bacteria and the organisms of nitrification behave as facultative heterotrophs. Indeed, Bejerinck held the view of the mutability of the nitrifiers.

A noteworthy finding in this connexion is that of Bömecke[3], who demonstrated the prevailing concept that the nitrifying bacteria possess no measurable metabolism other than the oxidation of ammonia and nitrite to be untenable, and that a heterotrophic dissimilation metabolism does exist, though only at a comparatively slow rate. From this point of view, the close correlation observed by Starkey[4] between the enhanced nitrification, carbon dioxide production and abundance of microbial population found in the regions of maximum root development is very significant. The profuse use of oxygen by the nitrifying bacteria, contrasted with the fact that the adsorbed or condensed oxygen in soil has an unfavourable effect on the ordinary heterotrophic microflora of the soil, emphasize the fact that organisms in their natural environments derive mutual benefit from one another. Fermentable organic matter is rapidly destroyed by the saprophytes of the soil, rendering conditions favourable for vigorous nitrification. Indeed, there is evidence to show that *Azotobacter chroococcum* could fix atmospheric nitrogen in the presence of different ammonium salts, and the enhanced nitrification observed in my experiments would be due to the greater amounts of ammonium salts present in the system as a result of nitrogen fixation by the *Azotobacter* fed by glucose. Here, unlike as observed by Imsenecki, there are no autolytic effects suffered by the *Nitrosomonas* cells to render organic matter for the growth of the associate organisms, and consequently not the intriguing point of doubt as to how long such a symbiosis can proceed if at every stage the synthesized *Nitrosomonas* cells have to be expended to provide energy material for the growth of the myxobacteria.

Thus it has to be recognized that a major part of nitrification occurring in Nature is brought about by bacteria which function in close association with the heterotrophic organisms of the soil, and the occurrence of a regulated chemomixotrophic metabolism seems established[5], at least for the organisms responsible for this moiety of nitrification. In other words, nitrification in Nature is at least in part due to symbiotic agencies.

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Formation of Hydrogen Peroxide by Spermatozoa and its Inhibitory Effect on Respiration

The biological formation of hydrogen peroxide has often been postulated in the past, particularly in connexion with the presumed function of catalase and peroxidase; but so far its actual detection has been successful only in cultures of some bacteria[1,2,3] and some moulds[4,5] and in certain enzymic oxidations catalysed *in vitro* and requiring molecular oxygen for the oxidation of their respective substrates. The chemical identification of H_2O_2 as a product of metabolic processes of animal tissues has up to now been unsuccessful. Indirect evidence for H_2O_2 formation during respiration of bovine spermatozoa in egg-yolk medium has been submitted in a previous communication[6], and some evidence of its possible formation in human sperm has been given[7]. Using suspensions of washed spermatozoa in presence of a substance separated from egg-yolk, we have now been able to demonstrate chemically the formation of hydrogen peroxide.

In the previous communication[6] we showed that during respiration of whole semen diluted with egg-yolk medium, the rate of oxygen absorption gradually decreases. As the cause of this inhibition of respiration we postulated a gradual formation of peroxide, since both catalase and peroxidase completely abolished this effect, while heat-inactivated catalase, cytochrome *c*, hæmatin and ferrous iron did not reverse it. Since then we have carried out a series of experiments using suspensions of washed spermatozoa, and we have found that inhibition of respiration develops in presence of egg-yolk or its dialysable portion. Furthermore, we have isolated in considerable purity from the dialysable portion the substance which on oxidation by spermatozoa yields as a metabolic product the inhibitor of sperm respiration which we now identify as hydrogen peroxide.

For the detection of H_2O_2 a suspension of washed spermatozoa containing 800 million cells in 3·925 ml. *M*/15 phosphate buffer (*p*H 7·4) and 0·075 ml. solution containing 1·8 mgm. of the purified substance (a quantity comparable with that in a corresponding amount of egg-yolk medium) was shaken in air at 37° in a Barcroft–Dixon manometer. After a certain time, when the inhibition of respiration had fully developed (1–1½ hr.), the suspension was centrifuged and the clear supernatant fluid examined for the presence of H_2O_2 by means of the benzidine – peroxidase reaction, the optimum conditions of which were carefully predetermined so as to allow a maximum colour development in low concentrations of H_2O_2. The concentration of H_2O_2 formed was determined, by comparing the intensity of colour with that formed with known concentrations, and was found to be of the order of 10^{-1} μmol. H_2O_2/1 ml., this amount corresponding to about 1 μl. oxygen.

That the inhibition is in fact caused by such low concentrations of H_2O_2 is supported also by the following evidence. (*a*) The addition of 6×10^{-1} μmol. H_2O_2 to 800 million washed spermatozoa suspended in 4 ml. *M*/15 phosphate buffer (*p*H 7·4) almost completely inhibited the endogenous respiration of the spermatozoa, and lower concentrations gave correspondingly lower inhibition. (*b*) In such experiments, if catalase was added at an early stage, before much damage was done to the spermatozoa, it almost completely reversed this inhibition, provided that all the intracellular substrates had not already been utilized. At the end of experiment (*a*) it was not possible to detect H_2O_2 chemically, because a large part of the H_2O_2 added was eliminated from the system by the sperm. However, H_2O_2 was detected at the end, if the original concentration added was 8×10^{-1} μmol. H_2O_2 or higher. The elimination of H_2O_2 by spermatozoa was quantitatively ascertained, in experiments with somewhat higher concentrations of H_2O_2, by estimating the amount of H_2O_2 with catalase before addition of the spermatozoa and after their incubation with H_2O_2 for an appropriate length of time. Thus, bovine spermatozoa must be equipped with a mechanism for the elimination of H_2O_2 from the system at a low rate.

