4.5, however, the antibody was shown to be present only in the most rapidly migrating subfraction (top fraction), while it was practically lacking in the others. These results agree substantially with those of SAIFER AND COREY and of HUMPHREY AND PORTER. The impossibility of separating γ-globulin subfractions from normal rabbit serum by the method used here is perhaps due to the fact that our method is relatively less sensitive compared with those used by the above authors. The fact that the separation was possible in the case of sera from rabbits submitted to long-time immunization probably shows that the "microheterogeneity" is more marked under these conditions. The hypothesis of HUMPHREY AND PORTER that in the immunization process different types of cells produce different types of γ-globulin is not in disagreement with the results obtained in these experiments, but the presence of the antibody in only one subfraction suggests that each cell produces antibodies with relatively identical properties.

SUMMARY

Rabbit serum γ-globulin was submitted to paper electrophoresis in two different buffers of pH 8.6 and 4.5, and of ionic strength 0.15 and 0.04, respectively. Only one spot was detected for the γ-globulin of normal animals, but the γ-globulin of animals treated for many months with guinea pig erythrocytes was separated into 3–4 subfractions. The specific antibody was present only in the most rapidly migrating subfraction and was completely absent in the others.

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BORATE AND PHOSPHOGLUCOSE ISOMERASE IN THE ASSAY OF PHOSPHOMANNOSE ISOMERASE

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Phosphomannose isomerase (PMI) and phosphoglucose isomerase (PGI) are widely distributed, but in every case so far examined the activity of PGI greatly exceeds that of PMI[1,2,3]. Moreover, PMI is usually far less stable than PGI. Consequently, fractionation procedures applied to several tissues have been successful in giving preparations of PGI free of PMI but not vice versa.

In the assay of PMI by the direct method of estimation of ketose formation (fructose-6-phosphate, F-6-P) from mannose-6-phosphate (M-6-P), the presence of

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PGI tends to decrease the amount of ketose accumulated because of partial transformation to another aldose ester, *i.e.*, glucose-6-phosphate (G-6-P) (reaction 3 in the scheme below).

$$\text{M-6-P} \underset{2}{\overset{1}{\rightleftharpoons}} \text{F-6-P} \underset{4}{\overset{3}{\rightleftharpoons}} \text{G-6-P}$$

SLEIN[4], working with rabbit muscle preparations, reported that an estimation of PMI activity without interference by PGI was possible at pH 5.5 "the optimum for PMI". This procedure cannot be generalized, since we have found that the pH optima for PMI of intestinal mucosa and testicle are in the pH range 7–8[2,3]. Moreover, an interference with the activity of contaminating PGI, unless it were strictly selective and complete, would be inadvisable. A partial interference would make the assay less reliable, since such an interference would tend to be irregular (see below). To decrease variability we have rather preferred to add a large excess of a purified preparation of PGI to approach the theoretical limit of the equilibrium between F-6-P and G-6-P catalyzed by this enzyme. Ketose accumulation in these conditions would be about 1/3 of ketose formation; but this procedure obviously decreases the

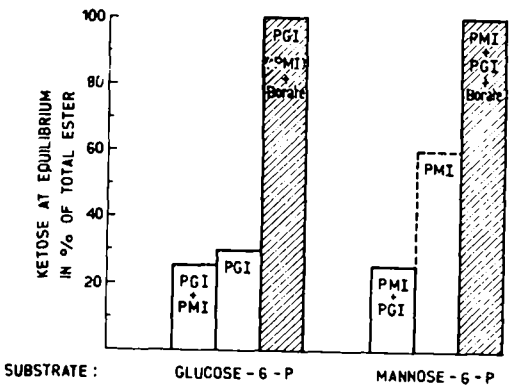

Fig. 1. Percentage of hexose phosphate as ketose at equilibrium catalyzed by phosphohexose isomerases, in the absence (open) and in the presence (shaded) of 0.1 *M* borate. The values without borate are taken from experiments carried out with preparations from several tissues of mammals as well as from yeasts and *Ascaris suis*[2,3]. The equilibrium for PMI alone, which could not be observed experimentally, is based on SLEIN's data[4]. The experiments with borate were carried out with a purified preparation of PGI from yeast free of PMI and a preparation of bull testicle containing both PMI and PGI. Ketose was estimated with resorcinol[6] or cysteine-carbazol[7].

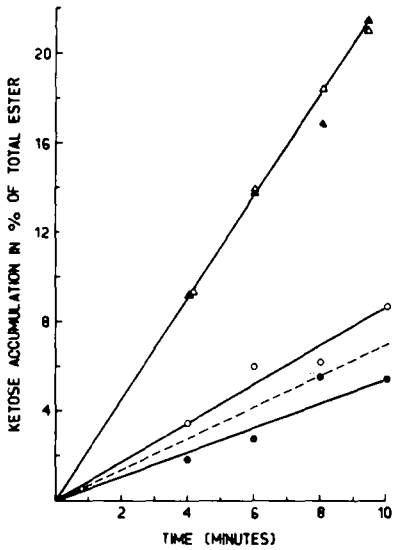

Fig. 2. Assay of PMI activity in the presence of variable amounts of PGI, with and without borate. A purified preparation of bull testicle containing PMI and PGI in the ratio 1:40, 1 μmole M-6-P, 10 μmoles potassium phosphate pH 7.2, and additions as follows: ○ no additions; ● a purified preparation of PGI[3] to change the PMI:PGI ratio to 1:1,200; △ 72 μmoles of borate; ▲ both the PGI preparation and borate, in a total volume of 0.6 ml. Ketose was estimated[7] after incubation at 30° for the stated times. The dotted line represents one third of the maximal PMI activity.

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sensitivity of the assay and its validity is open to question if assay conditions are changed.

To obviate the difficulties related to the presence of PGI it appeared highly desirable to find a method which could entirely eliminate its interference. This has been accomplished on the basis of the ability of borate to complex with F-6-P. Borate had been utilized by COHEN[5] and others to displace the equilibrium in the enzymic isomerization of pentoses.

Within the usual range of pH and substrate concentration 0.1 *M* borate selectively complexes with the F-6-P so completely that the isomerase reaction goes essentially to 100% ketose ester formation from the aldose ester added (Fig. 1). F-6-P is not appreciably isomerized by the phosphohexose isomerases in the presence of borate. Only reactions 1 or 4 in the scheme above are possible in these conditions.

When applied to the assay of PMI activity, borate eliminates interference by any accompanying PGI and gives maximal sensitivity. Both aspects are illustrated by the experiments presented in Fig. 2, which corresponds to the early course of F-6-P appearance from M-6-P catalyzed by PMI, in the presence of variable amounts of PGI with and without borate. The fact that the effect of a 40-fold excess of PGI is not maximal can be attributed to lack of saturation with substrate and perhaps competitive inhibition by M-6-P. On the other hand, borate, by preventing any backward reaction, can give a ketose accumulation appreciably greater than that which could be expected with PMI alone. This fact eliminates the necessity for correction to initial rate as recommended by BODANSKY[8].

It appears that a systematic utilization of borate could be very useful in the assay of isomerases.

SUMMARY

The isomerization catalyzed by phosphohexose isomerases is essentially irreversible in the presence of excess borate, which selectively complexes with fructose-6-phosphate. This trapping effect gives zero order kinetics as long as there is enough substrate to saturate the enzyme. It also makes it possible to assay phosphomannose isomerase without interference by any contaminating phosphoglucose isomerase, and vice versa. A systematic utilization of borate in the assay o isomerases is recommended.

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