FINAL REPORT

CONTRACT NAS2-4310

STUDY, STERILIZATION AND STORAGE COMPATIBILITY OF GROWTH MEDIA FOR EXTRATERRESTRIAL USE

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ABSTRACT

Because of the requirement of dry-heat sterilization for interplanetary spacecraft, an investigation was initiated to assess the scope of the problem presented by the thermal instability of substances important to extraterrestrial life detection experiments. Ninety-four substances were exposed to drastic thermal conditioning. Following heating, thirty-seven of the substances were found to pass preliminary screening tests designed to detect degradation. Twenty of the 57 substances not passing the screening tests were not grossly degraded.

The substances were individually packaged under vacuum in borosilicate glass ampoules. Before packaging most substances were dried in a vacuum. After drying the substances were placed in ampoules which were flushed with nitrogen, evacuated and sealed.

The thermal processing consisted of two separate 92-hour heating periods at 135°C. After the second thermal treatment, the specimens were examined by a preassigned sequence of physical and chemical tests designed to detect degradation. Once significant degradation was detected, the test sequence was terminated for that substance.

Candidate substances were selected on the basis of their importance in microbiological growth media, as spore germinating agents, as substrates for metabolic assays and as substances characteristic of major biochemical classes. The forms of the substances tested were chosen on the basis of physical properties and purity of commercially available forms.

Results of tests completed suggest further investigations will find additional evidence of degradation in the heated test substances, but they also suggest that choice of other forms of some of the degraded materials and other packaging conditions will permit them to survive the thermal process used.

FOREWORD

This report describes the technical approach and results of an investigation to explore the sterilization and storage compatibility problems of microbiological growth media substances of potential use in the biological exploration of the planets. The investigation was performed by the Space and Re-entry Systems Division of Philco-Ford Corporation under Contract NAS2-4310 with the Ames Research Center of the National Aeronautics and Space Administration. The program was directed by Dr. John B. Opfell of SRS. J. D. Albert, D. E. Gelvin, J. W. Mason, T. A. Oda, and E. R. Walwick of the Philco-Ford, Aeronutronic Applied Research Laboratories performed the laboratory investigations and are the principal authors of this report.

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SECTION I

INTRODUCTION

This report describes an investigation of the thermal stabilities of substances important to extraterrestrial life detection experiments. The program was a preliminary study designed to assess the problem presented by the inclusion of biological test materials in planetary-impacting spacecraft which require terminal dry-heat sterilization. The thermal conditions chosen for this study were more severe than would be applied in the sterilization of actual planet-bound material. Substances were sealed in glass *in vacuo* and exposed to a temperature of 135°C for 2 heating periods of 92 hours each.

Candidate substances were selected on the basis of their importance in microbiological growth media, particularly for growth of soil microorganisms, as spore germinating agents, as substrates for metabolic assays and as substances characteristic of major biochemical classes.

Since this was a preliminary investigation detailed descriptions of materials, equipment, and procedures, useful in subsequent investigations are reported.

The literature was reviewed to compile information on the thermal stability of the substances chosen for study. However, little applicable thermal degradation information was found, except for inorganic compounds.

After heating, the thermally treated specimens of each candidate substance were compared with the unheated control specimens by a sequence of both physical and chemical tests. The first group of tests were simple physical tests designed to identify those substance which had been grossly degraded by the thermal treatment. Subsequent tests were more specific and were designed to quantify the degradation. Sufficient time was not available to accomplish this goal in its entirety.

SECTION 2

SELECTION OF CANDIDATE SUBSTANCES

2.1 LIST OF SUBSTANCES FROM LITERATURE SURVEY

A review of the literature to determine those substances that are particularly useful in microbiological growth media was carried out. Those selected have been used extensively in growth media for soil microorganisms, in classical microbiological growth media, or in spore germinative media. A listing of the substances appears in Appendix A. Excluded from the list were substances used in enrichment media for selective culture.

2.2 CHOICE OF CANDIDATE SUBSTANCES

The substances listed in Appendix B were selected as candidates for testing from a compilation of substances used in microbiological growth media (Appendix A), or were included at the request of NASA Ames Research Center. The list of candidate substances was limited to that number which could be procured and handled adequately in terms of the present program. The selection of test substances was based on their: (a) extent of use in general growth media, (b) irreplaceable nature as growth requirements for microorganisms, (c) cost being reasonable in relation to funds available, and (d) estimated physical stability to thermal degradation under the thermal processing conditions. Inorganic salts which were estimated to be stable were not included (see Appendix C, Table I).

Selection of the most useful chemical form of each substance was made after evaluation of physical properties, purity, and cost of available forms. Preferred forms were anhydrous and had high water solubility, high melting point, purity grades of A.C.S., N.R.C., or U.S.P., and low cost.

SECTION 3

LITERATURE REVIEW

The original purpose of the literature survey was to collect applicable data from laboratory studies on thermal decomposition of the candidate substances. The information obtained was to be used as an aid in constructing test sequences to assess degradation. Even anticipating that little experimental work had been done under similar conditions (135°C and vacuum), it was surprising to find so little information useful to the present effort. The one exception was the data collected on inorganic compounds, which was useful in selecting procedures to detect degradation. The information collected for the organic compounds contributed little to the selection of test procedures. Most procedures which were found would have been proposed on the basis of a fundamental understanding of organic chemistry. The failure of the literature search to uncover much applicable information does serve to justify the need for the present research program.

The literature survey covered the period from 1920 to 1962. Key phrases, in addition to the candidate substances, included: decomposition of, heat of degradation of, heat effects of, thermal stability of. The literature search was limited to the Chemical Abstracts, from which more than 250 papers were located.

Information collected during the search included the identification of products and intermediates formed during pyrolytic, bacterial, oxidative, photolytic, and hydrolytic degradation studies, methods employed in detecting decomposition, and distinctive properties of degraded materials that could be utilized to detect the beginning stages of decomposition. However, most of the data encountered in the literature included decomposition temperatures of compounds, the catalytic effects of contaminants on degradation, thermal studies at a particular temperature and time period,

or the relative stabilities of various compounds at fixed temperatures. Thermal decomposition temperatures could not be used to predict the thermal behavior of candidate substances in the present study unless they greatly exceeded the temperatures of interest, 135°C.

3.1 AMINO ACIDS

No thermal degradation studies were found for amino acids, which yielded information concerning degradation products. Photolysis studies (1,2) were encountered that gave evidence indicating the following degradation products were produced: amines with one carbon less than parent amino acids, alcohols, aldehydes, CO_2, CO, and NH_3, but not hydroxy- or keto-acids. The photolysis studies could only be used as a rough guide in choosing tests for detection of degradation.

3.2 PROTEINS

Sodium caseinate was reported to be dehydrated below 153°C in 2 hours, and to degrade extensively above 153°C with loss of amine and basic groups (3). At approximately 300°C, CO_2, O_2 and CO were formed when casein was dry distilled (4). Loss of nitrogen was found to be a function of time and temperature. At 125°C and up to 16 hours, the loss of nitrogen was nearly linear (5). These data were useful in considering tests for caseinate and suggested that changes in solubility and aggregation would be important in evaluating degradation. They would also be useful in considering this protein as a source of amino acids after hydrolysis of the heated caseinate. However, because of the great effort required to determine the amount of each individual amino acid which survived the heating and hydrolysis it was considered expeditious to determine how well the caseinate survived the heating. If it were grossly degraded, further investigation would not be warranted.

3.3 CARBOHYDRATES

Pyrolysis studies of sucrose, glucose, and lactose reported that with temperatures up to 500°C degradation products included CO_2, CO, C_nH_{2n}, and CH_4 and H_2 (6). These data confirmed that chromatography would be a definitive test for this group of substances and that the screening tests (especially Differential Refractometry) would be appropriate. A chromatographic test for the detection of thermally produced changes in potato starch was described in the literature (7). Accuracy of the test was such that 0.6 mg of decomposed starch in 10 ml of water (0.006% solution) became visible on an alumina column.

3.4 ALCOHOLS AND POLYOLS

Acetaldehyde, hydrogen, and formaldehyde were reported as pyrolysis products of ethanol at 576-624°C (8). These results suggested degradation routes that could be evaluated by the test sequences.

3.5 LIPIDS

It was reported that when linoleic acid was heated in a vacuum in the presence of active nickel, the density and the refractive index increased and the iodine value decreased. Without active nickel there was very little change (9). Two of the analysis procedures employed for the tests reported above were included in the test sequence.

3.6 VITAMINS

Data on vitamins of even limited value was not encountered in the literature search.

3.7 INORGANIC SALTS

3.7.1 SODIUM THIOSULFATE

The thiosulfates of the alkaline metals, on being heated in the absence of air, are changed into sulfate and polysulfide, and the latter into sulfide and sulfur: $4Na_2S_2O_3 \rightarrow 3Na_2SO_4 + Na_2S_5 \rightarrow 3Na_2SO_4 + Na_2S + 4S$ (10).

3.7.2 POTASSIUM BICARBONATE

Thermal decomposition of $KHCO_3$ was studied at 140-200°C by means of a thermal balance. The results indicate that decomposition takes place according to $KHCO_3 \rightarrow 5KHCO_3 \cdot K_2CO_3 \rightarrow 2KHCO_3 \cdot K_2CO_3 \rightarrow KHCO_3 \cdot K_2CO_3 \rightarrow K_2CO_3 + H_2O + CO_2$ (11).

3.7.3 AMMONIUM MOLYBDATE

Differential thermal analysis studies on various forms of ammonium molybdates show that thermal dissociation begins with the removal of water, followed by removal of ammonium hydroxide. The end product in each case is MoO_3 (12).

3.7.4 MANGANOUS SULFATE

Manganous sulfate pentahydrate gave $MnSO_4 \cdot H_2O$ at about 300°C, $MnSO_4$ at 800°C and finally Mn_2O_4 at 900°C (13). Manganous sulfate yields MnS at 750-900°C, and MnO at 650°C on heating 15 minutes with 0-32.5% C in a current of Ar containing not more than 0.03% oxygen (14).

3.7.5 FERROUS SULFATE

This salt decomposed completely at 750°C (in Ar) to form Fe_2O_3, SO_2, and SO_3 (14).

3.7.6 SODIUM PHOSPHATE, DIBASIC

This substance is dehydrated at temperatures above 240°C directly to the metaphosphate with no indication of an intermediate (15).

3.7.7 AMMONIUM PHOSPHATE, DIBASIC

This substance decomposes with the loss of 2 moles of NH_3 and 2 moles of H_2O. Metaphosphoric acid is always isolated as the semi- or monohydrate (15).

3.8 MISCELLANEOUS

A kinetic study of the decomposition of sodium formate was reported (16). The results indicated two main reactions:

$$2HCOONa \rightarrow Na_2C_2O_4 + H_2$$

$$2HCOONa \rightarrow Na_2CO_3 + H_2 + CO$$

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SECTION 4

PROCESSING OF CANDIDATE SUBSTANCES

4.1 PROCUREMENT AND STORAGE OF MATERIALS

4.1.1 ALL GLASS CONTAINERS (AMPOULES)

A number of different types of glass containers were tested for durability and ease of handling. The container selected for use was the LG 6770, 10-ml drying ampoule, made by Lab Glass, Inc., Vineland, New Jersey. When received, the ampoules were marked with code numbers, washed, and stored in a covered container until used (refer to Appendix D for the configuration, composition, and preparation of the all glass container.)

4.1.2 CANDIDATE SUBSTANCES

Several sources were considered for each candidate substance. Final choice was based on cost and availability of substances of specified purity. Identification of the procured candidate substances consisted of inspection of the container labels to insure that the material received was that specified. Appendix B gives the source, cost, amount purchased, and manufacturer's lot number for each candidate substance.

Substances were stored in the original, sealed containers, in the dark at room temperature until they were prepared for packaging.

4.2 PREPARATION OF INDIVIDUAL SPECIMENS PRIOR TO THERMAL PROCESSING

4.2.1 PREPARATION OF CANDIDATE SUBSTANCES

Since it was considered a disadvantage to have water present in the test

substances, most of them were dried to constant weight in a vacuum oven or vacuum desiccator. Substances were dried at 80°C or at room temperature (23°C). Vacuum desiccators contained P_2O_5 and vacuum pumping was continuous on desiccators and oven during the drying process. Drying conditions for each substance are given in Appendix B. Liquids received no prepackaging treatment.

4.2.2 PACKAGING

Each candidate substance was assigned an identification number. The four replicate specimens of each substance were labeled A, B, C, and D.

Four containers (ampoules) with proper identification numbers were weighed on the Mettler H15 balance. Weights were recorded to the nearest 0.0001 g. A predetermined amount of the prepared test substance was placed in each of the ampoules. Whenever possible approximately 5 g were used. Use of smaller amounts was required when: (a) high cost necessitated purchase of some substances in amounts less than 20 g, (b) 5 g of a substance would have exceeded the capacity of the ampoule, or (c) difficulty was encountered in evacuating ampoules containing finely powdered substances. The weight of one of the heated specimens of each test substance is given in Appendix C, Table I.

Stems of the filled ampoules were heated and drawn out forming a constriction about 3 cm above the shoulder. The constricted section had an inside diameter of approximately 3 mm and a length of 2 cm. They were then connected to a vacuum manifold, evacuated to a pressure of 0.1 mm Hg and returned to atmospheric pressure by filling the manifold with dry nitrogen. After flushing twice with nitrogen the ampoules were evacuated, held at a pressure of 0.1 mm Hg for about 5 minutes, then sealed by melting the glass at the constriction in the stem. The time required to achieve equilibrium with the 0.1 mm Hg vacuum varied with the nature of the test substance. Finely powdered substances presented the greatest difficulty, due to a tendency to escape from the ampoule as the pressure was reduced. In some cases up to an hour was required to reach the 0.1 mm Hg pressure.

