position of the atoms attached to the carbon atom is one in which they are staggered with respect to each other.

If II is the favored configuration, the molecule is inactive because it possesses a center of symmetry. Molecules corresponding to III and IV are active, and if IV is inverted, it becomes the mirror image of III; that is, they are enantiomorphs. Accordingly if III and IV should happen to be the most stable configurations, equal amounts of each would lead to a racemic mixture. One would not expect to be able to resolve it into the active components, however, because the barrier preventing free rotation usually would be low, and a molecule having configuration III and passing through configuration I would have an equal chance of returning to its original configuration or to that of its enantiomorph.

From chemical evidence in solution, it appears, in the case of meso 1,2-diaminosuccinic acid,[3] and meso dihydrobenzoin,[4] that the configuration corresponds to that of Fig. II, that is, the staggered position in which like groups are at the greatest possible distance from each other. On the other hand, the dipole moments of meso and racemic stilbene dichloride are 1.27 and 2.75, respectively, and those of meso and racemic dihydrobenzoin are 2.0 and 2.6. If free rotation existed, both meso and racemic forms should have identical moments. Moreover, if the meso stilbene dichloride molecule had a completely trans configuration analogous to Fig. II, the calculated moment is 0.52, while for free rotation it is 2.31.[5] Hence not only is rotation restricted somewhat but a considerable proportion of the molecules must have the unsymmetrical configurations of III and IV.

In the solid state the results of an x-ray investigation of meso erythritol are interpreted as indicating that this molecule has a center of symmetry.[6] However, in the case of anhydrous meso tartaric acid and of the dihydrate of its potassium salt, double molecules are present and the individual molecules are considered to be unsymmetrical.[7]

Therefore it may be concluded that the fact that the molecule has two similar asymmetric carbon atoms of opposite configuration has nothing whatever to do with the inactivity of meso compounds; that is, they are not inactive because of "internal compensation." They are inactive either because the molecules have a center of symmetry as in Fig. II, or because the enantiomorphs corresponding to Figs. III and IV are readily interconvertible, that is, readily racemized.

In the light of the above considerations, it immediately becomes obvious that if the groups on the ethane carbon atoms were large enough, rotation should be restricted sufficiently to permit the isolation of stable forms having configurations III and IV as well as configuration II. An examination of Stuart-type models indicates that such might be the case for α,β-dibromo-α,β-diiodosuccinic acid. Hence when the ethane carbon atoms of this compound have opposite configurations, it should exist in one resolvable racemic form and one meso form having a center of symmetry. In addition there should be three racemic modifications, instead of the usual one, when the ethane carbon atoms have like configurations. Moreover, tetraiodosuccinic acid should exist in a racemic as well as a meso modification. The possibility that these compounds would be chemically stable, however, is remote. Space relationships appear to prevent α,β-di-*ter*-butylsuccinic acid from existing in any configuration except that having a center of symmetry. There is a possibility that the chemical stability of α,α-dibromo-β,β-diiodosuccinic acid may be greater than that of α,β-dibromo-α,β-diiodosuccinic acid, and while the restriction of rotation appears to be less in the first compound, it may be sufficient to permit resolution.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A GLUTAMINE-RICH PEPTONE FOR CULTIVATION OF HEMOLYTIC STREPTOCOCCI

GLUTAMINE has been shown by McIlwain *et al.*[1] and by Bernheimer and Pappenheimer[2] to be a growth factor for various strains of hemolytic streptococci. Lankford and Snell[3] have found glutamine to be of importance also for the cultivation of fastidious strains of gonococci.

Since glutamine is costly and its preparation rather cumbersome, a convenient substitute for this material is desirable. Such a substitute has been found in a peptone prepared by tryptic digestion of gliadin, a protein rich in glutamine. The process is as follows: 2 g of pancreatin (Parke, Davis and Co.) is suspended in 30 cc of water, kept at 37° for 1½ hours,

[1] H. McIlwain, P. Fildes, G. P. Gladstone and B. C. J. G. Knight, *Biochem. Jour.*, 33: 223, 1939.
[2] A. W. Bernheimer and A. M. Pappenheimer, Jr., *Jour. Bact.*, 43: 481, 1942.
[3] Ch. E. Lankford and E. E. Snell, *Jour. Bact.*, 45: 410, 1943.

