those for the effect on glucose utilization; this demonstrated itself in the relatively large standard deviations of ΔK compared with those of Δ glucose. The greater irregularity of the potassium shift may be due to the varying degree of tissue damage at the excision and the ensuing leakage of potassium from the injured muscle fibers.

Extremely small concentrations of insulin can be detected by this method. Even with insulin concentrations as low as 5×10^{-3} units/ml, significant effects on the glucose and potassium metabolism of the isolated rat diaphragm were observed. Lower concentrations appeared to have a potassium effect, but the level of significance was less than 1%. Apparently this technique makes it possible to detect insulin in amounts that are much smaller than those reported by previous observers (*1*, *6*). It should be borne in mind, however, that variations in sensitivity of the diaphragms may occur.

The sample of pure insulin used in these experiments contained 28 units/mg.[2] The smallest concentration of insulin that still produced a significant increase in glucose utilization was 5×10^{-6} units/ml, which, assuming a molecular weight of insulin of 48.000, amounts to 4.5×10^{9} molecules per flask. This number of insulin molecules enabled the diaphragm to utilize about 10^{18} more molecules of glucose than diaphragms in the control flask without insulin. This extra utilization of glucose was associated with an extra shift of about half as many atoms of potassium from the medium into the tissue. With further refinements of technique, the isolated diaphragm test may offer opportunities for the determination of minute amounts of insulin.

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[2] We are indebted to J. Lens (*4*) for a gift of pure insulin.

A Method for Silver Staining of Nerve Fibers in Whole-Mount Preparations of Blood Vessels

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Humphreys (*3*, *4*) demonstrated vascular nerve fibers on cerebral blood vessels, using a modification of the Bodian (*1*) technique. He obtained better and more consistent results than with either the modified Gros-Bielschowsky technique of Penfield (*5*) or Huber's (*2*) methylene blue technique.

On cerebral blood vessels of the pia mater we have obtained more satisfactory results by use of the following modification of the Bodian technique. Blood vessels of anesthetized animals were flushed with a physiological salt solution, followed by perfusion with neutral 10% formalin. The leptomeninges containing the blood vessels were dissected from the brain and brain stem, and pinned flat on paraffin for 24 hr in a Petri dish contain-

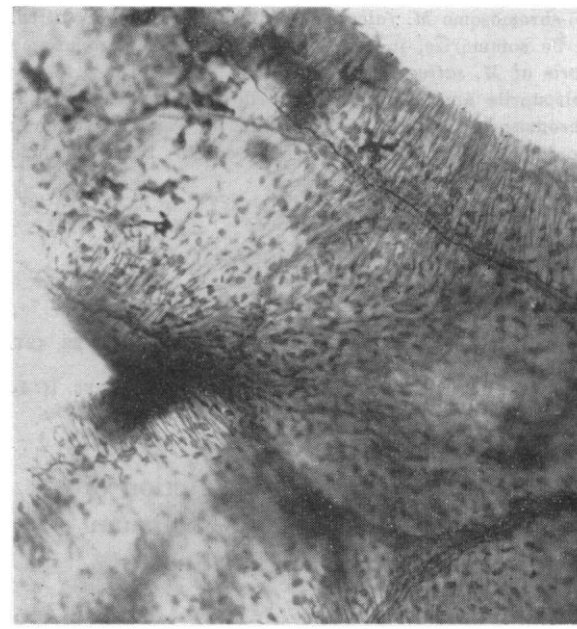

FIG. 1. Arterial branch of vertebral artery showing the crossing and branching of large mixed fiber bundles of the adventitia. The dark stellate cells are chromatophores. Objective, 16 mm; ocular, 10 ×. Protargol.

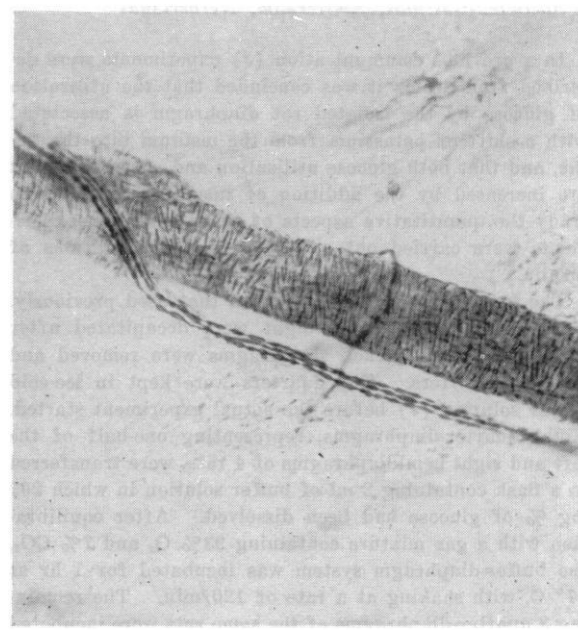

FIG. 2. Group of myelinated fibers becoming related to a branch of the basilar artery. Objective, 16 mm; ocular, 10 ×. Protargol.