Substances not packaged according to the above procedure were ethanol (44), glycerin (45), and beef extract (77). Ampoules containing ethanol were covered with a rubber cap and dipped in liquid nitrogen. When the ethanol was frozen the ampoules were quickly sealed by melting the glass in the stems. Glycerin was packaged in the usual manner except that evacuations were to a pressure of 3 mm Hg instead of 0.1 mm. Beef extract was frozen in liquid nitrogen prior to evacuation of the ampoules.

All specimens were stored in the dark at room temperature.

Pressure measurements during the packaging process were made with a McLeod Vacuum Gauge (Virtis Company, Inc., Gardiner, New York). The gauge and the ampoules being evacuated were attached to the vacuum manifold through stopcocks and rubber sleeves. Distance of the ampoules from the manifold was approximately 15 cm. The most severe constriction in the connecting tubing was the stopcock, with a bore 3 mm in diameter and 17 mm long.

4.3 THERMAL PROCESSING AND STORAGE OF PROCESSED SPECIMENS

4.3.1 PROCESSING LOT

Two of the four replicate specimens of each candidate substance were assigned to a processing lot. Each lot consisted of about 40 specimens which were exposed to the thermal process at one time. The other two replicate specimens remained in storage at room temperature. Appendix B shows which of the replicate specimens were exposed to the thermal process and the number of the processing lot to which they were assigned. Five heating lots were used to process the required number of specimens of the 94 candidate substances.

4.3.2 THERMAL PROCESS

The specimens of each processing lot were placed in an aluminum-block tube heater (see Appendix E) which had been stabilized at 135°C. The prescribed 92-hour heat soak period began when the temperature of the specimens reached 133°C. Specimens were held at 135°C ± 2°C for 92 hours ± 30 minutes. At the end of the first 92-hour heat soak, the processing lot was transferred to a test tube rack and allowed to cool to room temperature. Specimens were stored in the dark at room temperature between heat soaks. After about 2 days of storage the lot of specimens was placed in the temperature-stabilized heating block for a second heat soak at 135°C ± 2°C for 92 hours ± 30 minutes. At the end of the second heat soak the specimens were allowed to cool to room temperature in a test tube rack and were stored in the dark at room temperature.

4.3.3 OPENING SPECIMENS FOLLOWING THERMAL PROCESSING

The sealed specimens were weighed before opening to determine if the containers leaked during heating or storage. A possibility existed that containers which had been heated were under pressure resulting from decomposition of test substances. Therefore, all specimens were cooled in liquid nitrogen before opening to condense any decomposition gases which may have been present. A horizontal scratch about 2 mm long was made on each container about 0.5 cm below the shoulder, and a small drop of water applied to the scratch. A hot glass rod pressed firmly against the glass near the scratch caused a crack to form around the container at the level of the scratch. The top of the container was then removed by applying slight pressure with the fingers. After opening, the containers were

covered with a tight fitting rubber cap. During the opening procedure, the specimens were held in a stream of dry nitrogen to minimize entry of air into the opened containers. When the specimens had warmed to room temperature and condensed vapors had dried, the weighing to determine loss of volatiles was performed. Opened specimens were stored in the dark, at room temperature, in screw top jars containing a desiccant.

SECTION 5

TESTS

5.1 TEST PROTOCOLS

5.1.1 TEST I COLOR CHANGE

Specimens were inspected in their closed ampoules under illumination provided by 16 Sylvania Lifeline No. F30T12-CW-RS fluorescent lamps located in a 9-by-13-foot ceiling which is 6 feet above the inspection station. The color was measured by comparison of the specimen with standard color swatches in the Munsell Book of Color (Glossy Finish Collection)*. This set of standard swatches represent color in terms of 9 units of value, 18 units of chroma, and 40 units of hue. If the color of the heated specimen differed more than 0.5 units of value, 1 unit of chroma, or 2.5 units of hue from that of the unheated control, the material was considered degraded and no further tests were performed on it.

5.1.2 TEST II PHASE CHANGE

Specimens were inspected in their closed ampoules. If either of the following phase changes occurred as a consequence of the heating processes, the material was considered degraded and no further tests were performed on it:

a. The heated material was a liquid whereas the unheated control was a solid, or conversely, the heated material was a solid whereas the unheated control was a liquid.

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* Munsell Color Company, Inc., Baltimore, Maryland

b. The heated material was a slush or a solid cake (as distinct from cemented particles) whereas the unheated control was a freely flowing powder or mass of crystals.

5.1.3 TEST III LOSS OF VOLATILES

Specimens producing volatiles were considered degraded and no further tests were performed on them. Specimens were considered to have produced volatiles as a result of the heating processes if they showed one of the following effects:

a. The ampoule exploded spontaneously during or after the heating process or during the attempt to open it. (The sample was usually lost in the explosion.)

b. After opening the ampoule the specimen lost more than 0.3% of its weight. Corrections were made for weight changes due to buoyancy and the opening operation (notching).

5.1.4 TEST IV PARTICLES IN SOLUTION

Measured quantities (not more than 50% of the specimen) of the heated specimen and of the unheated control material were each dispersed in enough Millipore-filtered water (Purified Water, U.S.P.) to make solutions which were about 50% saturated. Both solutions were diluted to identical concentrations.

Both solutions were inspected under strong white light against both a flat-black and a flat-white background. If the number of particles visible in the solution from the heated specimen was more than 3 times that in the solution from the unheated control specimen, the material was considered to be degraded and no further tests were performed on it.

5.1.5 TEST V pH CHANGE

This protocol was proposed for use in this work but time did not permit its accomplishment.

Measured quantities of the heated specimen and of the unheated control material would be dispersed in enough Millipore-filtered water (Purified Water, U.S.P.) to make 50% saturated solutions. Both solutions would be diluted to identical concentrations and the pH determined either with a pH meter or pH indicator paper. Each solution would be sampled twice with the first sample diluted 50% with water and the second with 0.001 N HCl. If the pH difference between the samples prepared from the heated material differed from that of the samples prepared from the unheated control by more than 1 unit, the material would be considered degraded and no further tests performed on it.

5.1.6 TEST VI DIFFERENTIAL REFRACTOMETRY

The solutions prepared for detection of particles in solution (Test IV) served as samples for measurement of differences in index of refraction. The measurements were made in a Brice-Phoenix, Model BP-2000-V, Differential Refractometer. This instrument has a sensitivity in n_D of 0.0000013, and the sensitivity of the method is illustrated by the following list of changes in the refractive index of water as solutes are introduced or the temperature of measurement is changed:

Pure water, n_D^{20} 1.33303
1% Glycerol (aq), n_D^{20} 1.33416
1% Mannitol (aq), n_D^{20} 1.33440
1% Dulcitol (aq), n_D^{20} 1.33441
Pure water, n_D^{25} 1.33251
2.5% Acetic Acid (aq), n_D^{25} 1.33427

If the difference between the refractive indexes of solutions prepared from the heated and control specimens was greater than that between two controls, one which is 1% more dilute than the other, the material was considered to have been degraded and no further tests were performed on it.

5.1.7 TEST VII X-RAY DIFFRACTION ANALYSIS

The crystal structures of the heated and control specimens were studied with a Philips Norelco X-ray Diffractometer, which used copper Kα-rays. If the difference in the diffraction patterns indicated more than a 1% change between the heated and control specimens the material was considered degraded and no further tests were performed on it.

5.1.8 TEST VIII MELTING POINT DETERMINATION

Melting point differences between heated and control specimens were measured with an Electrothermal Melting Point Apparatus* consisting of an electrically heated block which accommodated three capillary melting point tubes. Simultaneous readings were made on samples of (a) heated specimen, (b) control specimen, and (c) a mixture of heated and control materials.

If there was a difference in melting ranges greater than $3^{\circ}C$, the material was considered degraded and no further tests were performed on it.

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* Electrothermal Engineering, Ltd., London, England

5.1.9 TEST IX BARIUM CARBONATE PRECIPITATION

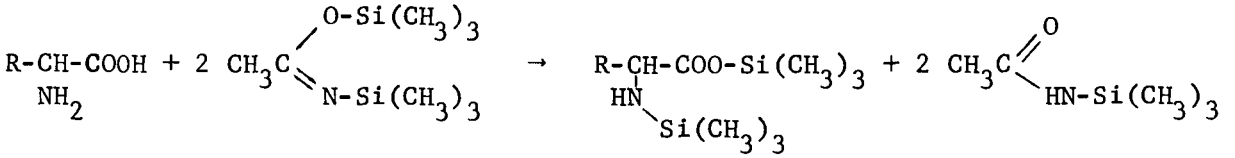
Tests for carbonate ion formation were made on ten-milliliter portions of 1% aqueous solutions of the heated and control specimens. Each solution was treated with six drops of 0.05 Normal barium chloride solution.

If the number of particles visible in the solution of the heated sample was more than twice that in the solution of the control sample, the material was considered to have been degraded and no further tests were performed on it.

5.1.10 TEST X GAS CHROMATOGRAPHY

This protocol was proposed for use in this work, but time did not permit its accomplishment.

Gas chromatography would be performed, either directly on test substances or their solutions, or after conversion of test substances to more volatile trimethylsilyl derivatives. In the case of the amino acids the latter route would be taken:



A sample of the test substance is dissolved in a nonaqueous solvent and treated with N,O-bis-(trimethylsilyl)acetamide (BSA), a silylating agent, and the reaction mixture is injected into the gas chromatograph column. By comparing chromatographic peaks of both control and heated specimens the presence of degradation products can be ascertained.

5.2 TEST SEQUENCE

The test sequences indicated in Appendix F for each of the candidate substances were designed principally to detect decomposition products but were not intended to identify these degradation products. Selection of these test sequences was based upon our scientific judgement or, where appropriate, the decomposition studies reported in the literature. However, since each type of test varies in sensitivity, one test in a given sequence may be more conclusive for one substance than another. Consequently, it occasionally could be necessary to complete the entire sequence of tests before conclusions regarding stability of a given substance can be made. Each test was conducted on samples from both control and heated specimens. Direct comparison of the test results for the heated and control specimens gave increased sensitivity of analysis over an attempt to make a judgement of purity of the heated samples alone.

Because the first three tests, for color change, phase change, and loss of volatiles are readily performed and permitted elimination of grossly decomposed substances from further testing, they were performed uniformly on specimens of all candidate substances. Subsequent tests were selectively assigned to various groups of substances. The sequences of these tests are discussed below in paragraphs under the respective headings of these groups.

5.2.1 AMINO ACIDS

The tests for Particles in Solution (IV) and Solution pH (V) were, perhaps, not as sensitive as subsequent tests for members of this series. Because aqueous solutions of these substances were needed in subsequent determinations, gross differences in solubilities between heated and control samples were noted. Buffering capacities, if altered by thermal processing, could also be observed. Differential Refractometry (VI) offered a simple but sensitive procedure to determine if small amounts of degradation had occurred. Gas chromatography offered an alternative sensitive procedure which would permit quantitation of the small amounts of degradation.

5.2.2 PROTEINS

Only the first three tests (I-III) were applied to the sodium caseinate specimens. However, subsequent testing should include amino acid analysis of hydrolyzates for both control and heated specimens. This will determine the appropriateness of proteins to act as a source of amino acids after heating.

Biological assay is also proposed for subsequent work to determine the effectiveness of the heated proteins in supporting growth.

5.2.3 CARBOHYDRATES

a. Monosaccharides. Because neither water-insoluble particles nor acidic products were expected in significant amounts until degradation was quite advanced, the tests for Particles in Solution (IV) and Solution pH (V) were not expected to be particularly sensitive. However, these tests offered simple means to determine intermediate levels of degradation. Solutions of substances used in the tests could also be used for Differential Refractometry (VI). The Differential Refractometry offered a simple but sensitive procedure for detecting changes between heated and control samples, owing partly to the fact that concentrated solutions could be prepared with the sugars. Gas Chromatography (X) (of the trimethylsilylated derivatives) is an established method for determining homogeneity and is included in "Reagent Chemicals", A.C.S. Specifications (1960) for all saccharides listed except salicin, but this substance also is expected to silylate with no difficulty.

b. Oligosaccharides and Polysaccharides. These substances were proposed as sources of monosaccharides and therefore were to be examined after acid hydrolysis. Tests for monosaccharides would be applied to the hydrolyzates.

5.2.4 ALCOHOLS AND POLYOLS

a. Ethanol and Glycerin. Differential Refractometry (VI) and Gas Chromatography (X) were considered to offer simple and sensitive procedures for the assessment of degradation of glycerin and ethanol.

b. Mannitol and Dulcitol. These crystalline solids have sharp melting points, therefore, Melting Point Determination (VIII) could be appropriately applied. Gas Chromatography (X) (after trimethylsilylation) would permit quantitation of slight degradation.

5.2.5 LIPIDS

a. Sodium Oleate. This is the salt of an unsaturated fatty acid and can polymerize at high temperatures to form water-insoluble products, therefore, Particles in Solution (IV) was appropriate. Iodine Value Determination could be used to measure the difference in unsaturation between the heated and control samples. This test would be appropriate for detection of intermediate levels of degradation. Gas Chromatography (X) could be performed on the trimethylsilyl derivative to give a very sensitive procedure for detecting degradation. The silylating agent in this case would be trimethylchlorosilane:

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COONa + Cl\ Si(CH_3)_3 \rightarrow\ CH_3(CH_2)_7CH{=}CH(CH_2)_7COOSi(CH_3)_3 + NaCl$$

b. Sodium Acetate. This could be investigated with the Permanganate Reduction to detect decomposition products which would be expected to be one- or two-carbon compounds. Any oxidizable products in the heated sample such as formaldehyde, sodium oxalate, ethanol, etc., would be detected by the consumption of permanganate. This test is included in "Reagent Chemicals", A.C.S. Specifications (1960), p. 437, for sodium acetate. The procedure is to add 5 ml of 10% sulfuric acid and 0.1 ml of 0.1 Normal potassium permanganate to 5 grams of sodium acetate in 50 ml of water. The pink color should not disappear in 1 hour.

c. Linoleic Acid. This substance is a liquid, unsaturated fatty acid. Differential Refractometry (VI), Iodine Value Determination, Gas Chromatography (X) (of trimethylsilylated derivatives), are all sensitive methods for detection of degradation.

