Nereis brandti, *Glycera rugosa*, and also in *Urechis caupo* indicated a slowly-hydrolyzable phosphate, unstable in acid at room temperature. *Glycera rugosa*, the most active species, gave the highest values for this labile phosphorus.

To study further the nature of annelid phosphagen the method of Meyerhof and Lohmann[4] for the isolation of phosphoarginine was applied to the body-wall muscle of *Nereis brandti*. From 160 gm. of muscle there was obtained 0.2 gm. of an impure barium salt. Qualitative tests indicated the presence of barium, phosphate, and some substance giving the Sakaguchi reaction. The phosphorus content was low, indicating that it was possible for only 21% of the isolated material to have been phosphoarginine. Although the Sakaguchi test was positive no trace of arginine could be detected by the use of arginase and xanthydrol. Concentrations of the hydrolyzed barium salt were used which should have given from 25 to 50 times the amount of urea necessary for detection with xanthydrol after treatment with a liver arginase preparation of demonstrated activity.

From these results we conclude that either (a) the procedure used for the isolation was unsatisfactory, or (b), in the light of the cumulative evidence, that the phosphagen of *Nereis* is not arginine phosphate. We favor the latter alternative.

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A New Apparatus and an Improved Method for Chromatographic Adsorption.

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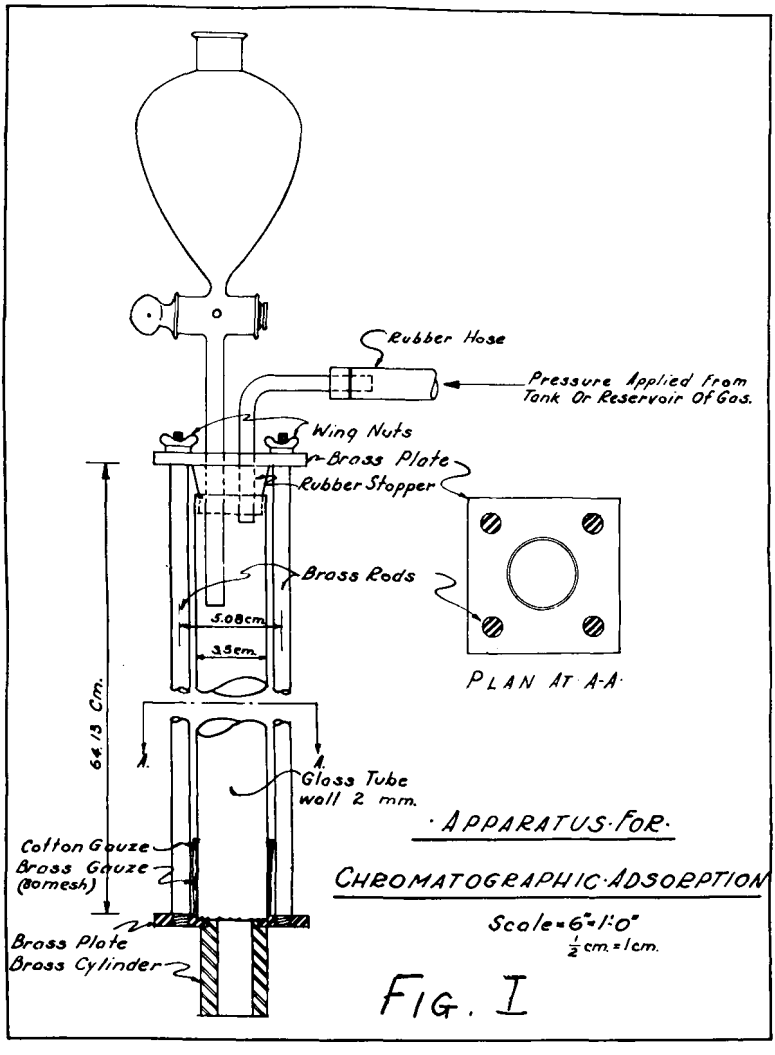
It is well known that chromatographic adsorption has been a very valuable method for the separation of organic compounds of biological importance. The principle involved in the use of our apparatus is essentially the same as in the older forms. The procedure, however, differs in that we filter our solution through the column of adsorbent by pressure and not by suction and we localize the area of specific adsorption of colorless compounds by means of a color reaction carried out on one side of the column of adsorbent or by the fluorescence observed with ultraviolet light. The method pro-

[4] Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1928, **196**, 49.

duces a more uniform column of the adsorbent; it is more rapid and prevents the irregular results produced by evaporation of the solvent.

