# SEROLOGICAL DIFFERENTIATION OF STERIC ISOMERS (ANTIGENS CONTAINING TARTARIC ACIDS)

## SECOND PAPER

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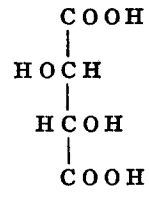
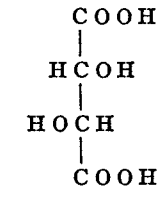
In experiments reported already (1) it has been demonstrated that the presence of optically isomeric groups in two otherwise identical antigens suffices to bring about a difference in their serological properties. The case examined was that of the levo- and dextro-phenyl (paraaminobenzoylamino) acetic acids which after diazotization were combined with proteins. The resulting antigens were distinctly different when tested with the corresponding precipitating immune sera. Considering that steric isomerism presumably is of significance for the specificity of natural antigens, it seemed desirable to extend the studies to other instances and also to proceed to the examination of compounds containing more than one asymmetric carbon atom.

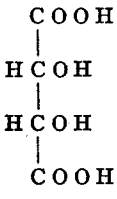
For this purpose the tartaric acids were chosen which, as is well known, contain two asymmetric carbon atoms and which exist in three isomeric forms, namely, levo-, dextro-, and mesotartaric acid, aside from the racemic mixture of *l*- and *d*-acid. The following formulae represent the three forms



levotartaric acid



dextrotartaric acid



mesotartaric acid

A method for attaching these substances to protein was found in

preparing first a compound with paraphenylenediamine in which one of the amino groups is linked to one of the carboxyls of the tartaric acid according to the following formula: $NH_2C_6H_4NHCO\ (CHOH)_2\ COOH$. This substance was diazotized and coupled to protein. After a few unsuccessful attempts to prepare this acyl derivative directly by condensation of tartaric acid with paraphenylenediamine, it was found possible to combine tartaric acid with paranitraniline and to reduce the resulting substance to the amino compound.

## EXPERIMENTAL

Levo- and mesotartaric acids were prepared according to the methods of Holleman (2) and Marckwald (3). The meso acid was obtained in the form of its calcium salt which forms characteristic square crystals easily distinguishable from those of the racemic mixture. From this calcium salt the free acid was isolated by adding less than the calculated amount of a dilute solution of sulfuric acid and allowing the material to stand at room temperature for 1 day. The filtered solution was concentrated to a small volume at 40° by vacuum distillation. The mesotartaric acid crystallized on cooling and was dried *in vacuo* at 45° (M.Pt. after two recrystallizations 142–143°C.)

Frankland and Slator (4) prepared a ditoluidide of tartaric acid $(C_7H_7NHCO)_2(CHOH)_2$ by heating *p*-toluidine (2 mols) and tartaric acid (1 mol) for 10 hours in an oil bath at 180–185°. They established by subsequent hydrolysis of the substance that the tartaric acid had not undergone any appreciable racemization during the process.

Applying a similar method to paranitraniline and using instead of 2 mols only 1 mol of paranitraniline for 1 mol of tartaric acid we found that besides a non-acid product (probably the dinitranilide of tartaric acid) paranitrotartranilic acid ($NO_2C_6H_4NHCO(CHOH)_2COOH$) in good yield was also obtained. Accordingly the derivatives of levo-, dextro-, and mesotartaric acid were prepared by the following process:

15 gm. of tartaric acid were ground with 13.8 gm. of paranitraniline and heated (in a test tube) in a paraffin bath at 170°C. When, after about 5 minutes, bubbles began to form in the molten mass the temperature was lowered to 155–160°. In the case of the levo- and dextro-tartaric acid but not in that of the meso-acid the fluid began to thicken after about 15 minutes and later solidified. After heating for about 40 minutes in all, the mass was broken up by heating with water and a little alcohol, the volume was brought to 600 cc. with water and a solution of NaOH was added until the liquid reacted neutral to litmus. The insoluble material was removed by filtration and the solution was concentrated on the steam bath to a volume of 200 cc. A little insoluble material which separated was filtered off after cooling to room temperature and enough 10 per cent hydrochloric acid was added to make the solution acid to congo red. The paranitrotartranilic acid pre-

cipitated as a light yellow powder which was filtered off, washed with water and dried *in vacuo*. The yield was approximately 12 gm. of the levo- and dextro-compounds and somewhat less of the meso-compound. Recrystallized from water the levo- and dextro acids form fine yellow needles. The meso acid crystallizes in pale yellow microscopic platelets.