5.2.6 VITAMINS

Thin Layer Chromatography, Ultraviolet Spectroscopy, Fluorimetry, and Melting Point Determination (VIII), are all sensitive tests for the vitamins listed. Particles in Solution (IV) was included as a general exploratory test because many of these substances have complex structures that upon heating would yield water-insoluble resinous degradation products. Differential Refractometry (VI) would be sensitive for those vitamins that are moderately to highly soluble.

5.2.7 PARTIAL HYDROLYSATES

Two separate points to be investigated for these substances are denaturation and amino acid destruction. Particles in Solution (IV) was proposed as an exploratory test to determine aggregation. Amino acid analysis, after complete hydrolysis, would be appropriate for determining whether individual amino acids were destroyed or partially destroyed by the heating. Thin layer chromatography could be used to determine destruction of peptides. Biological assay would be used to demonstrate whether toxic materials were produced by the heating.

5.2.8 NUCLEIC ACID BASES

Many of these substances are only sparingly soluble in water, and solutions for Particles in Solution (IV) would, therefore, be prepared with dilute hydrochloric acid for a comparison of heated with control. These acidified solutions could be used for Thin Layer Chromatography, Differential Refractometry (VI), and Ultraviolet Spectroscopy, all of which are sensitive tests for these compounds.

5.2.9 BIOLOGICAL EXTRACTS

Only biological assays appear to be appropriate additional tests to be performed on these substances.

5.2.10 INORGANIC SALTS

a. Sodium Thiosulfate. The literature states that this compound degrades under conditions of heat and no air in the following way:

$$Na_2S_2O_3 \rightarrow Na_2SO_4 + Na_2S + S$$

Particles in Solution (IV) would show any formation of free S. Solution pH (V), is a criterion in "Reagent Chemicals", A.C.S. Specifications (1960). Differential Refractometry (VI), is appropriate because of the solubility of sodium thiosulfate.

b. Sodium Phosphate, Dibasic, and Ammonium Phosphate, Dibasic. A report in the literature states that these salts convert to a mixture of metaphosphates, PO_3^-, and pyrophosphates, $P_2O_7^{4-}$, at elevated temperatures. X-Ray Diffractometry (VII) is sufficiently sensitive to detect a 0.5% change in the crystal structure.

c. Potassium Bicarbonate. When heated, this substance is reported to transform entirely to K_2CO_3. The transformation is stepwise, beginning with the intermediate product $5\ KHCO_3 \cdot K_2CO_3$. Barium Carbonate Precipitation (IX) would detect the formation of any carbonate ions.

d. Manganous Sulphate, Monohydrate. This salt is reported to form manganous oxide, MnO, and manganous sulfide, MnS, at temperatures above 650°C. If these water-insoluble products are present in the heated sample, Particles in Solution (IV) would detect their presence. Manganous sulfate in the presence of water can also hydrolyze in the following way:

$$Mn(SO_4)_2 + 3\ H_2O \rightleftarrows H_2MnO_3 + 2\ H_2SO_4.$$

Solution pH would thus also be appropriate.

5.2.11 MISCELLANEOUS

a. Dipicolinic Acid. Gas Chromatography (X) would be appropriate with the trimethylsilyl derivative.

b. Furfural. Differential Refractometry (VI) on the undiluted samples would be quick and conclusive. Gas Chromatography (X) would also give a sensitive test.

c. Sodium Citrate, Dihydrate. Gas Chromatography (X) would be used on the trimethylsilylated derivative.

d. Succinic Acid, Lactide and Urea. Melting Point Determination (VII) is simple, sensitive, quick and appropriate for all three compounds.

5.2.12 EXOBIOLOGICAL REAGENTS

a. Sodium Formate. This substance is reported to form sodium oxalate and sodium carbonate. Barium Carbonate Precipitation (IX) would detect any carbonate formed.

b. Sodium Pyruvate. Gas Chromatography (X) would be used on the trimethylsilyl derivative.

c. The Naphthylamides (3 substances). Fluorimetry would be performed on these compounds before and after peptidase hydrolysis. To pass this test, fluorescence must not be exhibited by the unhydrolyzed substrate.

SECTION 6

TEST RESULTS

Test results are given in Appendix G Table I and II. The data indicate that of the 94 candidate substances investigated, 57 failed one or more of the tests. The substances were categorized into one of 5 classes on the basis of the screening test results. The stability class of each substance is given in Table I.

Eleven of the 23 amino acids passed all of the tests with which they were screened. Five of those not passing the tests were only marginally degraded and thus still offer potential, if further effort were expended on finding procedures that would give slight improvement in their stability. No protein, partial hydrolysate, or biological extract passed the screening tests and all were extensively degraded.

Salicin, salicyl alcohol glucoside, was the only survivor of 12 candidate monosaccharides. With the exception of α-methyl-D-glucoside all of the other monosaccharides were extensively degraded. Of the 5 oligo- and polysaccharides 4 were extensively degraded. Starch survived the heating well and was only slightly discolored.

Three of the four substances in the alcohols and polyols passed all screening tests used. The fourth substance, galactitol, failed because of a slight darkening. However, there was no change in the melting point or mixed melting point (heated + control).

Two of the three lipids passed the screening tests. Linoleic acid, the third substance, was darkened slightly by the heating and failed the color test, but passed all of the other tests used.

Four of the 11 substances in the vitamin group passed all of the screening tests used. Of those not passing, four failed because of slight discolorations or fusing of crystals, but exhibited little deterioration otherwise.

Five of the 8 nucleic acid bases passed the tests imposed and the three that failed did so because of a slight discoloration.

Seven of the nine inorganic substances passed the screening tests used. X-ray diffraction disclosed that the crystal structures of both the heated and control samples of ammonium phosphate (83) were identical, but that the composition corresponded to a mixture of 25 percent $(NH_4)_2\ PO_4$ and 75 percent $(NH_4)\ H_2PO_4$.

Three of the substances in the miscellaneous category passed the tests applied. These were dipicolinic acid, succinic acid and sodium formate. Lactide failed because of slight discoloration and urea (m.p. 132) failed because it fused into a cake, sublimed, and lost weight, but remained white in color. None of the amino acid naphthylamides passed, but of these L-α-aspartyl-β-naphthylamide seemed least affected by the heating.

SECTION 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 THERMAL PROCESS SYSTEM

The equipment used in heating and monitoring of heating was found to be completely adequate for the task. If future work called for scaling up the operation, it could best be done by increasing the number of heating and monitoring units rather than increasing the size of the basic heating unit. In initiating this work some consideration was given to the use of an oil bath to achieve the heating process. The oil bath offered two important advantages. Heating of specimens would be more uniform and specimens would be visible during the heating process. Visibility of specimens would permit observation of changes in phase and rate of discoloration during heating. The major disadvantage of the oil bath was the danger presented if an ampoule exploded. Not only would hot oil be projected from the bath, but the exploding ampoule could break other ampoules resulting in loss of specimens. Explosion of ampoules during heating did occur, therefore, this was a real danger. Degradation of the oil in the oil bath also presents a disadvantage.

The drying ampoules used as specimen containers were satisfactory. No changes in ampoule configuration are recommended. The size of the sample of test substance accommodated by the container was found adequate to satisfy the needs of all the tests carried out with a considerable quantity to spare in most cases.

Definite advantages would be gained if an ampoule opening device were constructed to permit evaluation of the pressure developed in ampoules as a result of degradation of test material. Gas samples could be removed from this system and analyzed for degradation components. Packaging

of the test substances presented many problems and consumed a large portion of the experimental effort. The greatest difficulties arose when ampoules were filled with low density powders and with development of static charges on ampoule and substance. It is difficult to transfer the test substance into the ampoule, but even greater difficulties are encountered when the filled ampoule is evacuated prior to sealing. Simple solutions to these problems are not immediately apparent and patience is the most important factor. In future studies it would be an improvement to allow more time for packaging. In those cases where traces of water may decrease stability of the test substances, more time should be allowed on the vacuum manifold prior to sealing. In addition, a vacuum system capable of a better vacuum could be employed.

7.2 EXPERIMENTAL

The amino acids, as a class of compounds, are rather stable to the heating process. However, some amino acids were grossly degraded. Because of the extensive degradation of the proteins, it would not appear reasonable that polymers of individual amino acids will improve their stability. It may be possible to achieve acceptable stability by preparing derivatives of thermally unstable amino acids, even though, results of tests on amino acid-naphthylamides indicate that these derivatives are less stable than the constituent amino acids (Compare results of tests on aspartic acid and phenylalanine with their respective naphthylamides). Another approach that may be value is the investigation of the thermal stability of amino acids in aqueous solution.

The most disappointing group of substances, with respect to thermal stability, were carbohydrates. The stability shown by salicin indicates that the desired stability for monosaccharides can be achieved by making derivatives of them. This is further substantiated by the results obtained with α-methyl-D-glucoside. The thermal stability demonstrated by starch was also encouraging. This substance was selected for study because it could be used as a source of glucose after acid hydrolysis. Therefore, it can be said that glucose is available for growth media, but some processing (e.g., acid hydrolysis of starch) will be required after heat sterilization of the spacecraft. If the polyols, especially glycerin, are considered satisfactory substitutes for carbohydrates the situation is further improved. Both glycerin and mannitol, and even dulcitol, appear to be good candidates because of their stability to heat. While dulcitol changed color slightly, the lack of difference in melting point when compared with the unheated control, and the lack of change when a mixed melting point was taken, indicates that degradation could not be appreciable.

The vitamins did not fare well as a class, but several of those that failed still hold promise. One example was pimelic acid, although it was discolored by the heating, its crystalline nature was apparent when it solidified (m.p. 103-105^{o}C) upon cooling.

That high decomposition points and melting points of substances are not always good indicators of thermal stability, was demonstrated by the heating of cytosine and orotic acid. It is reported that cytosine has a decomposition point of 320-325°C and orotic acid has a melting point of 345-346°C. Degradation was indicated after heating for both of these substances by a change in color.

Substances in the partial hydrolysate and biological extract groups will require special processing to qualify for use in exobiological-life-detection-experiments. The possibility of finding procedures to stabilize them, as powders, to dry-heat sterilization would seem remote on the basis of their extensive degradation.

The results with inorganic salts showed that, except for molybdate, any of the ions considered, could be furnished. While ammonium molybdate was degraded, molybdate can not be eliminated on the basis of this work. The finding that the ammonium phosphate was 75% $(NH_4)H_2PO_4$ and 25% $(NH_4)_2HPO_4$ instead of 100% of the latter is attributed to the prepackaging process. As a prepackaging operation the salt was dried in a vacuum oven for 48 hrs at 80°C. The change in composition apparently resulted from loss of ammonia. This conclusion was further established by examining the product directly from the manufacturer's container. The results of the investigation showed that the diammoniun phosphate was present in roughly three times the quantity of the monoammonium phosphate. Therefore, the drying operation (vacuum oven - 80°C) had reversed the ratios of the two ammonium phosphates.

Lactide, in the miscellaneous group of substances, was chosen as a source of lactic acid because of its physical properties. It is formed from lactic acid by heating at 180-220°C in vacuo below 25 mm Hg, has a m.p. of 125°C and a b.p. of 255°C, and hydrolyzes to lactic acid even in cold water. It may be that the discoloration encountered as a result of heating was due to trace impurities. Because of the importance of lactate in intermediary metabolism and the poor thermal stability of sodium pyruvate, further work on improving the stability of lactide should be expended.

7.3 RECOMMENDATIONS FOR FUTURE WORK

7.3.1 GENERAL COMMENTS

This exploratory study placed primary emphasis on physical and chemical detection of thermal degradation of substances typical of microbiological media and representative of biochemical intermediates. Ultimately, the significance of this degradation must be established through suitable tests. Two avenues can be pursued. One would involve the extensive characterization of those compounds which can survive the terminal heat sterilization of the spacecraft and whose contaminants are identified. The other approach is to use the heated materials in the biological tests for which they are intended and determine how well they perform. For example, degradation of growth media components can result in stimulation, inhibition, or no effect

on microbial growth. It can be argued that classification of substances into which are stimulatory and which are inhibitory or inert cannot be made for extraterrestrial organisms because their metabolic requirements and biochemical processes are not known. By the same argument, the classification of substances into metabolites and nonmetabolites cannot be made. The use of growth media sterilized by dry-heat for exobiological studies will be formulated on the metabolic processes and requirements of certain specific terrestrial organisms.

The sum of the degradation products obtained from separate sterilization and storage of a set of growth medium components can differ in both quantity and number compared to degradation products resulting from sterilization of the same components as mixtures. This effect is the result of the increased possibilities for degradation reactions in complex mixtures. In an earlier study (under Contract NASw-1065) it was found that degradation products in dry-heat-sterilized complex growth media were generally inhibitory toward growth of microorganisms. This effect was not uniform with respect to all microbial species tested nor was it stable on extended storage. One other useful result of this earlier study was the observation that systems of substances can be fully effective for a particular intended purpose even though each of the separate components suffers degradation in the course of thermal sterilization or storage. As long as the greater part of the specific activity or material survives the sterilization, it is presumably useful in some permutation of the other components of a growth medium. This same concept, of testing systems before developing perfect parts, has been successfully applied in the development of the Saturn V. In successful application it is only necessary to know that none of the parts will fail catastrophically under the conditions of intended usage.

