Experiments with the tracer dilution when adding non-radioactive HGG and experiments on measuring absolute increase in protein when being added to a sorbent-antibody showed the combining capacity of the preparations used is 30-50 mg of antigens on 1 g of sorbent-antibody.

Further experiments showed those antigens which are specifically adsorbed by the sorbent antibody keep the capacity to react with corresponding antibodies from the solution added: this was proved by means of 14C-antibodies. Sorbent antibody also appeared to be capable of reacting not only with antigens but also with soluble antigen-antibody complex; it allows presence of radioactive antibodies masked by excess of antigen in the system to be revealed.

The data obtained testify to the possibility of the following reactions between antigens (Ag) and antibodies (Ab).

1 
$$C - Ag + Ab = C - Ag : Ab$$
  
sorbent-  
antigen antibody  
2  $C - Ag : Ab + Ag = C - Ag : Ab : Ag$   
3  $C - Ag : Ab : Ag + Ab = C - Ag : Ab : Ag : Ab$   
4  $C - Ag : Ab + Ag : Ab = C - Ag : Ab : Ag : Ab$   
soluble  
antigen-antibody  
complex

The method described here can be used for the detection of different radioactive antigens. At rather high specific activity one can reveal as much as 0.01-0.001 µg of radioactive antigens. This has certain advantages over that of co-precipitation since it can be applied not only to precipitating but also to non-precipitating systems. Another advantage is low non-specific adsorption and small labour consumption.

A. E. GURVICH G. I. DRIZLIKH Department of Immunology and Oncology, Gamaleja Institute,

Moscow, U.S.S.R.

<sup>1</sup>Gurvich. A. E., Kuzovleva, O. B., and Tumanova, A. E., *Biokhimija*, **26**, 934 (1961).

<sup>2</sup> Gurvich, A. E., Kuzovleva, O. B., and Tumanova, A. E., *Biokhimija*, 27, 246 (1962).

<sup>3</sup> Gurvich, A. E., and Drizlikh, G. I., D.A.N. U.S.S.R., 155, 482 (1964).

<sup>4</sup> Gurvich, A. E., and Sidorova, E. V., Biokhimija, 29, 556 (1964). <sup>5</sup> Stone, S. S., and Williams, R. R., Arch. Biochem. Biophys., 71, 386 (1957).

<sup>6</sup> McFarlane, A. S., Biochem. J., 62, 35 (1956).

Effect of the Unsaturated Bond on the Degradation of the Tetragalacturonic Acids by a Transeliminase

DURING the course of investigations of the degradation of the normal and unsaturated-tetragalacturonic acids by a transeliminase<sup>1,2</sup>, it was found that unsaturated tetragalacturonic acid was attacked about 1.4 times faster than the normal tetramer. Furthermore, it was observed that the unsaturated tetramer must be almost completely converted to unsaturated digalacturonic acid since there was an approximate doubling (96 per cent increase) in the optical density. All activities were determined by measuring the change in optical density at 232 mµ. Unsaturated galacturonic acid does not absorb at this wave-length, presumably because of isomerization to the keto-deoxy derivative<sup>3</sup>. The observation with unsaturated tetragalacturonic acid was confirmed with the aid of paper chromatography using the ethyl acetate-pyridinewater-acetic acid solvent<sup>1</sup>. Only a spot with an  $R_F$ identical to unsaturated digalacturonic acid was detected.

As indicated in a previous publication<sup>1</sup>, tetragalacturonic acid is attacked at the two glycosidic linkages farthest from the reducing end (designated bonds 2 and 3; bond 1 being that nearest the reducing group). That bond 1

is not attacked was shown by the complete absence of normal trigalacturonic acid. The relative rates of attack of the two bonds can be determined by comparing the amounts of normal galacturonic acid and normal digalacturonic acid produced since the latter arises only from attack at bond 2 and the former by attack at bond 3. Thus a sample of a 24-h reaction mixture was spotted on paper, developed with the previously mentioned solvent system, the strips cut out and eluted with water. The amount of uronide material was determined in each sample by the carbazole method<sup>4</sup>. In this manner it was determined that bond 3 was attacked about 1.4 times faster than bond 2. All the above results were reproducible.

Since the total cleavage of glycosidic linkages in tetragalacturonic acid is the sum of attack at bonds 2 and 3, it was concluded that the central glycosidic linkage of unsaturated tetragalacturonic acid was attacked about 3.4 times faster than was bond 2 of normal tetragalacturonic acid. Thus if one accepts the previous conclusion that only one enzyme is excreted by Bacillus polymyxa<sup>5</sup> one must conclude that the presence of the unsaturated bond somehow inhibits attack at bond 3 but enhances the rate of degradation of bond 2. Whether this is due to the change in configuration of the unsaturated unit (planar) or to other effects has yet to be determined.

This work was supported in part by research grant GM 08088 from the U.S. Public Health Service, National Institutes of Health.

> MARTIN M. ANDERSON CHARLES W. NAGEL

Food Sciences Program, Department of Horticulture, Washington State University, Pullman, Washington.

<sup>1</sup> Nagel, C. W., and Vaughn, R. H., Arch. Biochem. Biophys., 94, 328 (1961).
 <sup>2</sup> Hasegawa, S., and Nagel, C. W., J. Biol. Chem., 237, 619 (1962).
 <sup>3</sup> Preiss, J., and Ashwell, G. J., J. Biol. Chem., 238, 1571 (1963).
 <sup>4</sup> McComb, E. A., and McCready, R. M., Anal. Chem., 24, 1630 (1952).

<sup>5</sup> Nagel, C. W., and Vaughn, R. H., Arch. Biochem. Biophys., 93, 344 (1961).

## Paper Electrophoresis of Soluble Lens **Proteins in Lactose-fed Rats**

PROTEINS of the lens tissue of the eye have been extensively examined because of their relationship with cataract formation. In all forms of cataract a decrease was found in the total amount of lens proteins as compared with normal lens of the same age<sup>1</sup>. Changes in the proportion of the soluble proteins of the lens measured by electrophoresis have been reported in different kinds of cataract; but, according to van Heyningen<sup>2</sup>, no consistent patterns have emerged from these investigations. The work recorded here was carried out to determine the behaviour on paper electrophoresis of soluble lens proteins of the rat, as well as the possible changes in the proportion of the different fractions during lactose feeding.

Albino rats, weighing 70-80 g at the beginning of the experiment, were given a 40 per cent lactose diet, and the controls a 40 per cent cane sugar diet, as already described<sup>3</sup>. At appropriate intervals, the animals were killed and the lens homogenate prepared as already described<sup>3</sup>. Paper electrophoresis was performed with a volume of the supernatant of centrifuged lens homogenate, containing 1-1.5 mg of soluble lens proteins. A borate buffer (0.05 M, pH 8.6) was used; the potential gradient of electric field was of 5 V/cm for 10 h. The paper strips were stained with bromophenol blue, and the different fractions measured by densitometry.

The soluble lens proteins of normal and lactose-fed animals, separated by means of this method, gave four distinct moieties (Fig. 1), which will be labelled I, II, III and IV, fraction I being the most rapid one. After two months of lactose feeding, it was possible to observe (Table 1) an increase of fraction I (the most rapid one); and