Levo-paranitrotartranilic acid $C_{10}H_{10}O_7N_2$. M.Pt. 211–212°.

Analysis: calculated: C 44.44 H 3.70
found: " 44.76 " 3.40

270 mg. neutralized 9.95 cc. 1/10 N NaOH; calculated: 10 cc. A 1% solution in methyl alcohol gave at 22° a rotation of −2.40 in a 2 dm. tube with sodium light, $[\alpha]_D$ −120.

Dextro-paranitrotartranilic acid M.Pt. 212–213°.

270 mg. neutralized 9.93 cc. 1/10 N NaOH; calculated: 10 cc. A 1% solution in methyl alcohol gave at 22° a rotation of +2.40 in a 2 dm. tube, $[\alpha]_D$ +120.

Meso-paranitrotartranilic acid M.Pt. 193–194°.

270 mg. neutralized 9.95 cc. 1/10 N NaOH; calculated 10 cc. A 1% solution in methyl alcohol showed no rotation.

*Reduction of Nitrotartranilic Acid to the Amino Compound.*—20 gm. of crude finely ground para-nitrotartranilic acid were suspended in 1.5 liters of ⅓ normal hydrochloric acid. About 36 gm. of zinc dust were added in small portions over a period of 15 minutes with constant stirring. The paranitrotartranilic acid disappeared gradually within ½ hour. The excess of zinc was filtered off and to the solution which showed faint acidity to congo red, 70 gm. of sodium acetate were added and hydrogen sulfide was passed through until the zinc had been completely precipitated. After filtration the solution was neutralized with sodium hydroxide. In the case of the meso-paraaminotartranilic acid it was neutralized before filtering off the zinc sulfide to avoid a loss of substance by precipitation in the acid solution. The solution was concentrated at 40° by vacuum distillation to a volume of 200 cc. and after removing a trace of insoluble material it was cooled in a freezing mixture and enough concentrated hydrochloric acid was added to make it weakly acid to congo. The paraaminotartranilic acid came out of solution and was filtered off. It was washed with water, alcohol and ether and dried *in vacuo*. The yield was approximately 10 gm. By recrystallization of the levo- and dextro-compounds from 25 parts of water with the addition of some decolorizing carbon they were obtained in the form of microscopic white needles or platelets.

The meso-compound which was almost white was purified by dissolving in water and the required amount of sodium hydroxide, and reprecipitation with acid; white microscopic rectangular platelets after recrystallization from water.

Analyses:

Levo-paraaminotartranilic acid $C_{10}H_{12}O_5N_2$.

240 mg. neutralized 9.85 cc. 1/10 N NaOH; calculated 10 cc.

Kjehldahl nitrogen analysis: Found N. 11.76%; calculated 11.67%.

A water solution containing 480 mg. of the substance and 2.6 cc. of normal HCl in a volume of 15 cc. gave at 25°C. a rotation of −6.31 in a 2 dm. tube; $[\alpha]_D$ −98.6.

Dextro-paraaminotartranilic acid $C_{10}H_{12}O_5N_2$

240 mg. neutralized 9.90 cc. 1/10 N NaOH; calculated 10 cc.

Kjehldahl nitrogen analysis: Found N. 11.55%, calculated 11.67%.

A water solution containing 480 mg. of the substance and 2.6 cc. of normal HCl in a volume of 15 cc. gave at 25°C. a rotation of +6.35 in a 2 dm. tube; $[\alpha]_D$ +99.2.