Arguments should also be presented in favor of microbiological media and biochemical test systems composed solely of relatively undegraded constituents. The advantage of such systems is knowledge of all components in the system. This permits manipulation of variables to determine their effect on the system. In the case of degraded materials, lack of knowledge of the composition of the system limits the information derived from changing a variable.

7.3.2 PHYSICAL AND CHEMICAL SCREENING

The first investigation in this sequence will be concerned with the completion of the sequence of physical and chemical tests for those substances so far passing all tests applied to them. This investigation will include a quantitative chemical determination of the extent of degradation of those substances which failed but were not extensively degraded. These two tasks will identify those substances which require little or no further work to meet the requirements imposed by the dry-heat sterilization process. This investigation may also include addition of new candidate substrates.

7.3.3 BIOLOGICAL ASSAY SCREENING

The second investigation will be concerned with measurement of the biological usefulness of the thermally processed substances. The investigation will be applied first to the individual substances and then to increasingly complex mixtures of them. The biological usefulness test will be designed to assay the individual, heated, pure substances and mixtures of them for the presence of either growth inhibitory or stimulatory substances for a set of microorganism types representing the spectrum of metabolic systems of greatest interest in exobiological research.

7.3.4 DEGRADATION PROCESS STUDY

The third investigation will be concerned with the natures of the degradation process and the products produced by the thermal treatment and storage. A thermodynamic-analysis approach may yield clues about which degradation products to expect. The use of a residual gas analyzer, in concert with a high vacuum chamber in which open containers of the substances are heated, may yield information about the kinetics of the thermal degradation processes.

7.3.5 ALTERNATIVE FORMULATION STUDY

The fourth investigation will be concerned with alternative preparation, formulation, and packaging designs for substances essential for growth media for extraterrestrial use and not among the substances surviving the thermal regime imposed in the current study. Among these alternatives are those which keep separate the reactive groups of the molecules during the high temperature soak, e.g., by solvation, by adsorption on a thermally stable polymer, or by making inert the thermally activated groups.

7.3.6 ALTERNATIVE PROCESSING

The fifth investigation will be concerned with alternative methods for preparing essential growth media substances which are inherently thermolabile. This effort will involve finding other forms of the substances which are more stable and other processes and procedures which will improve the stability of the substances. For each substance, such studies will require extensive expenditure of effort. It is therefore important to establish an order of priority for candidate substances. Those substances considered essential and for which the greatest promise of achieving stability is present, should be given the highest priority. Procedures which may improve stability include: (a) seal substances under hard vacuum, (b) seal substances under pressure supplied by inert gas or gases which result from decomposition of test substances (e.g., ammonia in the ampoule containing ammonium phosphate), (c) process solutions of candidate substances and (e) adsorb test material on a thermostable polymer.

APPENDIX A

MICROBIAL GROWTH MEDIA SUBSTANCES

Substance	Reference to Medium Containing Component
AMINO ACIDS	
β-alanine	25,26,27
L-alanine	3,12,15,16,18,20,27,33,34,37
L-arginine	7,12,15,20
L-asparagine	2a,e,3,5,6,12,15,20,35,37
L-aspartic acid	15,20
L-cysteine	1,15,18
L-cystine	6,11,15,20
L-glutamic acid	1,3,7,12,15,20,26,37
L-glutamine	15,20,26
glycine	15,20
L-histidine	7,15,20
L-hydroxyproline	20
L-isoleucine	7,15,20
L-leucine	9,12,15,20,33
L-lysine	7,15,20
L-methionine	7,10,15,20
L-phenylalanine	10,15,20
L-proline	15,20
L-serine	12,15,20
L-threonine	12,15,20
L-tryptophan	10,15,20
L-tyrosine	15,20
L-valine	7,12,15,20,27
PEPTIDES	
glutathione	26
PROTEINS	
casein or sodium caseinate	2,6 21,23
CARBOHYDRATES	
Monosaccharides	
D-glucose	1,2,3,5,6,7,11,12,16,17,20,21,23,26,27,29,30,35,37,38
D-fructose	17,26,35
D-galactose	17,26

Monosaccharides (continued)

L-arabinose	26
D-xylose	25,26
D-mannose	17,26
L-rhamnose	26
N-acetylglucosamine	15,17
D-glucosamine	17
α-methyl-D-glucoside	17
2-deoxy-D-glucose	17
salicin	26

Oligosaccharides

sucrose	3,17,26
maltose	26
lactose	17,26
trehalose	26
melibiose	26
melezitose	26
gentianose	26
raffinose	26

Polysaccharides

starch	26
inulin	26
cellulose	26
dextrin	26
glycogen	26

ALCOHOLS AND POLYOLS

ethanol	16,29
glycerol	3,25,26
D-mannitol	2a,5,6,17,22,24,25,26
D-sorbitol	26
D-galactitol	26
adonitol	26

LIPIDS

oleic acid or sodium salt	9
acetic acid or sodium salt	3,7,26
linolenic acid	15
linoleic acid	9
arachidonic acid	9
mevalonic acid	15
cholesterol	15
lactobacillic acid	15

LIPIDS (continued)

vaccinic acid	15
9- or 10-hydroxy oleate	15
9- or 10-hydroxy stearate	15

VITAMINS

D- or (+)- biotin	1,3,7,15,25,26
thiamine	1,3,7,15,20,25,26
thiamine pyrophosphate	15
nicotinic acid	3,15,20,26,29
nicotinamide	15,26
riboflavin	3,15,20,26
flavin mononucleotide	15
flavin adenine dinucleotide	15
pyridoxine	3,15
pyridoxal	15,26
pyridoxamine	3,15,20
pyridoxine phosphates	15
para-aminobenzoic acid	3,15,20,25,26
folic acid	3,15,20,26
cobalamin	1,15,26
myo-inositol (L-)	3,15,26
choline	3,15,20,26
pantothenic acid	3,15,20,26
pantetheine	15
lipoic acid	15
vitamin K	15
iron porphyrins	15,25
hematin	26
heme	
ferrichrome	
coprogen	
pimelic acid	26
terregens factor	15

PARTIAL HYDROLYSATES

casitone	6,8,11
peptone	3,5,14,22,23
soypeptone	6d,14,31
tryptone	6d,7,14,31
tryptose	14,38
proteose peptone #3	38
casamino acids	12
multi-peptone	5,14

NUCLEIC ACID BASES

adenine	3,15,20,26
guanine	3,15,20,26
hypoxanthine	15
xanthine	3,15,20,26,29
cytosine	20,26
orotic acid	15,26
thymine	20,26
uracil	3,15,20,26

NUCLEOSIDES

adenosine	15,27
adenine deoxyriboside	15
guanosine	15
guanine deoxyriboside	15
inosine	
hypoxanthine deoxyriboside	10,15,27
xanthosine	15
cytidine	15
cytosine deoxyriboside	15
thymidine	15
uridine	15

NUCLEOTIDES

adenylic acid	26
adenosine-5'-phosphate	15
guanylic acid	15,26
inosine-5'-phosphate	15
cytidylic acid	15
uridine-5'-phosphate	15

NUCLEIC ACIDS

yeast RNA	20

BIOLOGICAL EXTRACTS

yeast	3,6d,e,8,11,28,29,36
beef	5,14,23,28
beef heart; infusion	8,14,38
malt	3
calf brain; infusion	14,38
soil	2,6,23

INORGANIC SALTS

sodium chloride	2,3,5,6,20,22,24,31,38
carbonate	3
nitrite	4,13,29
nitrate	3,8,29
molybate	3,20,22,29
sulfate	1,3,6,12,20,29
sulfite	14
thiosulfate	29
monohydrogen phosphate	6,14,37,38
dihydrogen phosphate	6,20,37
bicarbonate	1
silicate	3
pyrophosphate	34
sulfide	3,29
potassium chloride	3,20
nitrite	1,4
nitrate	2,5,23
monohydrogen phosphate	1,3,4,5,6,8,14,20,22,23,24,29,37
dihydrogen phosphate	1,3,4,6,12,22,29,30,37
sulfate	22,24
bicarbonate	1
ammonium nitrate	3,12,29,30
chloride	1,3,6,12,20,29,37
monohydrogen phosphate	3,6
paramolybdate	3
sulfate	1,20
calcium chloride	1,2,3,5,6,12,20,29,37
carbonate	1,20,23,29
sulfate	2,6
magnesium carbonate	6
sulfate	1,2,3,5,6,20,22,23,24,29,30
chloride	6,12,20,37
boric acid	3,20
ferrous chloride	12
sulfate	1,3,6,18,29
ferric chloride	1,2,3,5,6,20,22
manganous chloride	3,12,29
sulfate	1,3,20,22,37
cobaltous nitrate	3
sulfate	20
cupric sulfate	1,3,20
zinc sulfate	1,3,20
chloride	3

MISCELLANEOUS

dipicolinic acid	19
furfural	32
lactic acid or sodium salt	2,6,15,26,29
sodium citrate	3,20,26
2-furfuryl diacetate	32
2-furfuryl-n-butyrate	32
sodium malate	3,26,29
succinic acid or sodium salt	3,26
oxalic acid	26
betaine	26
ammonium tartrate	3
putrescine	3,26
spermidine or spermine	15
Tris(hydroxymethylamino)-methane	48
λ-pyran-2,6-dipicolinate	19
urea	29

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   i. Jensen's streptomycete medium, 30 b.

   j. Starkey's sulfur oxidation, 30 c.

   k. Beijerinck's thiosulfate oxidation medium, 30 d.

   l. Van Delden's sulfate reduction medium, 30 e.

   m. Ammonification medium, 30 f.

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Peptone + tryptone
Peptone + soy peptone, bacteriological
Peptone + NaCl

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Media for growing facultative anaerobes
Heart infusion broth (Difco)
Brain-heart infusion broth (Difco)

