about 80% picrolonic acid, whereas this constituent comprises only a small portion of the Hammarsten preparation. The "dog threshold dose" for our secretin base is 14 gamma.[5] (d) The preparation, Pancreotest, is about half as potent as our standard SI material, which we have considered to be too imperfect for use in humans. The secretin used by us[7] on human subjects is 10 times as potent as our SI and 20 times as potent as Pancreotest.

Conclusions. The commercial secretin preparation, Pancreotest, has been assayed on dogs and found to be half as potent as our standard material. An apparent discrepancy in the expression of units of secretin has been explained.

11918 P

Electrolytic Splitting of Liver Albumin.

J. MURRAY LUCK, C. NIMMO AND C. ALVAREZ-TOSTADO.

From the Biochemical Laboratory, Stanford University.

The albumin used in these studies was prepared from dog liver perfused *in situ* until free from blood. In all cases the animals had been well fed. The organ was excised, frozen in liquid air, powdered, and stored at −13°C. Preparation of the liver albumin followed upon extraction of the powdered liver with 0.5 *M* $(NH_4)_2SO_4$, and salting out of the globulins in 2.1 *M* $(NH_4)_2SO_4$. The albumin was salted out by increasing the concentration of ammonium sulphate to 3.5 *M* and was rendered free from any detectable amount of globulin by reprecipitation with ammonium sulphate. After 3 or 4 reprecipitations, the amount of material salted out in 2.1 *M* $(NH_4)_2SO_4$ was reduced to the vanishing point. The pH was maintained at 6.5 to 7.0 throughout this treatment. The amount of albumin obtained from 100 g of liver was 0.9 to 1.5 g.

Electrophoretic studies were carried out in the Tiselius apparatus, using for observation of the boundaries the Toepler "Schlieren" method as modified by Longsworth. In a medium consisting of 0.1 *M* NaCl and 0.02 *M* KH_2PO_4/K_2HPO_4, following upon dialysis against a fluid of the same composition, electrophoretic analysis (pH 5.8 to 7.3) revealed 2 fractions; both moved towards the positive electrode. One fraction was considerably larger than the other and

[7] Voegtlin, Greengard and Ivy, *Am. J. Physiol.*, 1934, **110**, 198.

moved more slowly.* The other fraction, small in quantity, has shown much variability; in some cases it was practically absent. This fraction is isoelectric at pH 4.7 and we suspect it may consist of traces of serum albumin not washed out during perfusion of the liver.

The major fraction has always displayed much stability and homogeneity in media consisting of 0.1 *M* NaCl and 0.02 *M* in either $PO_4^{\equiv}$, Ac^-, or $PO_4^{\equiv}$ and Ac^-, according to the pH; many runs have been made over a wide range of pH (3.9 to 8.0) without evidence of heterogeneity. After about 7 hours of electrophoresis at 2.0 to 2.5 volts/cm the band tends to become somewhat diffuse at the edges but there is no evidence of splitting.

In potassium phosphate buffers of ionic strength equal to or slightly less than that used in the sodium chloride experiments ($\mu = 0.2$) the stability of the albumin is much reduced. In 8 experiments on albumin prepared from livers from 3 different dogs, after electrophoresis was continued for 3 to 4 hours at pH 5.4 to 6.7 and 2.0 to 2.5 volts/cm, the major band split suddenly and rapidly so that within 30 minutes as many as 4 or 5 rapidly moving components arose from the albumin.

In our opinion this is a true splitting of the protein, although admittedly there is much to be done to further elucidate the phenomenon. The appearance of multiple bands is not due to convection since control experiments with sucrose[1] have shown that convection bands do not appear unless the input of power be 3 to 5 times greater than was employed in these experiments. Individual variations can also be ruled out, since albumin prepared from any given liver was homogeneous in the low phosphate medium and produced multiple bands in the high phosphate buffer. It deserves mention that this splitting is electrolytic in character. Though dependent upon the presence of phosphate, prolonged standing of the protein in the phosphate medium is not in itself sufficient to ensure dissociation of the protein. An electrical field seems to be essential. Under the conditions of our experiments the passage of current for 3 to 4 hours is required.

Finally, there is some indication that the occurrence of this splitting depends on the nutritional state of the animal. Three liver albumin preparations did not show the splitting but the dogs from which these have been prepared had been starved 3 days and given epinephrine (0.2 mg/kilo) one to two hours before removal of the liver. Liver

* Isoelectric point, pH 5.6 to 5.8.

1 Alvarez-Tostado, C., *J. Biol. Chem.*, 1940, **135**, 799.

albumin from 2 dogs which we had supposed normal did not give a completely characteristic splitting, yet did not give the single band which the liver albumin from starved epinephrinized dogs showed. We have reason to believe from other evidence that these 2 dogs were not in a normal nutritional state.

11919 P

An Electrophoretically Homogeneous Component of Ragweed Producing Hay Fever.*

HAROLD A. ABRAMSON, D. H. MOORE AND H. H. GETTNER.

From the Electrophoresis Laboratory, Department of Physiology, College of Physicians and Surgeons, Columbia University, and the Laboratories, Mount Sinai Hospital, New York City.

It has been shown[1, 2] that in extracts of giant ragweed pollen there is a major component which is unpigmented and which moves slowly in the electrical field at pH 7.4 in phosphate buffer. In addition to this unpigmented, slow component (designated as USG) as many as 6 other components, probably pigments, were observed by means of the electrophoretic technic. These electrophoretic studies have now been extended to dwarf ragweed extract. Ultracentrifugal and diffusion data have also been obtained.

Figs. 1a and 1b represent the electrophoretic patterns obtained with crude giant and crude dwarf ragweed extract in M/6 phosphate buffer at pH 7.4. Note the similarity of the two sets of curves which were obtained with the Philpot-Svensson technic. The migration time was 2 hours. The base line on the right side of each of the figures has been obliterated. In both ascending and descending parts of the electrophoresis cell, the major component and pigments correspond fairly well. Not all of the pigments are shown.

The giant component† (USG) and the dwarf component† (USD) were isolated in the Tiselius cell and studied in the ultracentrifuge.

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

1 Abramson, H. A., Moore, D., Gettner, H., Gagarin, J., and Jennings, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 311.

2 Abramson, H. A., and Moore, D. H., *J. Lab. and Clin. Med.*, 1940, **26**, 174.

† The names Trifidin and Artefolin are suggested for the USG and USD components respectively.