Meso-paraaminotartranilic acid $C_{10}H_{12}O_5N_2$

240 mg. neutralized 9.90 cc. 1/10 N NaOH; calculated 10 cc.

Kjehldahl nitrogen analysis: Found N. 11.62%; calculated 11.67%.

The amino compounds became dark but did not melt when heated to 285°C.

The meso-paraaminotartranilic acid obtained must consist of a racemic mixture of two optically active compounds since the combination with paranitraniline destroys the symmetry of the molecule. This circumstance does not interfere at all with the conclusions to be drawn from the following experiments and consequently no attempt was made to resolve the product into its components.

The levo-, dextro-, and meso-paraaminotartranilic acids will be designated as *l*-, *d*-, and *m*-acid and likewise the azoproteins prepared from these amino acids and the immune sera obtained by immunization with the azoproteins will be referred to as *l*-, *d*-, and *m*-antigens, and *l*-, *d*-, and *m*-immune sera, respectively.

*Preparation of the l-, d-, and m-Antigens for Immunization.*—The *l*- and *d*-paraaminotartranilic acids were coupled to protein in the following manner:

7.6 gm. were dissolved in 200 cc. water and 85 cc. of normal HCl and diazotized with the required amount of sodium nitrite at a temperature of 0–5°C. with starch iodide paper as indicator. The diazo solution was diluted with ice water to a volume of 1200 cc.

800 cc. of this solution were added to a cold mixture of 500 cc. horse serum and 100 cc. normal sodium carbonate and the mixture, chilled with ice, was kept weakly alkaline to phenolphthalein by adding frequently small quantities of sodium carbonate solution. The coupling proceeded slowly (test with alkaline R salt solution for the presence of free diazo compound) and the diazo compound was used up after about 1½ hours. Then the remaining 400 cc. of diazo solution and 50 cc. of normal sodium carbonate were added (further addition of sodium carbonate and cooling as above). The coupling was finished after about 1 hour. Under the conditions described the meso compound coupled faster but for the sake of uniformity the operations were carried out in exactly the same manner as with the *l*- and *d*- substances.

By acidification with hydrochloric acid the azoprotein was precipitated and after filtration it was dissolved in a small volume of water by addition of a little normal sodium carbonate. It was reprecipitated from this solution with a large quantity of alcohol. The precipitated azoprotein was ground in a mortar to a thin paste, brought up to a volume of 950 cc. with water and the necessary amount of a salt solution to make the salt concentration approximately 1%. As a preservative 50 cc. of 5% phenol solution were added.

*Immunization.*—Three batches of six rabbits each were injected intraperitoneally at weekly intervals. Each rabbit received 12 cc. of the antigen per injection. Test bleedings were made 1 week after the third and the fourth injections. Three to four sera of sufficient strength were obtained in each lot after three or four injections.

*Antigens for the Tests.*—These were prepared in the same way as the antigens for immunization, chicken serum being used instead of horse serum. The azoproteins were precipitated with acid, washed with water and brought into solution by means of sodium carbonate. The quantity of antigen in the solution was determined by precipitation with alcohol and weighing the dried substance. The dilutions given in the tables are in terms of a 5% stock solution.

The intensity of the reaction is indicated as follows: o, f. tr. (faint trace), tr. (trace), ±, +, +±, etc.

The experiments presented in Tables I to III show an almost complete specificity of the *l*- and *d*- antigens. The *l*- and *d*- immune sera give rather weak group reactions with the *m*-antigen; the *m*-immune sera gave practically no group reactions.

Tests were also made with an antigen made from racemic tartaric acid. It was found that this substance, as was to be expected, reacts like a mixture of *l*- and *d*-antigens, *i.e.*, it is precipitated by both the *l*- and *d*-immune sera like the homologous antigens only somewhat weaker according to the lower concentration of the respective homologous antigens. With the *m*-immune sera there were only faint reactions similar to those of the *l*- and *d*-antigens. These results with the racemic preparation are not tabulated.