APPENDIX B

CANDIDATE SUBSTANCES; PROCUREMENT, PREPARATION AND THERMAL PROCESSING DATA

Substance and Quality*	Empirical Formula	Mol. Wgt.	Code No.	Source	Mfg. Lot No.	Cost, dollars/gram	Date Recd.	Date Opened	Date Pdgd.	Heating Lot	Heating Specimen	Prepackaging drying	°C	hrs
AMINO ACIDS														
β-Alanine, M.A.	$C_3H_7NO_2$	89.1	1	MRL	S2321	3.50/100	9-20	9-25	9-28	3	A,B	vac oven	23	48
L-α-Alanine, N.R.C.	$C_3H_7NO_2$	89.1	2	GBI	57251	12.50/50	9-13	9-25	9-28	3	A,B	vac oven	23	48
L-Arginine hydrochloride, N.R.C.	$C_6H_{14}N_4O_2 \cdot HCl$	210.7	3	GBI	59323	3.25/50	9-13	9-14	9-18	1	B,D	vac oven	23	20
L-Asparagine, N.R.C.	$C_4H_8N_2O_3$	132.1	4	GBI	59419	4.00/50	9-13	9-25	9-29	3	A,B	vac oven	23	48
L-Aspartic acid, N.R.C.	$C_4H_7NO_4$	133.1	5	GBI	59859	3.50/50	9-13	9-14	9-22	2	C,D	vac oven	80	20
L-Cysteine hydrochloride monohydrate, N.R.C.	$C_3H_7NO_2S \cdot HCl \cdot H_2O$	175.8	6	GBI	80193	3.25/50	9-13	9-14	9-22	2	A,B	vac oven	80	40
L-Cystine dihydrochloride	$C_6H_{12}N_2O_4S_2 \cdot 2HCl$	313.2	7	GBI	808	6.50/50	9-13	9-14	9-18	1	A,B	vac oven	80	20
L-Glutamic acid hydrochloride, C.P.	$C_5H_9NO_4 \cdot HCl$	183.6	8	MRL	R2966	4.50/100	9-20	9-25	9-29	3	A,B	vac oven	23	48
L-Glutamine, N.R.C.	$C_5H_{10}N_2O_3$	146.6	9	GBI	58142	11.00/50	9-13	10-10	10-16	5	A,B	vac oven	23	144
Glycine, N.R.C.	$C_2H_5NO_2$	75.1	10	GBI	59264	1.25/50	9-13	9-25	9-29	3	A,B	vac oven	23	48
L-Histidine, N.R.C.	$C_6H_9N_3O_2$	155.2	11	GBI	59184	10.00/50	9-13	9-25	9-29	3	A,B	vac oven	23	48
4-Hydroxy-L-proline, N.R.C.	$C_5H_9NO_3$	131.1	12	GBI	44452	18.75/25	9-13	9-25	9-29	3	A,B	vac oven	23	48
L-Isoleucine, N.R.C. (allo free)	$C_6H_{13}NO_2$	131.2	13	GBI	80717	21.25/25	9-13	9-14	10-9	4	A,B	vac desicc	23	48
L-Leucine (methionine free)	$C_6H_{13}NO_2$	131.2	14	GBI	59656	4.75/50	9-13	9-25	9-29	3	A,B	vac oven	23	48
L-Lysine hydrochloride, N.R.C.	$C_6H_{14}N_2O_2 \cdot HCl$	182.7	15	GBI	80745	3.25/50	9-13	9-14	9-22	2	C,D	vac oven	80	20
L-Methionine, N.R.C.	$C_5H_{11}NO_2S$	149.2	16	GBI	82532	8.00/50	9-13	9-25	9-28	3	A,B	vac oven	23	48
L-Phenylalanine, N.R.C.	$C_9H_{11}NO_2$	165.2	17	GBI	57104	7.50/25	9-13	9-25	10-2	3	A,B	vac oven	23	48
L-Proline, N.R.C. (hydroxy-L-proline free)	$C_5H_9NO_2$	115.1	18	GBI	651421	8.75/25	9-13	9-14	10-20	4	A,D	vac desicc	23	48
L-Serine, N.R.C.	$C_3H_7NO_3$	105.1	19	GBI	58270	15.00/25	9-13	9-18	9-22	2	C,D	vac oven	80	20
L-Threonine, N.R.C. (allo free)	$C_4H_9NO_3$	119.1	20	GBI	59657	15.00/25	9-13	10-3	10-6	5	A,B	vac oven	23	48
L-Tryptophan, N.R.C.	$C_{11}H_{12}N_2O_2$	204.2	21	GBI	59858	14.50/25	9-13	10-3	10-19	5	C,D	vac oven	23	48
L-Tyrosine, N.R.C.	$C_9H_{11}NO_3$	181.2	22	GBI	80819	3.25/50	9-13	10-3	10-6	5	A,B	vac oven	23	48
L-Valine, N.R.C.	$C_5H_{11}NO_2$	117.2	23	GBI	80719	13.00/50	9-13	9-22	9-27	3	A,B	vac oven	23	72
PROTEINS														
Sodium caseinate (soluble casein)			24	GBI	82243	.75/50	9-13	10-3	10-16	5	A,B	vac oven	23	192
CARBOHYDRATES														
Monosaccharides														
N-Acetyl-α-D-glucosamine	$C_8H_5NO_6$	211.1	25	ACC	022461	16.00/50	9-20	10-10	10-16	5	B,D	vac oven	23	48
L-Arabinose, N.R.C.	$C_5H_{10}O_5$	150.1	27	GBI	82582	6.00/50	9-13	9-14	9-21	2	D	vac oven	80	20
2-Deoxy-D-glucose, N.R.C.	$C_6H_{12}O_5$	164.2	28	GBI	82975	36.25/25	9-28	10-3	10-11	4	A,B	vac oven	23	48
Levulose (β-D-fructose), N.R.C.	$C_6H_{12}O_6$	180.2	29	GBI	81043	1.50/50	9-13	9-14	9-27	3	A,B	vac oven	23	72
D-Galactose, N.R.C.	$C_6H_{12}O_6$	180.2	30	GBI	81030	3.00/50	9-13	9-14	9-22	2	C	vac oven	80	20
D-Glucosamine hydrochloride	$C_6H_{13}NO_5 \cdot HCl$	179.2	31	GBI	59004	4.00/50	9-13	9-14	9-21	2	C,D	vac oven	80	20
Dextrose (α-D-glucose), reagent, A.C.S.	$C_6H_{12}O_6$	180.2	32	B&A	Z091	1.96/227	9-11	9-18	9-27	3	A,B	vac oven	23	72
D-Mannose, N.R.C.	$C_6H_{12}O_6$	180.2	33	GBI	57126	5.00/50	9-13	9-18	9-27	3	A,B	vac oven	23	72
α-Methyl-D-glucoside	$C_7H_{14}O_6$	194.2	34	MRL	J1595	3.50/100	9-20	10-3	10-10	5	A,B	vac oven	23	48
L(+)Rhamnose monohydrate, M.A.	$C_6H_{12}O_5 \cdot H_2O$	182.2	35	MRL	S3918	13.00/50	9-20	10-3	10-6	5	A,B	vac oven	23	48
D-Salicin (Bact.), Puriss	$C_{13}H_{18}O_7$	286.3	36	PCC	379100	24.00/50	10-4	10-10	10-16	5	A,B	vac oven	23	48
D-Ribose, N.R.C.	$C_5H_{10}O_5$	150.1	37	GBI	81545	18.00/50	9-28	10-3	10-11	4	A,B	vac oven	23	48
Oligosaccharides														
α-Lactose monohydrate, U.S.P.	$C_{12}H_{22}O_{11} \cdot H_2O$	342.3	38	SIG	36B-1830	1.00/100	8-31	9-5	9-15	1	A,B	vac oven	23	120
β-D-Maltose monohydrate, N.R.C.	$C_{12}H_{22}O_{11} \cdot H_2O$	360.3	39	GBI	56830	1.75/50	9-13	9-14	10-6	5	A,B	vac oven	23	96
D(+)Sucrose, reagent, A.C.S.	$C_{12}H_{22}O_{11}$	342.3	40	B&A	Z118	1.37/454	8-4	9-5	9-6	1	A,B	vac oven	80	48
Polysaccharides														
Starch, soluble powder, reagent, A.C.S.			41	B&A	Z133	3.00/227	8-4	9-5	10-5	5	A,D	vac desicc	23	96
Inulin	$(C_6H_{10}O_5)_n$	500	42	CBC	63156	2.50/25	10-18	10-18	10-22	4	A,B	vac oven	23	120
ALCOHOLS AND POLYOLS														
Dulcitol (D-galactitol), N.R.C.	$C_6H_{14}O_6$	182.2	43	GBI	57085	19.50/50	9-13	9-14	9-21	2	A,C	vac oven	80	20
Ethyl alcohol, pure (ethanol), U.S.P.	C_2H_6O	46.1	44	USI	53298	36.03/2986	9-11	9-14	10-28	4	A,B	none		
Glycerine (glycerol), reagent, A.C.S.	$C_3H_8O_3$	92.1	45	B&A	Z215	2.59/596	8-4	9-15	9-15	1	A,B	none		
D-Mannitol, N.R.C.	$C_6H_{14}O_6$	182.2	46	GBI	80960	1.50/50	9-13	9-18	9-23	2	C,D	vac oven	80	20
LIPIDS AND RELATED SUBSTANCES														
Sodium acetate, reagent	$C_2H_3O_2Na$	82.0	47	B&A	X113	2.47/454	8-31	9-5	9-15	1	A,B	vac oven	80	48
Linoleic acid, Puriss (99%)	$C_{18}H_{32}O_2$	280.4	48	PCC	0352920	17.50/25	10-4	10-25	10-25	4	A,B	none		
Oleic acid sodium salt (sodium oleate), practical	$C_{18}H_{33}O_2Na$	304.4	50	MCB	OX170	2.85/1000	8-31	9-5	9-15	1	A,B	vac oven	80	48
VITAMINS														
Para-aminobenzoic acid, U.S.P.	$C_7H_7NO_2$	137.1	51	GBI	81885	1.50/50	9-13	9-18	9-25	2	C,D	vac oven	80	20
Biotin, crystalline	$C_{10}H_{16}N_2O_3S$	244.3	52	GBI	82433	120.00/10	9-13	10-3	10-7	5	B,D	vac oven	23	48
Choline chloride, crystalline (99%)	$C_5H_{14}ClNO$	139.6	53	SIG	106B-2170	.90/100	8-31	9-5	9-15	1	A,B	vac oven	80	48
i-Inositol (meso)	$C_6H_{12}O_6$	180.2	55	GBI	82034	1.50/50	9-13	9-14	9-18	1	A,B	vac oven	80	20
Niacin (nicotinic acid) U.S.P.	$C_6H_5NO_2$	123.1	56	CBC	71794	2.50/100	8-31	9-5	9-21	2	C,D	vac oven	23	120
Calcium-D-pantothenate, U.S.P.	$(C_9H_{16}NO_5)_2Ca$	476.5	57	SIG	105B-1660	4.50/50	8-31	9-5	10-27	4	A,B	vac oven	23	120
Pimelic acid, C.P.	$C_7H_{12}O_4$	160.2	58	MRL	L2196	16.00/50	9-20	10-3	10-7	5	A,B	vac oven	23	48
Pyridoxine monohydrochloride (Vitamin B6)	$C_8H_{11}NO_3 \cdot HCl$	205.6	59	SIG	105B-1030	15.00/50	8-31	10-3	10-13	4	A,B	vac oven	23	48
Riboflavin (Vitamin B2)	$C_{17}H_{20}N_4O_6$	376.4	60	SIG	77B-0300	4.20/50	8-31	9-5	9-21	3	A,C	vac oven	23	72
Thiamine hydrochloride, (Vitamin B1), U.S.P.	$C_{12}H_{17}ClN_4OS \cdot HCl$	337.3	61	MRL	S3393	7.00/50	9-20	10-3	10-9	5	A,B	vac oven	23	48
Menadione sodium bisulfite (water soluble Vitamin K)	$C_{11}H_8O_2 \cdot NaHSO_3 \cdot 3H_2O$	330.3	62	GBI	57910	3.50/50	9-13	9-14	9-27	2	D	vac oven	23	72
PARTIAL HYDROLYSATES														
Casitone** vitamin free, dehydrated			63	DIF	496024	3.50/114	10-4	10-13	10-13	4	B,D	none		
Proteose Peptone No. 3**			65	DIF	496490	2.55/114	10-4	10-13	10-13	4	A,D	none		
Bacto-soytone**			66	DIF	510127	2.20/114	10-4	10-18	10-18	5	A,B	none		
NUCLEIC ACID BASES														
Adenine, N.R.C.	$C_5H_5N_5$	135.1	69	GBI	80460	13.50/50	9-13	9-18	9-25	2	A,C	vac oven	80	20
Cytosine, N.R.C.	$C_4H_5N_3O$	111.1	70	GBI	54535	38.00/25	9-13	9-18	9-25	2	A,C	vac oven	80	20
Guanine	$C_5H_5N_5O$	151.1	71	GBI	82388	17.00/50	9-13	9-22	10-16	5	B,D	vac oven	23	96
Hypoxanthine, N.R.C.	$C_5H_4N_4O$	136.1	72	GBI	81727	24.50/25	9-13	9-18	9-25	2	C,D	vac oven	80	20
Orotic acid (uracil 4-carboxylic acid)	$C_5H_4N_2O_4$	156.1	73	NBC	6512	24.50/50	8-31	9-5	10-24	4	A,B	vac oven	23	96
Thymine (5-methyluracil)	$C_5H_6N_2O_2$	126.1	74	NBC	6404	7.45/50	8-31	9-5	10-24	4	A,B	none		
Uracil	$C_4H_4N_2O_2$	112.1	75	GBI	59551	5.00/50	9-13	10-10	10-17	4	A,B	vac oven	23	48
Xanthine	$C_5H_4N_4O_2$	152.1	76	NBC	1963	13.38/50	8-31	9-5	9-22	2	B,D	vac oven	80	48
BIOLOGICAL EXTRACTS														
Beef extract			77	DIF	493821	5.25/114	10-4	10-30	10-30	4	C,D	none		
Beef heart for infusion			78	DIF	511085	13.00/454	10-4	10-27	10-28	4	A,B	none		
Malt extract			79	DIF	491155	1.05/114	10-4	10-18	10-18	4	A,B	none		
Yeast extract			80	DIF	493665	3.70/114	10-4	10-25	10-25	4	A,B	none		
INORGANIC SALTS														
Ammonium chloride, granular, reagent, A.C.S.	NH_4Cl	53.5	81	B&A	A086	1.55/454	8-4	9-5	9-12	1	A,B	vac oven	23	120
Ammonium molybdate, crystal, reagent, A.C.S.	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1236.0	82	B&A	Y364	2.46/114	8-31	9-5	9-15	1	A,B	vac oven	80	48
Ammonium phosphate, dibasic, crystal, reagent, A.C.S.	$(NH_4)_2HPO_4$	132.1	83	B&A	Z040	2.46/454	8-4	9-5	9-15	1	A,B	vac oven	80	48
Ferrous chloride, crystal, reagent	$FeCl_2$	126.8	84	B&A	Y309	2.09/114	8-31	10-18	10-19	4	A,B	vac desicc	23	48
Ferrous sulfate, crystal, reagent A.C.S.	$FeSO_4 \cdot 7H_2O$	278.0	85	B&A	A025	1.82/454	8-4	9-15	9-23	2	C,D	vac desicc	23	168
Manganous sulfate monohydrate, powder, reagent, A.C.S.	$MnSO_4 \cdot H_2O$	169.0	86	JTB	34454	2.98/227	8-4	9-5	9-21	2	C,D	vac oven	80	48
Potassium bicarbonate, crystal, reagent, A.C.S.	$KHCO_3$	100.1	87	JTB	34035	1.96/454	8-4	9-5	9-12	1	A,B	vac oven	80	48
Sodium phosphate, dibasic, reagent, A.C.S.	Na_2HPO_4	142.0	88	B&A	X366	2.74/227	8-4	10-10	10-18	5	A,B	vac oven	23	96
Sodium thiosulfate, reagent	$Na_2S_2O_3$	158.1	89	B&A	Z138	1.98/454	8-31	9-5	9-14	1	A,B	vac oven	80	48
MISCELLANEOUS														
Dipicolinic acid (2,6-pyridine dicarboxylic acid)	$C_7H_5NO_4$	167.1	90	ACC	022051	10.00/100	9-20	10-10	10-16	5	A,B	vac oven	23	48
Furfural, reagent	$C_5H_4O_2$	96.1	91	B&A	Z235	2.84/526	8-31	9-16	9-16	1	A,B	none		
Lactide, reagent	$C_6H_8O_4$	144.1	92	K&K	2031	42.00/30	10-4	10-19	10-24	4	A,B	vac desicc	23	120
Sodium citrate dihydrate, crystal, reagent	$Na_3C_6H_5O_7 \cdot 2H_2O$	294.1	93	B&A	X126	1.52/114	8-31	9-5	9-11	1	A,B	vac oven	80	48
Succinic acid, crystal, reagent	$C_4H_6O_4$	118.1	94	B&A	Z116	2.32/114	8-31	9-5	9-25	2	C,D	vac oven	23	72
L-α-Aspartyl-β-naphthylamide, M.A.	$C_{14}H_{14}N_2O$	258.3	95	MRL	S2881	30.00/1	9-20	9-28	9-29	3	A,B	vac desicc	23	27
L-Histidyl-β-naphthylamide, M.A.	$C_{16}H_{16}N_4O$	280.3	96	MRL	S1257	30.00/1	9-20	9-28	9-29	3	A,B	vac desicc	23	27
L-Phenylalanyl-β-naphthylamide, M.A.	$C_{19}H_{18}N_2O$	290.4	97	MRL	S4611	30.00/1	9-18	9-28	9-29	3	A,B	vac desicc	23	27
Sodium formate, crystal, reagent	$NaCHO_2$	68.0	98	B&A	Z278	4.20/454	8-31	9-5	9-15	1	A,B	vac oven	80	48
Sodium pyruvate, reagent	$C_3H_3O_3Na$	110.0	99	GBI	80550	20.00/50	9-13	9-14	10-6	5	A,B	vac oven	23	96
Urea, crystal, reagent, A.C.S.	$(NH_2)_2CO$	60.1	100	B&A	Y318	2.72/454	8-31	9-5	9-11	1	A,B	vac oven	80	48

* Except where indicated by A.C.S., N.R.C., or U.S.P., the quality specification is unique for the manufacturer.

