# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A GLASS ASSEMBLY FOR SEITZ BACTERIOLOGICAL FILTERS

BACTERIOLOGICAL filters are so made that the filtered liquid comes in contact with metal surfaces which usually contain copper or some other heavy metal. On one occasion a drop of water suspended from the stem of a well-washed and freshly sterilized Berkefeld filter contained enough copper to have a distinct blue color and give a flame test for copper. In view of the known static action of very small amounts of heavy metals for bacteria, even in protein media, it seems possible that contamination by copper or other metals may sometimes be sufficient to cause erratic variation in the growth of bacteria in synthetic media.[1] Such

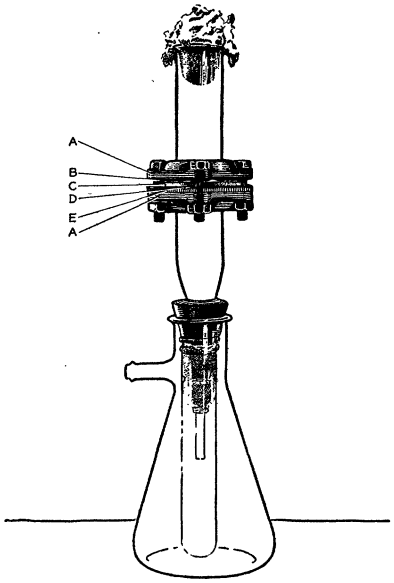


FIG. 1. A. Iron flanges. B. Filter paper. C. Seitz filter disc. D. Aluminum or platinum support. E. Asbestos interface gasket.

variation was in fact observed in this type of medium when the usual filters were used in sterilization, but not when a glass filter assembly was used.

In order to escape such an obvious possibility of contamination by heavy metals, a modified Seitz filter has been devised in which the liquid comes in contact with no metal surfaces other than aluminum or platinum. In addition to being easily constructed and allowing full vision of the material being filtered, this device has the advantage of being much less expensive than the usual filter of this type.

The heavy pyrex glass piping which has recently become available in a variety of sizes can be used to construct such a filter. For the filtration of 50 ml or less, two straight 4 in. lengths of 1 in. flanged piping with the metal flanges, bolts and asbestos interface gaskets to fit may be used. One of the straight lengths is cut in two, and to each half is sealed an appropriate funnel tube. If suction alone is required, only one of these need be used. The flanges and gaskets are then put in place, and between them is inserted a Seitz filter pad. This is supported on a sheet of aluminum foil, which may be 5/1000 in. thick or more, patterned after the gasket and perforated by about 50 pin-holes. The bolts and nuts are adjusted loosely, and after the usual preparation for filtration, the apparatus is sterilized in the autoclave. The bolts are tightened with a small wrench before the apparatus is used.

The filter pad requires support, since atmospheric pressure is sufficient to break through the wet pad. The aluminum support here described has given satisfactory service over a period of six months. There is no visible corrosion in the central part, and but slight corrosion at the exposed edges. Platinum gauze has been used, but no great advantage over aluminum has been discovered. The apparatus assembled for filtration by suction only is shown in Fig. 1.

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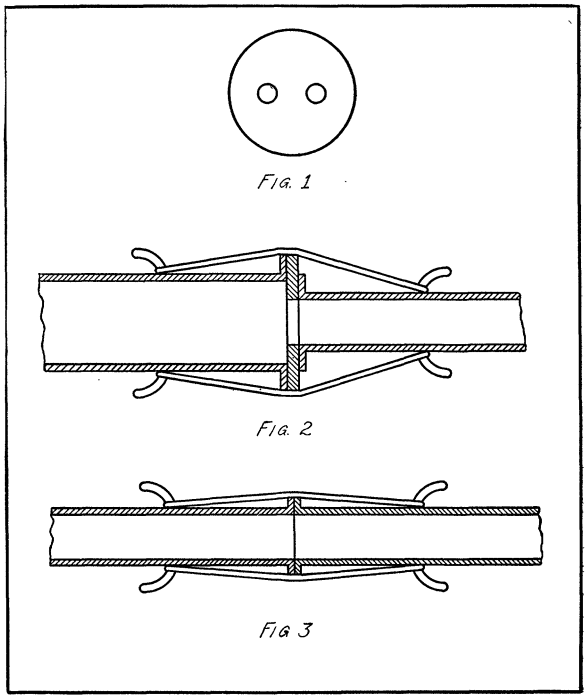
## A SIMPLE GLASS CONNECTION

AN easily made glass to glass connection which will serve satisfactorily in many glass apparatuses where




[1] E. O. Jordan and I. S. Falk, "Newer Knowledge of Bacteriology and Immunology," University of Chicago Press, 1928, p. 284.

only small pressures or vacua are developed is shown in Plate 1.