Other immune sera, namely, two *l*-, three *d*- and three *m*- sera gave results entirely in agreement with those presented in the tables.

In the following experiments presented in Tables IV,*a*, IV,*b*, and IV,*c*, the inhibiting effect on the precipitin reaction of the tartaric acids, the paraaminotartranilic acids and some other substances used for

TABLE I, *a*

To 0.2 cc. of the diluted antigens (prepared with chicken serum) were added 2 capillary drops of *l*-immune serum.

| Readings taken after: | *l*-Antigen Dilution 1: | | | | *d*-Antigen Dilution 1: | | | | *m*-Antigen Dilution 1: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 |
| 1 hr. at room temperature | ± | + | + | tr. | 0 | 0 | 0 | 0 | 0 | tr. | 0 | 0 |
| 3 hrs. at room temperature | + | +± | +± | tr. | 0 | 0 | 0 | 0 | f. tr. | tr. | f. tr. | 0 |
| Night in ice box | +± | ++ | ++ | + | 0 | 0 | f. tr. | 0 | ± | ± | tr. | 0 |

TABLE I, *b*

To 0.2 cc. of the diluted antigens were added 4 capillary drops of *l*-immune serum.

| Readings taken after: | *l*-Antigen Dilution 1: | | | | *d*-Antigen Dilution 1: | | | | *m*-Antigen Dilution 1: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 |
| 1 hr. at room temperature | + | +± | + | tr. | 0 | 0 | 0 | 0 | f. tr. | tr. | f. tr. | 0 |
| 3 hrs. at room temperature | + | ++ | +± | ± | 0 | 0 | 0 | 0 | tr. | tr. | tr. | 0 |
| Night in ice box | ++± | +++ | ++± | + | 0 | f. tr. | 0 | 0 | ± | + | tr. | 0 |

TABLE II, *a*

To 0.2 cc. of the diluted antigens were added 2 capillary drops of *d*-immune serum.

| Readings taken after: | *l*-Antigen Dilution 1: | | | | *d*-Antigen Dilution 1: | | | | *m*-Antigen Dilution 1: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 |
| 1 hr. at room temperature | 0 | 0 | 0 | 0 | tr. | + | + | tr. | 0 | tr. | f. tr. | 0 |
| 3 hrs. at room temperature | 0 | 0 | 0 | 0 | tr. | + | + | ± | f. tr. | tr. | f. tr. | 0 |
| Night in ice box | 0 | 0 | f. tr. | 0 | ± | ++ | ++ | +± | ± | ± | tr. | 0 |

TABLE II, *b*

To 0.2 cc. of the diluted antigens were added 4 capillary drops of *d*-immune serum.

| Readings taken after: | *l*-Antigen Dilution 1: | | | | *d*-Antigen Dilution 1: | | | | *m*-Antigen Dilution 1: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 |
| 1 hr. at room temperature | 0 | 0 | 0 | 0 | + | +± | + | tr. | tr. | tr. | f. tr. | 0 |
| 3 hrs. at room temperature | 0 | 0 | 0 | 0 | + | +± | + | tr. | tr. | tr. | tr. | 0 |
| Night in ice box | 0 | 0 | 0 | 0 | ++± | +++ | ++± | + | + | + | tr. | 0 |

TABLE III, *a*

To 0.2 cc. of the diluted antigens were added 2 capillary drops of *m*-immune serum.

| Readings taken after: | *l*-Antigen Dilution 1: | | | | *d*-Antigen Dilution 1: | | | | *m*-Antigen Dilution 1: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 |
| 1 hr. at room temperature | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ± | + | + | ± |
| 3 hrs. at room temperature | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ± | + | +± | ± |
| Night in ice box | 0 | 0 | f. tr. | 0 | 0 | 0 | 0 | 0 | ++ | ++± | ++± | +± |

TABLE III, *b*

To 0.2 cc. of the diluted antigens were added 4 capillary drops of *m*-immune serum.