ing 10% formalin. The tissues were then washed in water, dehydrated in alcohol, and cleared in cedarwood oil. After clearing, they were hydrated and placed in distilled water for 1–2 hr. This procedure seemed to give the tissues a greater clarity and stainability. The connective tissue elements took less silver stain, and small nerve fibers appeared finer and darker. Individual types of fibers could be distinguished in the roots of cranial nerves that lay in the pia-arachnoid. Subsequently the standard Bodian (*1*) technique for staining paraffin sections was employed. The length of time the tissues were left in the various solutions varied with the tissue under study and was determined by direct observation. In general, they were left in the Protargol solution for 32–48 hr, and in the other solutions for approximately the same period as suggested by Bodian for section-staining. It was necessary, however, to flush the solutions from within the vessels each time a change was made. This was easily accomplished by gentle pressure on the blood vessels exerted with a round-tipped glass rod. After staining, the tissues were dehydrated and cleared in cedarwood oil and xylene. Mounting in clarite and permount proved satisfactory, and no fading was seen after 6 months.

The technique consistently demonstrated the nerve fibers along blood vessels. Small and large fibers, singly or in bundles, were readily distinguished (Figs. 1, 2). These preparations made it possible to follow nerve fibers along blood vessels for relatively long distances.

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Molluscan Shells as a Practical Source of Uroporphyrin I

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To obtain a supply of uroporphyrins for laboratory work has always presented difficulties. Human cases of porphyrinuria have been the main source, but large quantities of urine must be handled to extract the uroporphyrin, and the cases themselves are uncommon. Extraction from urine by the simplified method of Sveinsson, Rimington, and Barnes (*6*) considerably shortens the procedure, but where large amounts of uroporphyrin are needed an alternative source is most desirable. Possible animal sources (the bones of *Sciurus*; the feathers of *Turacus* which yield turacin) involve scarce and expensive material. Very large amounts of uroporphyrin in an easily manageable form have, however, been found in molluscan shells.

Shell porphyrins were originally recognized by Fischer and Jordan (*3*), and have since been extracted by several workers (Fischer and Haarer [*2*], Waldenström [*9*], and Tixier [*7*, *8*]). The supposed pentacarboxylic conchoporphyrin of Fischer and Jordan was found by Nicholas and Comfort (*5*) to be a mixture of uro- and coproporphyrin. In most of the porphyrin-containing forms, uroporphyrin I occurs in an almost pure state. Many species are suitable for use as a source: Porphyrins have been shown to occur only in marine genera, and in largest quantity among the pearl oysters (*1*). The most convenient material for large-scale extraction is the Persian lingah oyster (*Pinctada vulgaris*), which is an article of commerce, and may yield as much as 10 mg per g of shell. *Trochus niloticus*, also used in the button industry, is an inferior source, owing to the thickness of the shell and the large amount of unpigmented mother-of-pearl.

The technique described by Tixier (*7*), in which shells are extracted with methanolic HCl to esterify the pigment, has the drawback of expense, since several liters of solvent are required per kg of shell. The following technique gives satisfactory results.

Shells of *Pinctada vulgaris* are selected under the ultraviolet lamp for their porphyrin content. They are coarsely broken, and the powder is added in small amounts to concentrated aqueous HCl in a very large beaker, allowing 500 ml of solvent to each 50 g of shell. Octanol and other antifoam agents should not be added, since these contaminate the product. The extraction is left to proceed at room temperature for 24 hr, the mixture brought to pH 1 approximately, and the debris removed by filtering over glass wool.

A chromatographic column is prepared by shaking pure acid-washed talc with *N* HCl, and sedimenting it over suction in a large tube (2.5-in. diameter) plugged with cotton wool. The filtered porphyrin solution is passed through this column by suction, and the chromatogram washed with 20% acetone in tap water. Partial development takes place, with separation of red, blue, and violet nonporphyrin bands. The extent of the porphyrin zone is checked by fluoroscopy. Red fluorescence in the subsidiary blue band should be ignored, as it appears to be due to an unidentified nonporphyrin pigment (*1*).

The column is extruded and the main porphyrin zone extracted with acetone containing 10% concentrated aqueous HCl. The filtered extract is concentrated *in vacuo* until it no longer smells of acetone, then diluted with an equal volume of distilled water, and adjusted to pH 3.2 by addition of saturated sodium acetate solution until precipitation of the uroporphyrin occurs. It is left overnight in the ice chest, the precipitate collected on a sintered glass filter, redissolved in concentrated aqueous HCl, and reprecipitated by neutralization. The final precipitate is then dried, esterified in a small volume of methanolic HCl, transferred to chloroform by dilution with water, chromatographed once or twice on alumina, and crystallized from chloroform-ethyl acetate mixture. The yield is approximately 1 mg of ester per g of shell.

The homogeneity of the sample should be checked by paper chromatography, using the method of Nicholas and Rimington (*4*). Uroporphyrin III has not been detected so far in molluscan shells.