Apparatus. The adsorption tube proper (Fig. 1) is a plain piece of Pyrex tubing 3.5 cm. in bore and 60 cm. long. The bottom is covered by a pad of absorbent cotton 1 cm. thick, held in a cup of



brass screening. The screening with cotton under it is wired to the tube to give ease in handling and added protection against leakage of adsorbent. This bottom end rests in the countersunk depression in the lower plate. This plate contains a central outlet hole into

which the outlet tube is screwed and is pierced at the corners by 4 holes into which the rods are screwed.

The top of the adsorption tube is closed during operation by a rubber stopper which is held in against the pressure by the top plate. This plate has 4 holes through which the rods pass. It is held down against the stopper by 4 wing nuts which screw on the ends of the rods. Both the top plate and the stopper have 2 holes at the center through which pass a separatory funnel for admission of the solution and a glass tube for the pressure inlet.

Pressure is obtained from a tank of compressed gas—N_2 or CO_2 is often desirable for easily oxidized compounds. The pressure line also contains a manometer, a drying tube, and a side-tube fitted with a stopcock.

In another modification of the apparatus we provided a reservoir of reasonable capacity below the separatory funnel and above a tube of narrow diameter containing the adsorbent. The reservoir is very convenient in passing larger volumes of solution through the narrow column.

The proper amount of adsorbent is suspended in the solvent to be used until the paste first formed gives way to a slow flowing suspension and this material is poured into the absorption tube. The stopper is inserted in the top and the plate screwed down. The pressure is raised and the excess solvent is forced through. Usually a pressure of one atmosphere is sufficient to give satisfactory packing. When the line of solvent just reaches the level of the top of the adsorbent, pressure is released by opening the stopcock in the pressure line. The stopper and top plate are removed and a pad of glass wool is introduced into the top of the column. A cork of the same diameter as the column is inserted and the packing is completed by applying pressure on the cork with a piece of dowel pin. The cork is then removed by means of a wire previously attached for the purpose. The glass wool is left to keep the top layer of adsorbent from being stirred up by the addition of liquid.

The solution to be adsorbed is poured in, the stopper and plate are replaced, and the pressure is raised. When the solution has passed through, the pressure is released, the developer solution is added through the separatory funnel, and the pressure is raised again. In the experiments conducted so far, the volume of the empty tube over the adsorbent has been about 200 cc., and since only 600 cc. of developer was used, the release of pressure 3 times was quite feasible. For larger volumes of solution it might be advantageous to have a method of adding liquid without interrupting the

course of adsorption. It would not be difficult to arrange an auxiliary pressure line to the separatory funnel by means of a T-tube, and add liquid from a smaller funnel into the large one. Thus the large separatory funnel could be filled, and a slight increase in pressure would force the liquid into the adsorption tube.

After development is finished (and not before) the liquid is allowed to fall below the top of the adsorbent and all excess solvent is forced out. The tube is removed from the apparatus, the bottom screen is taken off, and the column of adsorbent is pushed out by the cork and dowel pin used previously.

A fresh surface of column is obtained by cutting away a small groove along the whole length of the cylinder, and this surface is observed under ultraviolet light if there are any fluorescent compounds whose location is desired. In the case of a non-fluorescent compound that gives a color reaction, the reagent is dropped along the fresh surface by means of a pipette. The column is again viewed under the ultraviolet lamp because it has been frequently noted that the reaction may create new compounds that fluoresce. It is obvious that a color is not essential here; the reagent need only convert the colorless non-fluorescent compound to a colorless fluorescent one. With the aid of the data thus obtained the bands are cut as desired, the parts contaminated by the reagent are discarded, and each band is eluted separately. Zechmeister, Cholnoky, and Ujhelyi[1, 2] have also detected the location of colorless adsorbed substances by applying a color reaction on the side of the column.

Summary. A new apparatus whose advantages are increased speed and uniformity of column has been described. A new method of working with colorless, non-fluorescent compounds by use of a color reaction has also been reported.

[1] Zechmeister, L. M., Cholnoky, L. V., and Ujhelyi, E., *Bull. Soc. chim. Biol.*, 1936, **18**, 1885.

[2] Zechmeister, L., and Cholnoky, L. V., *Die Chromatographische Adsorptionsmethode*, Vienna, 1936, Julius Springer.