** Trademark

ABBREVIATIONS

A.C.S.	American Chemical Society Specification
U.S.P.	United States Pharmacopeia Specification
N.R.C.	National Research Council Specification
MRL	Mann Research Laboratories, Inc., New York, N. Y.
GBI	General Biochemicals, Chagrin Falls, Ohio
B&A	Baker and Adamson, General Chemicals Division, Allied Chemical Corporation, Morristown, New Jersey
ACC	Aldrich Chemical Company, Inc., Milwaukee, Wisconsin
PCC	Pierce Chemical Company, Rockford, Illinois
JTB	J. T. Baker Chemical Company, Phillipsburg, New Jersey
USI	U. S. Industrial Chemicals Co., New York, N.Y.
CBC	California Corporation for Biochemical Research (Calbiochem), Los Angeles, California
MCB	Matheson Coleman & Bell Division, The Matheson Company, Inc., East Rutherford, New Jersey
NBC	Nutritional Biochemicals Corporation, Cleveland, Ohio
DIF	Difco Laboratories, Detroit, Michigan
SIG	Sigma Chemical Company, St. Louis, Missouri
K&K	K&K Laboratories, Inc., Plainview, New York
M.A.	Mann Assayed
C.P.	Chemically Pure

APPENDIX C

THERMAL STABILITIES OF SELECTED CANDIDATE INORGANIC SALTS

Thermal stability of selected candidate inorganic salts was estimated on the basis of either their low vapor pressures at 135°C and 20 μ Hg or their melting point, or boiling point, or decomposition point being in excess of 135°C. The vapor pressures were calculated from thermodynamic free energies of formation of potential gaseous decomposition products at equilibrium with the conditions specified above. The purpose of this evaluation was to identify those inorganic salts which would readily withstand sterilization conditions, and could therefore be eliminated from further testing. Those substances with questionable stability would be selected for testing on the basis of their priority for use in microbiological growth media.

Those compounds judged to be stable were investigated for availability. The ones that were found to be commercially available in the anhydrous or stable hydrated form and in reagent grade or meeting A.C.S. specifications were eliminated from testing. The compounds falling in this category are listed in Table I. The remaining compounds which required laboratory experimentation to establish their ability to meet the heating requirements are listed in Table II.

TABLE I

INORGANIC SALTS - ESTIMATED TO BE STABLE AT 135°C,
20 μ Hg ABSOLUTE PRESSURE

		mp, °C	Calculated Vapor Pressure μ Hg (135°C)	bp, °C
calcium chloride	$CaCl_2$	772		>1600
carbonate	$CaCO_3$	d.825	10^{-10}	
cobaltous sulfate	$CoSO_4$	989		
cupric sulfate	$CuSO_4$	200		d.650
ferric chloride	$FeCl_3$	282		315
magnesium carbonate	$MgCO_3$	d.350		$-CO_2$,900
sulfate	$MgSO_4$	d.1124		
potassium chloride	KCl	776		sub1.1500
nitrite	KNO_2	d.350		
nitrate	KNO_3	334		d.400
sulfate	K_2SO_4	588		
sodium chloride	NaCl	801	10^{-12}	1413
nitrite	$NaNO_2$	271		d.320
nitrate	$NaNO_3$	307		d.380
sulfite	Na_2SO_3		10^{-36}	d.
sulfate	Na_2SO_4	884		
sulfide	Na_2S	1180		
carbonate	Na_2CO_3	851		d.
pyrophosphate	$Na_4P_2O_7$	880		
zinc chloride	$ZnCl_2$	262		732
sulfate	$ZnSO_4$	d.740		

TABLE II

INORGANIC SALTS REQUIRING LABORATORY DATA TO ESTABLISH STABILITY

AT 135°C, 20 μ Hg ABSOLUTE PRESSURE

		mp, °C	Calculated Vapor Pressure μ Hg (135°C)	bp, °C
ammonium chloride	NH_4Cl	subl.335	21	
paramolybdate	$(NH_4)_6Mo_7O_{24}\cdot 4\ H_2O$	d.		
monohydrogen phosphate	$(NH_4)_2HPO_4$	d.		
nitrate	NH_4NO_3	170		d.210
sulfate	$(NH_4)_2SO_4$			
boric acid	H_3BO_3	d.185		$-1\frac{1}{2}\ H_2O$, 300
calcium sulfate dihydrate	$CaSO_4\cdot 2\ H_2O$	$-1\frac{1}{2}\ H_2O$, 128		$-2\ H_2O$, 163
cobaltous nitrate hexahydrate	$Co(NO_3)_2\cdot 6\ H_2O$	<100		$-3\ H_2O$, 50
ferrous chloride tetrahydrate	$FeCl_2$			
sulfate heptahydrate	$FeSO_4\cdot 7\ H_2O$	64; $-6\ H_2O$, 100	(>20)	$-7\ H_2O$, 300
magnesium chloride hexahydrate	$MgCl_2\cdot 6\ H_2O$	d.116-8		d.
manganous chloride tetrahydrate	$MnCl_2\cdot 4\ H_2O$	58; $-H_2O$, 106		$-4\ H_2O$, 198
sulfate monohydrate	$MnSO_4\cdot H_2O$	stable 57-117		
potassium monohydrogen phosphate	K_2HPO_4	d.		
dihydrogen phosphate	KH_2PO_4	253	100	
bicarbonate	$KHCO_3$	d.100-200	(>0.02)	
sodium monohydrogen phosphate	Na_2HPO_4			
dihydrogen phosphate monohydrate	$NaH_2PO_4\cdot H_2O$	$-H_2O$, 100		d.204
molybdate	$Na_2MoO_4\cdot 2\ H_2O$	$-H_2O$, 100		
bicarbonate	$NaHCO_3$	$-CO_2$, 270	11.6	
thiosulfate	$Na_2S_2O_3$			
silicate nonahydrate	$Na_2SiO_3\cdot 9\ H_2O$	40-48		$-6\ H_2O$, 100

APPENDIX D

CONFIGURATION, COMPOSITION AND PREPARATION OF THE ALL-GLASS CONTAINER

1. CONFIGURATION AND COMPOSITION

The all-glass containers were LG 6770, 10-ml drying ampoules, made of Kimble KG-33 low expansion borosilicate glass, and were made by Lab Glass, Inc., Vineland, New Jersey.

1.1 PHYSICAL DESCRIPTION

The LG 6770 containers were round bottom drying ampoules with the following dimensions:

overall length	184 mm ± 3 mm
stem length	95 mm
base length	89 mm
stem O.D.	10 mm ± 0.5 mm
stem wall thickness	1.0 mm ± 0.2 mm
base O.D.	15 mm ± 0.5 mm
base wall thickness	1.2 mm ± 0.2 mm

1.2 CHEMICAL COMPOSITION

The containers were made entirely of Kimble KG-33 glass (Owens-Illinois, Inc., Vineland, New Jersey). KG-33 glass is a low-expansion borosilicate glass.

1.3 PHYSICAL PROPERTIES

The physical properties of KG-33 glass are:

strain point	515°C
annealing point	555°C
softening point	820°C
linear coef. of expansion	$32 \times 10^{-7}/^{\circ}C$ (0-300°C)
density (g/ml)	2.23
refractive index	1.47
transmission (2 mm)	92%
spec. heat (average for range 25°C to 175°C)	0.205 (cal/g °C)
thermal cond. (20°C)	0.0028 (cal/sec cm °C)
Young's modulus psi	8.9×10^{6}

1.4 BURSTING PRESSURE

Water was sealed into two containers according to the packaging procedure used for test substances except that the containers were held in liquid nitrogen during evacuation. The sealed containers were then placed in an aluminum-block tube heater and the temperature increased until they burst. By recording the temperature of the block it was possible to establish that the containers withstood pressures in excess of 700 psi before bursting.

1.5 U.S.P. TYPE

Ten containers, selected at random, were subjected to the U.S.P. Powdered Glass Test described in the United States Pharmacopia, seventeenth

revision, 1965, p.900. The test was carried out by Truesdail Laboratories, Inc., Los Angeles, California. Test results showed that glass used in the containers met requirements for U.S.P. Type I glass.

1.6 SINGLE BATCH CONTROL

All tubing used to construct the containers was Kimble K80200 standard wall glass tubing. Container stems were made of 10-mm tubing from Kimble lot number 124122866. Bases were made of 15-mm tubing from Kimble lot number 3121367. Kimble K80200 tubing is made of KG-33 glass.

2. PREPARATION FOR STORAGE AND USAGE

2.1 PRELIMINARY WASH

The ampoules were inverted in stainless steel baskets, flushed with distilled water in the Heinicke model HW-5000E dishwasher (Heinicke Instruments Co., Hollywood, Florida) for two minutes, and then dried in an oven.

2.2 MARKING

Identification numbers were marked on the ampoules using a Glass Marker Grinder. The numbers were marked on the stem about 2 cm from the open end, and on the body about 3 cm below the shoulder of the ampoule.

2.3 WASHING AND ANNEALING

2.3.1 ACID WASH

The ampoules were immersed in A.C.S. grade, 90% Nitric Acid at about 70°C for 25-35 minutes.

2.3.2 FIRST RINSE

The ampoules were rinsed four times with purified water (U.S.P.). (This water was deionized water which met the U.S.P. test requirements.) The ampoules were filled completely and then drained on each rinse.

2.3.3 ANNEALING

The ampoules were annealed at 560°C for 15 minutes. The cooling rate was 2°C/min for the first 150°C.

2.3.4 FINAL RINSE

The ampoules were rinsed twice with purified water (U.S.P.), completely filled and then drained on each rinse. They were then rinsed twice more

with purified water (U.S.P.) which had been passed twice through Millipore Filter disks, pore size 0.22 μ. After the final rinse the ampoules were dried in an oven.

2.4 STORAGE

The ampoules were stored in a large, covered, polyethylene bucket until used.

APPENDIX E

THERMAL PROCESSING EQUIPMENT

1. TUBE HEATERS

The thermal process environment was provided by two aluminum-block tube heaters. Each tube heater consisted of a heating base, an aluminum block, and a lid, made of Transite (an insulating material).

1.1 HEATING BASES

The heating bases were RSCo model 2127-A (Research Specialties Company, Richmond, California). They are heated electrically and rated by the manufacturer to cover the temperature range 25-300°C ± 1°C.

1.2 ALUMINUM BLOCKS

Each aluminum block had a capacity of forty-two, 15 mm tubes. The blocks were 11-5/8" long, 4-3/16" wide, and 3-1/2" deep. A sheet of Transite, 9/16" thick, was fastened to the top of each block. Forty-two holes, 5/8" in diameter, were drilled through the aluminum and Transite. Holes were 1 inch apart, measured center to center.

1.3 TEMPERATURE CONTROL

There were two principal sources of temperature variation in the heating blocks. At any one time a tube position near the center of the block could be warmer than one near the edge. Also, the temperature at a single tube position could vary over time. Calibration of the tube heaters (Section 2.2) showed that the difference in temperature between any two tube positions at one time was less than 2°C, and that the temperature at

any one tube position did not vary more than 1°C from its mean during the heat soak.

2. CALIBRATION OF EQUIPMENT

2.1 RECORDING POTENTIOMETER WITH TEMPERATURE SENSORS

A Foxboro model ERB12-30ML12-123 recording potentiometer (Foxboro Company, East Bridgewater, Massachusetts) and iron-constantan thermocouples were used to measure and monitor temperatures within the heating blocks. The recorder and thermocouples were calibrated against a National Bureau of Standards certified thermocouple by the Aeronutronic Division Standards Laboratory. Thermocouples were selected for similarity of voltage produced over the range of temperature measured in the investigation.

2.2 TUBE HEATERS

At the start of each heat soak all specimens of a particular processing lot were placed in one of the tube heaters at one time. Because of a wide range of physical properties among the test substances, all specimens did not reach the specified temperature at the same time. The following calibration procedures were carried out to determine when to begin timing the 92-hour heat soak, and to determine if the difference in warmup times of specimens exceeded the ± 30 minutes allowed for variation in heat soak time. Stability and uniformity of temperature in the heating blocks were also determined.

2.2.1 WARMUP TIMES

The aluminum block and heating base were adjusted to a temperature near 135°C and allowed to equilibrate for 24 hours before final adjustment to the specified temperature was made. Iron-constantan thermocouples were placed in ten 15-mm test tubes. The tubes were filled with different test materials. Two contained 1 g sand, two contained 5 g sand, two were filled to a point five centimeters from the bottom with bentonite, two were filled to five centimeters with oil, and two were empty. These ten tubes were distributed in the heating block in such a manner that extremes of temperature would be indicated. Their temperatures were recorded for a 3-hour period. The entire set of tubes was then transferred to a test tube rack and cooled for one hour. This heating and cooling process was repeated five times. The mean warmup time (33 minutes) was used to determine the start of the 92-hour heat soak in the exposure of the test substances. The fastest warmup time was 18 minutes, the slowest was 52 minutes. The difference in warmup times was, therefore, within the range allowed for variation in heat soak time.