Pieces of tubing, mouths of flasks, etc., are simply flared and given a flat lip, and then this lip is grounded in the usual manner by emery on a flat glass surface. The customary small glass arms for anchorage of rubber bands for holding pieces together can be easily fused to the main element.

The writer has built "T's," "L's," and straight lengths of tubing of varying lengths which are all surprisingly interchangeable. Figs. 1 and 2 give a simple method for reducing the diameter of a tube—*i.e.*, by the insertion of a ground glass disk with a hole in it. The ground glass disk idea is also satisfactory where it is desired to have two tubes connect with one vessel or another tube. Fig. 3 shows a straight connection involving the same size tubing.

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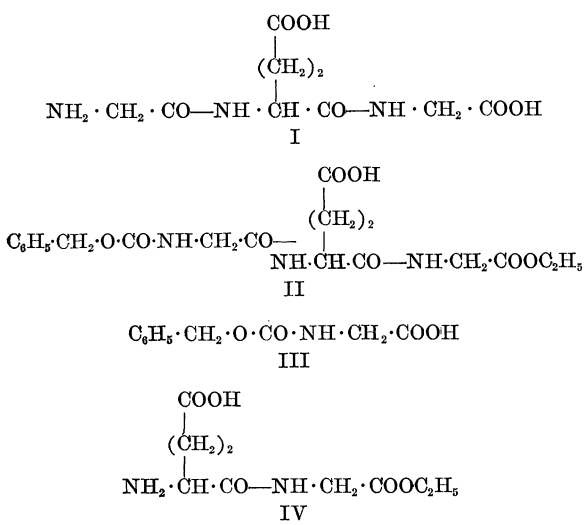
# SPECIAL ARTICLES

## A SYNTHETIC PEPTIDE AS SUBSTRATE FOR TRYPTIC PROTEINASE

LITTLE is known regarding the mechanism and the specificity of those enzymes which split true proteins —the proteinases. This is due to the fact that until recently it was not possible to obtain a proteinase substrate of known structure.

By means of the carbobenzoxy method[1] a peptide-like substrate for tryptic proteinase was synthesized. It is a derivative of the tripeptide glycyl glutamyl glycine (I). The amino group at one end of the molecule was blocked with the carbobenzoxy group, and the glycine carboxyl on the other end was esterified with ethyl alcohol (II). Thus the only free reactive group was the γ-carboxyl of the glutamic acid.



Substance II was split quite rapidly by pancreatin Merck as well as by a preparation of crystalline trypsin (tryptic proteinase) kindly placed at our disposal by Dr. John H. Northrop. The products of the splitting were carbobenzoxy glycine (III) and glutamyl glycine ester (IV). The latter product is rapidly transformed to a diketopiperazine under the conditions of the experiment.

[1] M. Bergmann, SCIENCE, 79: 439, 1934.

This experiment shows that tryptic proteinase does not require for its action linkages of unknown nature, but is able to split ordinary peptide linkages if the rest of the molecule fulfils certain structural requirements. In II one of these requirements is the presence of the free γ-carboxyl, which combines with the tryptic proteinase and thus enables it to split the peptide.

It is probable that the other amino dicarboxylic and diamino carboxylic constituents of the proteins play a rôle similar to that of glutamic acid in combining with proteinases by means of their extra acid or basic groups. From the work of Gurin and Clarke[2] it is to be expected that the ε-amino group of lysine in a protein combines with pepsin. By means of the carbobenzoxy method we are preparing peptides of lysine and aspartic acid and shall report on their behavior towards proteinases in the near future. The theoretical significance of these results as well as the interesting experiments of Matsui,[3] Ishiyama[4] and Shibata[5] on the splitting of diketopiperazines will be discussed in a future publication.

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## THE ELECTRICAL RESPONSE OF THE VESTIBULAR NERVE DURING ADEQUATE STIMULATION

A STRIKING characteristic of the vestibular nystagmus which is produced in virtually all vertebrate species by the angular retardation incident to the termination of a prolonged period of uniform bodily rotation (also by the acceleration incident to the onset of such a period of rotation) is that this response ordinarily persists for a considerable time—often 20 or 30 seconds—after the cessation of its

[2] S. Gurin and H. T. Clarke, *Jour. Biol. Chem.*, 107: 395, 1934.
[3] J. Matsui, *Jour. Biochem.*, 17: 163, 253, 1933.
[4] T. Ishiyama, *Jour. Biochem.*, 17: 285, 1933.
[5] K. Shibata, *Acta Phytochimica*, 8: 173, 1934.