and then centrifuged. The supernatant liquid is added to a suspension of 10 g gliadin (prepared from wheat gluten by extracting it with alcohol of 70 per cent.[4]) in 200 cc of water. The mixture is kept for one hour under toluene at 37°, then adjusted to pH 7–8 by addition of 0,1 N ammonia, and left over night at 37°. The next day the pH of the mixture is again brought to 7–8 by addition of ammonia. After being kept at 37° for an additional 4–5 hours, the mixture is heated for 15 minutes in a boiling water bath and is then filtered over night in the ice box. The filtrate is concentrated *in vacuo* to a volume of 50–70 cc. It is turbid at first, presumably due to presence of emulsified toluene, and becomes clear during the concentration. In order to avoid foaming during the concentration step, alcohol is constantly added dropwise from a dropping funnel whose tap has been opened to a suitable degree. To the concentrated solution absolute alcohol is added until a strong turbidity is produced. The turbid solution is then poured with stirring into ten volumes of absolute alcohol. The white flocculent precipitate is allowed to settle and is then filtered by suction, washed with absolute alcohol and dried in a vacuum desiccator over sulphuric acid. Yield, 4–4.5. The peptone is free of ammonium salts. On acid hydrolysis it yields 4.61–4.74 per cent. ammonia, which corresponds to a total glutamine content of about 40 per cent. if the small asparagine content of gliadin is neglected).

Gliadin peptone in a concentration of 20–40 mg per cent. effectively replaced glutamine as a growth factor for hemolytic streptococci in the medium of McIlwain *et al.* It has also been found that the gliadin peptone effectively replaces Bacto-peptone "Difco" employed by McIlwain *et al.* The following medium, which is entirely suitable for the growth of the streptococcus, has been adopted by us: glucose 0.5 per cent.; gliadin peptone 1.0 per cent.; NaCl 0.03 per cent.; Na_2HPO_4 12 H_2O 0.5 per cent.; KH_2PO_4 0.035 per cent.; $MgSO_4$ 7 H_2O 0.03 per cent. The substances should be dissolved in the order given, and the solution heated to boiling for ten minutes, filtered and then autoclaved at 15 pounds for 30 minutes. pH = 7.6. After cooling, the following sterile ingredients are added to the basal mixture: riboflavine, calcium pantothenate and thiamine in amounts of 100 micrograms per cent. The results of a typical experiment are shown in Table 1.

It has also been found by us[5] that α-methylamide and α-ethylamide of glutamic acid[6] fail to act as growth factors for hemolytic streptococci in the medium of McIlwain *et al.* McIlwain[7] has previously shown that N-acetylglutamine and a number of glutamine containing dipeptides (leucylglutamine, cysteylglutamine, glutaminylglycine, glutaminylcysteine and glutaminylglutamic acid) fail to replace glutamine as a growth factor for hemolytic streptococci. The results obtained with our peptone indicate therefore either that it contains free glutamine or that it contains peptides of glutamine which can be split hydrolytically by the proteases of the streptococci.

TABLE 1

GROWTH OF STREPTOCOCCUS HAEMOLYTICUS "RICHARDS." GROWTH AFTER 24 HOURS AT 37°

Medium	Photometer reading
McIlwain's medium without glutamine	97*
" " + glutamine 0.03 mg†	64
" " + " 0.1 "	58
" " + gliadin peptone‡ 0.2 "	96.5
" " + " " 1.0 "	75
" " + " " 2.0 "	48
" " + " " 4.0 "	45
Gliadin peptone medium	54

* Growth was measured photometrically. The photometer reading of tubes containing water was adjusted to 100. Sterile medium then gave a reading of 97. Increasing growth is reflected by a decreasing reading.
† Amounts per 10 cc medium.
‡ The pure gliadin peptone solution was autoclaved at 15 pounds for 30 minutes.

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ESTIMATION OF CATHEPSIN ACTIVITY

RECENTLY Plentl and Page,[1] in a study of renin preparations, have stated that the hydrolysis of benzoylargininamide was not followed quantitatively by an increase in titratable carboxyl groups since, with the concentrations of protein required, the large amount of precipitate formed during the titration masked the color change of the indicator. The procedure referred to[2] is that of Grassmann and Heyde.[3] Since this method has become so important in the kinetics of proteinase action,[4] and in order that others might not be discouraged, it seems worth while to point out that the above-mentioned difficulty was encountered in a similar case and circumvented not long ago.[5]

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