2.2.2 STABILITY AND UNIFORMITY OF TEMPERATURE

The aluminum block and heating base were adjusted to 135°C and allowed 24 hours to equilibrate. Iron-constantan thermocouples were placed in ten, 15-mm test tubes containing about 5 g sand. These tubes were placed in representative holes in the heating block and their temperatures recorded for 48 hours. The temperature variation at any tube position was less than ± 1°C during the 48 hours. The temperature at each tube position in the heating block was measured to determine the uniformity of temperature in the block. The steady-state temperatures were measured ten holes at a time. The arrays were so chosen that all holes were included in four trials. The difference between the highest and lowest temperatures in any group of ten positions was less than 2°C.

3. MONITORING THE THERMAL PROCESS ENVIRONMENT

The temperatures of the heating blocks were monitored and recorded throughout each 92-hour heat soak period, including the associated warmup period.

APPENDIX F

TEST SEQUENCE

Test No.	I Color Change	II Phase Change	III Loss of Volatiles	IV Particles in Solution	V pH Change	VI Differential Refractometry	VII X-Ray Diffraction Analysis	VIII Melting Point Determination	IX Barium Carbonate Pptn.	X Chromatography	XI Spectrophotometry	XII Fluorimetry	XIII Iodine Value Determination	XIV Amino Acid Analysis	XV Biological Assay	XVI Permanganate Reduction	XVII Colorimetry	XVIII Ferrocyanide Test
AMINO ACIDS																		
β-Alanine	1	2	3	4	5	6				7								
L-α-Alanine	1	2	3	4	5	6				7								
L-Arginine Hydrochloride	1	2	3	4	5	6				7								
L-Asparagine	1	2	3	4	5	6				7								
L-Aspartic Acid	1	2	3	4	5	6				7								
L-Cysteine Monohydrate Hydrochloride	1	2	3	4	5	6				7								
L-Cystine Dihydrochloride	1	2	3	4	5	6				7								
L-Glutamic Acid Hydrochloride	1	2	3	4	5	6				7								
L-Glutamine	1	2	3	4	5	6				7								
Glycine	1	2	3	4	5	6				7								
L-Histidine	1	2	3	4	5	6				7								
L-Hydroxyproline	1	2	3	4	5	6				7								
L-Isoleucine	1	2	3	4	5	6				7								
L-Leucine	1	2	3	4	5	6				7								
L-Lysine Monohydrochloride	1	2	3	4	5	6				7								
L-Methionine	1	2	3	4	5	6				7								
L-Phenylalanine	1	2	3	4	5	6				7								
L-Proline	1	2	3	4	5	6				7								
L-Serine	1	2	3	4	5	6				7								
L-Threonine	1	2	3	4	5	6				7								
L-Tryptophan	1	2	3	4	5	6				7								
L-Tyrosine	1	2	3	4	5	6				7								
L-Valine	1	2	3	4	5	6				7								
PROTEINS																		
Sodium Caseinate	1	2	3											4a	4b			
MONOSACCHARIDES																		
Dextrose (α-D-Glucose)	1	2	3	4	6	5				7								
Levulose (β-D-Fructose)	1	2	3	4	6	5				7								
D-Galactose	1	2	3	4	6	5				7								
L-Arabinose	1	2	3	4	6	5				7								
D-Mannose	1	2	3	4	6	5				7								

Test No.	I Color Change	II Phase Change	III Loss of Volatiles	IV Particles in Solution	V pH Change	VI Differential Refractometry	VII X-Ray Diffraction Analysis	VIII Melting Point Determination	IX Barium Carbonate Pptn.	X Chromatography	XI Spectrophotometry	XII Fluorimetry	XIII Iodine Value Determination	XIV Amino Acid Analysis	XV Biological Assay	XVI Permanganate Reduction	XVII Colorimetry	XVIII Ferrocyanide Test
MONOSACCHARIDES, Contd.																		
L-(+)Rhamnose Monohydrate	1	2	3	4	6	5				7								
N-Acetyl glucosamine	1	2	3	4	6	5				7								
α-Methyl-D-Glucoside	1	2	3	4	6	5				7								
D-Glucosamine Hydrochloride	1	2	3	4	6	5				7								
2-Deoxy-D-Glucose	1	2	3	4	6	5				7								
Salicin	1	2	3	4	6	5				7								
D-Ribose	1	2	3	4	6	5				7								
OLIGOSACCHARIDES																		
D(-) Sucrose	1	2	3	4						5*								
β-D-Maltose Monohydrate	1	2	3	4						5*								
α-Lactose Monohydrate	1	2	3	4						5*								
POLYSACCHARIDES																		
Starch	1	2	3	4						5*								
Inulin	1	2	3	4						5*								
ALCOHOLS, POLYOLS																		
Ethanol	1	2	3			4				5	6							
Glycerin	1	2	3			4				5	6							
D-Mannitol	1	2	3	4		7		5		6								
Dulcitol (D-Galactitol)	1	2	3	4		7		5		6								
LIPIDS																		
Oleic Acid Sod. Salt	1	2	3	6		7				4			5					
Sodium Acetate	1	2	3		4											5		
Linoleic Acid	1	2	3			4				5	7		6					

* after hydrolysis

Test No.	I Color Change	II Phase Change	III Loss of Volatiles	IV Particles in Solution	V pH Change	VI Differential Refractometry	VII X-Ray Diffraction Analysis	VIII Melting Point Determination	IX Barium Carbonate Pptn.	X Chromatography	XI Spectrophotometry	XII Fluorimetry	XIII Iodine Value Determination	XIV Amino Acid Analysis	XV Biological Assay	XVI Permanganate Reduction	XVII Colorimetry	XVIII Ferrocyanide Test
VITAMINS																		
Biotin	1	2	3	5		6		4		7								
Thiamine Hydrochloride	1	2	3	4		5				6		7						
Niacin (nicotinic acid)	1	2	3	4		5				6	7							
Riboflavin	1	2	3	4		5				6		7						
Pyridoxine Hydrochloride	1	2	3	4		5				6		7						
p-Aminobenzoic Acid	1	2	3	5		6		4		7								
i-Inositol (meso)	1	2	3	5		6		4		7								
Choline chloride	1	2	3	4		5				6							7	
Calcium Pantothenate	1	2	3	4		5				6							7	
Menadione Sodium Bisulfite	1	2	3	4		5				6		7						
Pimelic Acid	1	2	3	5		6		4		7								
PARTIAL HYDROLYSATES																		
Casitone	1	2	3	4						5				6a	6b			
Bacto-soytone	1	2	3	4						5				6a	6b			
Proteose Peptone #3	1	2	3	4						5				6a	6b			
NUCLEIC ACID BASES																		
Adenine	1	2	3	5		6				4	7							
Guanine	1	2	3	5		6				4	7							
Hypoxanthine	1	2	3	5		6				4	7							
Xanthine	1	2	3	5		6				4	7							
Cytosine	1	2	3	5		6				4	7							
Orotic Acid	1	2	3	5		6				4	7							
Thymine	1	2	3	5		6				4	7							
Uracil	1	2	3	5		6				4	7							
BIOLOGICAL EXTRACTS																		
Yeast extract	1	2	3												4			
Beef extract	1	2	3												4			
Beef Heart for infusion	1	2	3												4			
Malt extract	1	2	3												4			

	Test No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
		Color Change	Phase Change	Loss of Volatiles	Particles in Solution	pH Change	Differential Refractometry	X-Ray Diffraction Analysis	Melting Point Determination	Barium Carbonate Pptn.	Chromatography	Spectrophotometry	Fluorimetry	Iodine Value Determination	Amino Acid Analysis	Biological Assay	Permanganate Reduction	Colorimetry	Ferrocyanide Test
INORGANIC SALTS																			
Sodium Thiosulfate, Anhyd.		1	2	3	4	5	6												
Sodium Phosphate, Dibasic, Anhydrous		1	2	3				4											
Potassium Bicarbonate		1	2	3		5	6			4									
Manganous Sulfate, Monohydrate		1	2	3	4	5	6												
Ferrous Chloride		1	2	3	4														5
Ferrous Sulfate		1	2	3	4														5
Ammonium Chloride		1	2	3		4	5												
Ammonium Molybdate		1	2	3		4													
Ammonium Phosphate, Dibasic		1	2	3				4											
MISCELLANEOUS																			
Dipicolinic Acid		1	2	3							4								
Furfural		1	2	3			4				5								
Sodium Citrate, Dihydrate		1	2	3							4								
Succinic Acid		1	2	3					4										
Lactide		1	2	3					4										
Urea		1	2	3					4		5								
EXOBIOLOGICAL EXPERIMENT REAGENTS																			
Sodium Formate		1	2	3	4					5	6								
Sodium Pyruvate		1	2	3	4						5								
L-α-Asparty1-β-Naphthylamide		1	2	3									4			5			
L-Histidyl-β-Naphthylamide		1	2	3									4			5			
L-Phenylalanyl-β-Naphthylamide		1	2	3									4			5			

APPENDIX G

LABORATORY DATA

Laboratory data for all screening tests which have been completed are given in Tables I and II.

Candidate substances have been categorized into stability classes, based on results of tests which have been completed. Stability classes are:

Class 1: Passed all tests.

Class 2: Passed first three tests and failed one or more subsequent tests.

Class 3: Failed the color test, because of a slight discoloration, and may have undergone only minimal damage (example: #41, Starch).

Class 4: Failed the phase-change and loss of volatiles tests, but may have undergone only slight chemical change (example: #100, Urea).

Class 5: Showed extensive decomposition.

Table I gives the stability class of each substance, indicates which tests have been completed, and reports the data from Tests I, II, and III. The data for Test I (Color Change) given in Munsell Color Values, are used to compare the color of heated and control specimens. The numbers represent Hue Value/Chroma. For example, 10 YR9/1 represents a color with Hue 10YR, Value 9 and Chroma 1. Chroma for neutral colors is zero and is not printed. The number N9.25/, therefore, represents neutral Hue, Value 9.25 and Chroma 0.

Descriptions of the phase of control and heated specimens are given as data for Test II (Phase Change). Under Test III (Loss of Volatiles), the weights of the heated specimens which were opened are given as sample weight. The weight changes reported are the differences in weight change between the control and heated specimen.

The data for Tests IV, VI, VII, VIII and IX are given in Table II. The appearance of solutions made from the heated and control specimens is reported under Test IV (Particles in Solution). Differential Refractometry (Test VI) data are given in terms of Δd and $\Delta d'$. These values are the differences between two numerical readings taken with the differential refractometer, and are proportional to the differences in the indexes of refraction. The Δd notation was the difference between the control and heated specimens and $\Delta d'$ was the difference between the control and a 1% dilution of the control. Data for X-ray Diffraction Analysis (Test VII) are given as the percentages of candidate substances in the test sample. Samples were taken from control and heated specimens. Melting ranges of control and heated specimens are reported under Test VIII (Melting Point Determination). Melting ranges given are uncorrected. Data for Test IX (Barium Carbonate Precipitation) are in terms of the clarity of test solutions of control and heated specimens.

The results of all tests are reported as P or F for pass or fail. Test results which were marginal are enclosed in parentheses. The + symbol beside a P or F under test results indicates that a strong umpleasant odor was detected when the heated specimen was opened. The ++ symbol indicates that the odor was that of ammonia.