| Readings taken after: | *l*-Antigen Dilution 1: | | | | *d*-Antigen Dilution 1: | | | | *m*-Antigen Dilution 1: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 | 20 | 100 | 500 | 2500 |
| 1 hr. at room temperature | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ± | + | + | tr. |
| 3 hrs. at room temperature | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ± | ++ | +± | ± |
| Night in ice box | 0 | f. tr. | f. tr. | 0 | 0 | 0 | 0 | 0 | ++± | +++ | +++ | + |

TABLE IV, *a*

*l-Antigen and l-Immune Serum*

| Readings taken after: | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 mins. at room temperature | f. tr. | ± | tr. | 0 | ± | f. tr. | + | ± | ± | + | + | + | + |
| 3 hrs. at room temperature | ± | + | ± | 0 | + | ± | +± | + | + | +± | +± | +± | +± |
| Night in ice box | + | ++ | + | 0 | + | ± | ++ | +± | +± | ++ | ++ | ++ | ++ |

TABLE IV, *b*

*d-Antigen and d-Immune Serum*

| Readings taken after: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 mins. at room temperature | ± | 0 | tr. | tr. | 0 | f. tr. | + | ± | tr. | ± | + | + | + |
| 3 hrs. at room temperature | + | ± | + | ± | 0 | tr. | +± | + | ± | +± | +± | +± | +± |
| Night in ice box | +± | + | ++ | ++ | 0 | ± | ++ | ++ | +± | ++± | ++± | ++± | ++± |

TABLE IV, *c*

*m-Antigen and m-Immune Serum*

| Readings taken after: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 mins. at room temperature | + | + | tr. | tr. | ± | 0 | + | ± | ± | ± | ± | + | + |
| 3 hrs. at room temperature | +± | +± | ± | + | + | 0 | +± | +± | +± | +± | ++ | +± | ++ |
| Night in ice box | ++± | ++± | +± | ++± | ++ | 0 | ++± | ++ | ++ | +++ | ++± | +++ | ++± |

*The numbers 1 to 12 refer to the substances tested for inhibition.

comparison was tested. The compounds are enumerated as follows: (1) levo-tartaric acid, (2) dextro-tartaric acid, (3) meso-tartaric acid, (4) levo-paraaminotartranilic acid, (5) dextro-paraaminotartranilic acid, (6) meso-paraaminotartranilic acid, (7) succinic acid, (8) l-malic acid, (9) d-l-malic acid, (10) lactic acid, (11) benzoic acid, (12) acetic acid.

0.2 cc. of the antigen (diluted 1:500) were mixed with 0.05 cc. of a neutral solution containing 1 millimol in 10 cc. of the substances indicated. To this 4 capillary drops of the homologous immune serum were added. The control tube contains only antigen and immune serum.[1]

TABLE V

The inhibiting substances used were: (4) *l*-paraaminotartranilic acid, (5) *d*-paraaminotartranilic acid, (6) *m*-paraaminotartranilic acid, (13) *l*-paranitrotartranilic acid, (14) *d*-paranitrotartranilic acid.

| | Readings taken after | 4 | 5 | 6 | 13 | 14 | Control |
|---|---|---|---|---|---|---|---|
| *l*-antigen and *l*-immune serum | 15 mins. at room temperature | .0 | ± | tr. | 0 | ± | + |
| | 3 hrs. at room temperature | 0 | + | ± | 0 | + | +± |
| | Night in ice box | 0 | +± | +± | 0 | +± | ++ |
| *d*-antigen and *d*-immune serum | 15 mins. at room temperature | ± | 0 | tr. | tr. | 0 | + |
| | 3 hrs. at room temperature | + | 0 | ± | ± | 0 | +± |
| | Night in ice box | +± | 0 | +± | +± | 0 | +± |
| *m*-antigen and *m*-immune serum | 15 mins. at room temperature | ± | ± | 0 | tr. | ± | ± |
| | 3 hrs. at room temperature | + | + | 0 | + | + | +± |
| | Night in ice box | +± | +± | 0 | +± | +± | ++ |

Table V represents inhibition tests with *l*-, *d*- and *m*-paraaminotartranilic acid and *l*- and *d*-paranitrotartranilic acid where 0.05 cc. of a neutral solution containing in 10 cc. 0.25 millimol of these substances was added to the antigen. The *m*-paranitrotartranilic acid could not be used since a precipitation occurred on mixing with saline solution.