So far we have not been able to detect chemically H_2O_2 as a metabolic product of spermatozoa when their oxygen uptake was measured in presence of: (*a*) phosphate buffer alone, (*b*) egg-yolk medium, (*c*) the dialysable portion of the egg-yolk, (*d*) seminal plasma, (*e*) seminal plasma after yeast-fermentation, or (*f*) media containing either fructose or glucose. Since in both egg-yolk medium (*b*) and the dialysable portion (*c*) the substance was present which gives rise to H_2O_2, and yet the latter was not detectable, we suggest as a possibility that some constituent of the egg-yolk obscures detection of H_2O_2 by the benzidine peroxidase reaction.

The concentration of H_2O_2 present at any stage of respiration of the spermatozoa is, therefore, a result of two simultaneous yet diametrically opposed reactions, those of biological formation and elimination of H_2O_2. Its actual detection by the benzidine – peroxidase reaction is possible only if the rate of formation exceeds the rate of elimination by an amount which permits not less than 3×10^{-2} μmol. H_2O_2 to accumulate in 3 ml. of the supernatant fluid, provided that substances which interfere with the detection of H_2O_2 are absent from the medium in which the benzidine – peroxidase reaction is tried.

A positive benzidine – peroxidase reaction is definite proof of H_2O_2 in concentrations at least as high as 3×10^{-2} μmol. H_2O_2/3 ml. of supernatant fluid, but a negative reaction need not necessarily mean that H_2O_2 is not formed during the metabolic processes of the spermatozoa.

Although we have proof of the formation and elimination of H_2O_2 by spermatozoa we cannot yet specify all the conditions which affect its detection. We can tentatively, at least, say, however, that the following are some of the necessary factors for its chemical detection: (*a*) an adequate concentration of active spermatozoa; (*b*) presence of a substrate, in sufficient concentration, which on oxidation by spermatozoa yields H_2O_2; (*c*) a ratio between the rates of formation and elimination of H_2O_2 by spermatozoa such that not less than 3×10^{-2} μmol. H_2O_2 in 3 ml. of the supernatant fluid accumulates by the time the chemical test is made, and (*d*) absence of interfering substances present in complex organic media (for example, egg-yolk, seminal plasma) which may obscure detection of hydrogen peroxide by the benzidine–peroxidase reaction.

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Vernalization of Sponge Gemmules

Sponge gemmules were collected at Cambridge in September 1945 and brought to Glasgow in a small bottle of water from the River Cam. On September 29, being impatient to make some observations on developing gemmules, I placed some of them in water from Loch Lomond in a refrigerator working at 50° F. The rest were kept in Cam water on my laboratory bench, where the temperature varied roughly between 55° and 65° F.

On December 2, that is, after about two months had elapsed, single gemmules were cleaned so far as possible, placed each in the centre of a coverslip lying in a Petri dish of water from Loch Lomond, and so left on the laboratory bench.

Dish *A* contained 9 *Spongilla* gemmules from the refrigerator; dish *B* contained 8 *Ephydatia* gemmules from the refrigerator; dish *C* contained 17 *Spongilla* and 9 *Ephydatia* gemmules which had been kept all the time on the laboratory bench.

A week later a white halo appeared around two of the *Spongilla* gemmules in dish *A*, and after another two days round two of the *Ephydatia* gemmules in dish *B*.

By December 21, development had begun in 7 of the 9 *Spongilla* gemmules in dish *A*, and in 7 of the 8 *Ephydatia* gemmules in dish *B*; while in dish *C* there was no sign of development in any of the gemmules of either genus. Some of these, however, did develop later, for when the dishes were next inspected on February 24, 1946, one of the *Spongilla* and all the *Ephydatia* excepting three very small ones had evidently hatched out.

Similar results were obtained in the second half of March, when the time of natural activity would be much nearer. All 26 gemmules (20 *Spongilla* and 6 *Ephydatia*) from the refrigerator had hatched ten days after being planted out in Petri dishes in natural water as before, while only 3 (all *Spongilla*) hatched out in that time of 28 gemmules (21 *Spongilla* and 7 *Ephydatia*) from the bench. The time taken for development to have begun in all the vernalized gemmules (ten days) is less than in December (two to three weeks); perhaps because the natural date of hatching was imminent, and/or because the temperature was a degree or two higher. Another batch of gemmules also, all *Ephydatia*, from a loch near Glasgow (for which I am indebted to Dr. Harry D. Slack of this Department), refrigerated only since December 2, gave 20 out of 24 gemmules hatching in the same ten days.

This method of vernalization is extremely simple and may well be more widely applicable to provide active material at desired times