cm.^{3}, absorbing 0.3 cm.^{3} of $n/5$ $Ba[OH]_2$, in average of several determinations. VanSlyke's gave with $T = 23°$ C. and barometer = 734 mm., non-absorbing 0.34 g. nitrogen and absorbing the same. The samples were in all cases positive to ninhydrin, showing that some alpha-amino acids were present in the filtrates, but in such small amounts that they were not recognizable by the methods used.

It may be well argued, as it was in the earlier work with amino-acids in the blood, that these compounds are so quickly removed from the sphere of action that at any one time they are present in only minute quantities. It is well known that phagocytes crowd into the tissues of the metamorphosing organs after the earlier stages of dissolution are under way and it may be through their agency that the products of proteolysis are removed. Mercier[1] has been able to trace the circulation of phagocytes throughout the metamorphosing organs and the body by causing the cells to engulf carmin granules and it may be that the end products of the action of proteolytic enzymes which we must imagine to be developed at the beginning of dissolution of the muscles, etc., are taken up and carried to the body proper by these cells. This conception, however, does not give any support to the so-called phagocytic theory of involutionary phenomena, for it is quite certain that dissolution has begun before the wandering phagocytes have entered the tissues affected.

In vitro studies of autolysis of normal and involuting larvæ are in progress.

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A simplified method for cultivating spirochaetes on liquid media.

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The method I wish to describe is a modification of the original method of Noguchi[2] for cultivating spirochaetes, which I think

[1] *Arch. zool. expér. et gén.*, T. 5, Ser. IVe, 1, p. 151, 1906.

[2] *Journ. Exp. Med.*, 1912, XV, p. 211.

might be of interest to those who are called upon to cultivate these organisms. Noguchi's method, although very efficient, offers one great disadvantage, namely that the rubber stopper connection between the upper and lower parts of the apparatus often becomes a source of contamination of the culture. The method I propose consists in utilizing the principles of anaerobic cultivation in general as well as those special features which were worked out by Noguchi. This new method can be used in two different ways. Firstly, one can use the tube as shown on Fig. 2 which instead of having the rubber connection of Noguchi joining its two parts as shown in Fig. 1, is made entirely out of one glass tube; but otherwise can be used exactly as Noguchi's apparatus, namely the lower part in which the piece of rabbit kidney is put before the tube is drawn out, is filled with the ascitic broth or sheep serum water up to the point where the tube broadens out again; another piece of tissue is placed in the upper portion of the tube and this tube is filled with the ascitic agar into which the spirochaetæ culture is placed.[1] Sterile paraffine oil in a thin layer is placed above the agar and the tube is incubated. Spirochaetes during their growth filter through into the lower portion of the tube exactly as in Noguchi's method. This method is especially convenient when one intends to open the tube many times to examine its contents. The other and better way however of cultivating spirochaetes which does away entirely with the upper part of the tube,[2] is the following: I put a piece of tissue at the bottom of the tube; draw it out as before; introduce by means of a capillary pipette the spirochaetæ culture and ascitic broth in the lower tube; connect the tube with the vacuum pump, as shown in Fig. 6, warming the lower part of the tube in a water bath at 37° to facilitate the exhaustion of the air; cover the ascitic broth, after exhaustion, with sterile paraffine oil by means of a special arrangement taking advantage of the negative pressure in the tube, and finally seal the lower part of the tube at the point of strangulation

[1] It was found that spirochaetes penetrate into the lower tube more readily if 1 per cent agar is used for the ascitic-agar mixture instead of 2 per cent, as recommended by Noguchi.

[2] A Florence flask with a long neck can be used in place of the tube if a larger quantity of culture is wanted.

as shown in Fig. 7. The paraffine oil in this tube makes it possible to preserve spirochaetes alive even after the tube is once opened. By this simple method I have been carrying successfully my subcultures of spirochaetes for the last three months.