilled water, which is allowed to stand a few hours to permit the heavier particles to sink. The fine suspension is then standardized optically with a suspension of the micro-organism equivalent to the desired amount of dried bacteria.

In standardizing the pneumococcus, suspensions were used of washed living pneumococci of the types Rockefeller 1, 2 and 3, cultures of which had been kindly sent by the Rocke-

1. Hirschfelder, J. O.: The Production of Active and Passive Immunity to the Pneumococcus with a Soluble Vaccine, THE JOURNAL A. M. A., Oct. 12, 1912, p. 1373.