## DISCUSSION AND SUMMARY

The experiments reported confirm the results of our previous studies and demonstrate again the striking influence of the steric constitution

[1] In the details given for the tests in a previous paper (1), Table IV *c*, it should read "0.5 millimol in 10 cc."

on serological properties. It would seem that in this respect the specificity of serum reactions is analogous to that of ferments. However, while in the investigations with ferments one is limited to those found in nature, by our method antibodies can be produced at will, which act on chosen substances.

In particular one sees that the change in the spatial configuration with regard to one asymmetric carbon atom is sufficient to cause a pronounced serological difference, as appears from a comparison of the reactions of either the *l*- or *d*-antigen with those of the *m*-antigen. Since the change of the levo- into the dextro-acid would involve a rearrangement of the groups around both asymmetric carbon atoms one may suppose that there is a greater serological difference between these two acids than between either and the *m*-tartaric acid. This reasoning is supported by the fact that the *l*-immune sera showed almost no reactions on the *d*-antigen and vice versa while there were weak group reactions of the *l*- and *d*-immune sera with the *m*-antigen. A question which requires further investigation is whether the marked serological distinction between steric isomers depends on the nature of the radicals connected with the asymmetric carbon atom, *i.e.*, whether any radical will produce an effect of the same order as polar groups like COOH or OH (*c.f.* Reiner (5)).

The fact that the specificity of the reactions is entirely determined by the substances linked to the protein and not by the protein molecule itself is in the first place due to the method of testing, namely, the use of a protein different from that employed for immunization. Besides one has to consider that, owing to the considerable number of tyrosine and histidine groups with which the azocompounds can combine, one may visualize the protein molecules, studded, as it were, with the groups artificially introduced.

The application of the findings presented, to the problem of specificity in natural antigens, especially those of carbohydrate nature, is suggested because the chemical constitution of tartaric acid is closely allied to that of the sugar acids which, from the work of Avery, Heidelberger and Goebel are known to form essential parts of the bacterial carbohydrate haptens. Since these haptens are high molecular compounds consisting of structurally different combinations of sugars and various carbohydrate acids it is easily conceivable

that there exists an almost unlimited number of such specific substances.

A special point is brought out by the inhibition tests. These tests distinguish definitely between the uncombined *l*- and *d*-tartaric acids themselves, while in contrast to the direct precipitin reactions there is no definite distinction between *l*- or *d*- and the *m*-tartaric acid, in the tests with *l*- or *d*-immune serum. The *m*-immune serum however differentiates clearly the *m*-tartaric acid from the two other acids. In comparison with the simple tartaric acids a considerably greater inhibiting effect and a more marked specificity similar to that of the precipitin tests is exhibited by the amino- or nitrotartranilic acids. This indicates that the antibodies involved do not act upon the tartaric acid part of the molecule only but attach themselves also to the aromatic nucleus and hence may be supposed to possess several distinct binding groups. This view suggests a further study of substances of complicated chemical structure.

In the present connection it is pertinent to state that in general the conclusions drawn from the inhibition tests are based upon reactions with substances whose chemical constitution is known in its entirety, and that of course proteins play no part whatsoever in the specificity of these phenomena.

## SUMMARY

In continuation of studies on the stereochemical specificity of serum reactions, antigens were examined containing the acyl radicals of the levo-, dextro-, and meso-tartaric acids. It was found that in this case also, immune sera can readily be obtained which differentiate sharply the three antigens identical in every other respect but possessing stereoisomeric groups.

Since the tartaric acids by their chemical constitution belong to the same class of substances as sugar acids the results have a bearing upon the question of the specificity of natural antigens containing carbohydrates such as have been described by Avery and Heidelberger.

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