TABLE I

LABORATORY DATA FOR TESTS I, II AND III

Substance and Quality	Stability Class	Tests Completed	Code No.	Test I. Color Change			Test II. Phase Change*			Test III. Loss of Volatiles		
				Control	Heated	Result	Control	Heated	Result	Sample wt. gm	Wt.Change mg	Result
AMINO ACIDS												
β-Alanine, M.A.	5	I,II,III	1	N9.5/	N9.25/	P	ff powd	wet cake	F	4.0499	+0.7	P
L-α-Alanine, N.R.C.	1	I,II,III,IV,VI	2	N9.5/	N9.5/	P	ff cryst	cryst adhesion;not ff even after loosening; subl	P	4.0377	+0.5	P
L-Arginine hydrochloride, N.R.C.	1	I,II,III,IV,VI	3	N9.5/	N9.25/	P	cryst	cryst subl	P	4.3411	-2.5	P
L-Asparagine, N.R.C.	5	I,II,III	4	N9.5/	10YR2/1	F	powd	polym; some subl	F	4.5558	-98.9	F
L-Aspartic acid, N.R.C.	1	I,II,III,IV,VI	5	N9.5/	N9.25/	P	cryst	cryst	P	4.2523	-0.8	P
L-Cysteine hydrochloride monohydrate, N.R.C.	5	I,II,III	6	-	-	F	-	-	F	5.0381	exploded	F
L-Cystine dihydrochloride	3	I,II,III	7	N9.25/	10YR9/1	(F)	powd	powd; some subl	P	4.0661	+3.7	P
L-Glutamic acid hydrochloride, C.P.	1	I,II,III,IV,VI	8	N9.25/	N9.25/	P	coarse granl	coarse granl	P	5.0569	-0.5	P
L-Glutamine, N.R.C.	5	I,II,III	9	N9.5/	5Y8.5/2	F	powd	fused cake	F	2.9376	-0.2	P
Glycine, N.R.C.	5	I,II,III,IV,VI	10	N9.5/	5Y9/1	F	powd	powd; liq on walls	P	3.9346	+0.6	P
L-Histidine, N.R.C.	3	I,II,III,IV,VI	11	N9.25/	5Y9/1	(F)	loose cryst, not ff	cryst adhesion;no change in cryst shape	P	3.3502	+0.3	P
4-Hydroxy-L-proline, N.R.C.	1	I,II,III,IV,VI	12	N9.25/	N9.25/	P	ff granl	cryst adhesion; ff after loosening	P	5.0419	-0.5	P
L-Isoleucine, N.R.C. (allo free)	2	I,II,III,IV,VI	13	N9.25/	N9.25/	P	ff cryst	ff cryst; some subl	P	1.5879	0	P
L-Leucine (methionine free)	1	I,II,III,IV,VI	14	N9.25/	N9.25/	P	ff leafs	cryst adhesion;not ff after loosening; no change in cryst shape	P	3.1312	+9.1	P
L-Lysine hydrochloride, N.R.C.	1	I,II,III,IV,VI	15	N9.25/	N9.25/	P	granl	granl	P	3.4503	-0.8	P
L-Methionine, N.R.C.	1	I,II,III,IV,VI	16	N9.5/	N9.25/	P	powd	powd; some subl	P	2.3269	-1.8	P+
L-Phenylalanine, N.R.C.	1	I,II,III,IV,VI	17	N9.5/	N9.5/	P	ff granl	ff granl; some subl	P	2.9497	+0.3	P
L-Proline, N.R.C. (hydroxy-L-proline free)	5	I,II,III	18	N9.5/	5Y9/4	F	powd	fused cake & liq	F	1.8623	-2.8	P
L-Serine, N.R.C.	5	I,II,III	19	-	-	F	-	-	F	3.6414	exploded	F
L-Threonine, N.R.C. (allo free)	3	I,II,III,IV,VI	20	N9.25/	5Y9/1	(F)	powd	powd	P	3.9475	-0.3	P
L-Tryptophan, N.R.C.	2	I,II,III,IV,VI	21	N9.25/	N9.25/	P	powd	powd	P	1.3933	-0.7	P
L-Tyrosine, N.R.C.	1	I,II,III,IV,VI	22	N9.25/	N9.25/	P	powd	powd	(P)	1.476	-0.4	P
L-Valine, N.R.C.	1	I,II,III,IV,VI	23	N9.5/	N9.5/	P	ff cryst	ff cryst; some subl (white & yellow)	(P)	2.2055	+0.8	P
PROTEINS												
Sodium caseinate (soluble casein)	5	I,II,III	24	5Y9/1	10YR8/6	F	powd	caked powd	F	1.2200	-8.7	F+
CARBOHYDRATES												
Monosaccharides												
N-Acetyl-α-D-glucosamine	5	I,II,III	25	10YR9/1	10YR2/1	F	powd	polym	F	2.8114	-91.9	F
L-Arabinose, N.R.C.	5	I,II,III	27	N9.25/	10YR2/1	F	powd	polym	F	4.2215	-39.9	F
2-Deoxy-D-glucose, N.R.C.	5	I,II,III	28	N9.25/	10YR2/2	F	powd	polym; some liq on walls	F	1.8971	-26.8	F
Levulose (β-D-fructose), N.R.C.	5	I,II,III	29	N9.5/	10YR2/2	F	ff granl	polym; some liq on walls	F	4.2670	-201.3	F
D-Galactose, N.R.C.	5	I,II,III	30	N9.5/	10YR2/1	F	powd	polym	F	2.9344	-13.3	F
D-Glucosamine hydrochloride	5	I,II,III	31	-	-	F	--	--	F	4.1840	exploded	F
Dextrose (α-D-glucose), reagent, A.C.S.	5	I,II,III	32	N9.5/	10YR2/2	F	ff granl	polym	F	5.0706	-22.8	F
D-Mannose, N.R.C.	5	I,II,III	33	N9.25/	10YR2/1	F	powd	polym	F	2.0622	-68.8	F
α-Methyl-D-glucoside	3	I,II,III	34	N9.5/	5Y9/1	F	powd	powd	P	2.6495	-2.2	P
L(+)Rhamnose monohydrate, M.A.	5	I,II,III	35	N9.5/	5Y2/2	F	powd	polym layer & liq layer	F	5.0088	-	-
D-Salicin (Bact.), Puriss	1	I,II,III	36	N9.5/	N9.25/	P	powd	powd; some subl	P	1.5374	-1.0	P
D-Ribose, N.R.C.	5	I,II,III	37	N9.5/	10YR2/1	F	powd	polym	F	1.9664	-39.7	F
Oligosaccharides												
α-Lactose monohydrate, U.S.P.	5	I,II,III	38	N9.25/	2.5Y4.5/4	F	powd	polym	F	4.5649	-16.5	F
β-D-Maltose monohydrate, N.R.C.	5	I,II,III	39	N9.5/	10YR2/2	F	powd	polym	F	1.8067	-58.4	F
D(+)Sucrose, reagent, A.C.S.	5	I,II,III	40	N9.25/	5YR2/1	F	large granl	polym	F	4.9581	-193.0	F
Polysaccharides												
Starch, soluble powder reagent, A.C.S.	3	I,II,III	41	N9.25/	2.5Y8.5/2	F	powd	powd	P	3.2827	+0.4	P
Inulin	5	I,II,III	42	N9.5/	10YR2/2	F	powd	polym	F	2.9657	-146.1	F
ALCOHOLS AND POLYOLS												
Dulcitol (D-galactitol), N.R.C.	3	I,II,III,VIII	43	N9.5/	5Y9/1	F	powd	caked powd; some decomp on walls	(P)	4.6114	-0.7	P
Ethyl alcohol, pure (ethanol), U.S.P.	1	I,II,III	44	colorless	colorless	P	liq	liq	P	4.7475	-0.8	P
Glycerine (glycerol), reagent, A.C.S.	1	I,II,III	45	colorless	colorless	P	liq	liq	P	6.7788	+3.2	P
D-Mannitol, N.R.C.	1	I,II,III,VIII	46	N9.5/	N9.25/	P	powd	caked powd	P	2.9350	-0.3	P
LIPIDS AND RELATED SUBSTANCES												
Sodium acetate, reagent	1	I,II,III	47	N9.5/	N9.5/	P	cryst	cryst	P	4.8153	+2.9	P
Linoleic acid, Puriss (99%)	3	I,II,III	48	5Y9/2	5Y8.5/10	F	liq	liq	P	4.8427	+0.3	P
Oleic acid sodium salt (sodium oleate), practical	1	I,II,III	50	2.5Y9/4	2.5Y9/4	P	fl	fl	P	2.8346	+4.2	P
VITAMINS												
Para-aminobenzoic acid, U.S.P.	5	I,II,III	51	-	-	F	--	--	F	3.2237	exploded	F
Biotin, crystalline	1	I,II,III,VIII	52	N9.25/	N9.25/	P	cryst	cryst	P	1.2532	-0.5	P
Choline chloride, crystalline (99%)	1	I,II,III	53	N9.5/	N9.5/	P	large granl	large granl	P	3.3969	-0.8	P
i-Inositol (meso)	1	I,II,III	55	N9.25/	N9.25/	P	cryst	cryst	P	5.0966	-8.6	P
Niacin (nicotinic acid), U.S.P.	4	I,II,III	56	N9.5/	N9.25/	P	powd	caked powd;considerable subl	(F)	1.8271	+0.5	P
Calcium-D-pantothenate, U.S.P.	3	I,II,III	57	N9.5/	5Y9/1	(F)	powd	caked powd; some liq on walls	P	1.5268	-1.9	P
Pimelic acid, C.P.	5	I,II,III	58	5Y9/1	10YR7/2	F	powd	fused cake	F	3.0229	-0.5	P
Pyridoxine monohydrochloride (Vitamin B6)	3	I,II,III	59	N9.5/	2.5Y9/2	(F)	powd	powd	P	2.7986	-0.2	P
Riboflavin (Vitamin B2)	1	I,II,III	60	10YR8/16	10YR8/16	P	powd	powd	P	1.3441	-3.6	P
Thiamine hydrochloride, (Vitamin B1), U.S.P.	3	I,II,III	61	N9.5/	5Y9/1.5	F	powd	powd; some subl	P	1.2976	+2.3	P
Menadione sodium bisulfite (water soluble Vitamin K)	5	I,II,III	62	N9.25/	5GY2/1	(F)	powd	polym	F	5.0076	-9.9	P
PARTIAL HYDROLYSATES												
Casitone vitamin free, dehydrated	5	I,II,III	63	5Y9/1	10YR4/6	F	powd	slush	F	1.9147	-37.3	F+
Proteose Peptone No. 3	5	I,II,III	65	2.5Y8.5/4	10YR2/2	F	large cryst	polym	F	2.9961	-79.4	F
Bacto-soytone	5	I,II,III	66	2.5Y8.5/4	10YR2/2	F	powd	polym	F	1.9516	-101.6	F
NUCLEIC ACID BASES												
Adenine, N.R.C.	1	I,II,III	69	N9.5/	N9.5/	P	powd	powd; some subl	P	2.7798	-0.3	P
Cytosine, N.R.C.	3	I,II,III	70	N9.5/	5Y9/1	F	powd	powd	P	3.3319	-2.5	P+
Guanine	1	I,II,III	71	N9.5/	N9.5/	P	powd	powd; some subl	P	2.9804	-1.0	P
Hypoxanthine, N.R.C.	3	I,II,III	72	5Y8.5/1	5Y9/2	F	powd	powd	P	3.3381	-6.7	(P)
Orotic acid (uracil 4-carboxylic acid)	3	I,II,III	73	N9.5	10YR9/1	F	powd	caked powd	P	1.6159	-3.5	P
Thymine (5-methyluracil)	1	I,II,III	74	5Y8.5/1	5Y8.5/1	P	fl	fl	P	0.9469	-2.0	P
Uracil	1	I,II,III	75	5Y9/1	5Y9/1	P	powd	powd	P	1.4641	-0.9	P
Xanthine	1	I,II,III	76	N9.5/	N9.5/	P	powd	powd	P	3.4893	-0.9	P
BIOLOGICAL EXTRACTS												
Beef extract	4	I,II,III	77	10YR2/1	10YR2/1	P	paste(foamy)	paste (no foam); some subl	P	4.7279	-59.3	F
Beef heart for infusion	5	I,II,III	78	2.5Y8/2	10YR7/4	F	powd	caked powd	P	1.4905	-25.6	F+
Malt extract	5	I,II,III	79	2.5Y9/2	10YR2/2	F	powd	polym	F	2.9503	-	F
Yeast extract	5	I,II,III	80	10YR9/2	10YR2/1	F	powd	slush	F	1.9908	-84.5	F
INORGANIC SALTS												
Ammonium chloride, granular, reagent, A.C.S.	1	I,II,III	81	N9.5/	N9.5/	P	granl	granl; some subl	P	5.0215	-1.0	P
Ammonium molybdate, crystal, reagent, A.C.S.	5	I,II,III	82	10Y9/1	7.5YR9/2	(F)	loose granl	solid cake;some liq but reabsorbed	F	5.0857	-1.8	P
Ammonium phosphate, dibasic. crystal, reagent, A.C.S.	1	I,II,III,VII	83	N9.5/	N9.25/	P	ff granl	granl adhesion	P	4.9048	-3.9	P
Ferrous chloride, crystal, reagent	5	I,II,III	84	5Y8.5/4	2.5GY7/4	(F)	granl	solid cake; some liq	F	2.5397	-2.8	P
Ferrous sulfate, crystal, reagent A.C.S.	1	I,II,III	85	N7/	N7/	P	granl	granl	P	3.4890	+3.0	P
Manganous sulfate monohydrate, powder, reagent,A.C.S.	1	I,II,III	86	N9.5/	N9.5/	P	powd	powd	P	5.0577	+0.2	P
Potassium bicarbonate, crystal, reagent, A.C.S.	1	I,II,III,IV,IX	87	N9.5/	N9.5/	P	cryst	cryst	P	5.0438	-0.9	P
Sodium phosphate, dibasic, reagent, A.C.S.	1	I,II,III,VII	88	N9.5/	N9.5/	P	powd	powd	P	4.9839	-0.1	P
Sodium thiosulfate, reagent	1	I,II,III	89	N9.5/	N9.5/	P	granl	granl	P	5.0647	+0.3	P
MISCELLANEOUS												
Dipicolinic acid (2,6-pyridine dicarboxylic acid)	1	I,II,III	90	N9.25/	N9.25/	P	powd	powd	P	1.7716	-1.0	P
Furfural, reagent	5	I,II	91	5Y9/2	10YR2/2	F	liq	liq	P	6.5265	-	-
Lactide, reagent	3	I,II,III	92	10YR9/2	2.5YR3/4	F	granl	granl adhesion	P	1.9824	+0.9	P
Sodium citrate dihydrate, crystal, reagent	5	I,II	93	N9.25/	N9.25/	P	ff granl	solid cake;liq on walls;reabsorbed liq & broke ampoule	F	5.0547	-	-
Succinic acid, crystal, reagent	1	I,II,III,VIII	94	N9.25/	N9.25/	P	loose granl	granl adhesion	P	4.9472	+1.4	P
L-α-Aspartyl-β-naphthylamide, M.A.	3	I,II,III	95	N9.5/	5Y9/1	F	powd	powd	P	0.1914	-0.4	P
L-Histidyl-β-naphthylamide, M.A.	5	I,II,III	96	5Y9/1	2.5YR6/2	F	fine powd	fused cake; some subl	F	0.1760	+0.2	P
L-Phenylalanyl-β-naphthylamide, M.A.	5	I,II,III	97	5Y9/1	10YR3/6	F	fine powd	fused cake	F	0.1909	-1.9	F
Sodium formate, crystal, reagent	1	I,II,III,IV,IX	98	N9.5/	N9.5/	P	fine cryst	cryst adhesion	P	4.8056	+1.3	P
Sodium pyruvate, reagent	5	I,II,III	99	5Y9/2	10YR5/8	F	powd	particles adhesion	P	2.4403	-49.7	F
Urea, crystal, reagent, A.C.S.	4	I,II,III	100	N9.5/	N9.5/	P	coarse granl	fused cake; some subl	F	5.0544	-59.3	F++

* Abbreviations used to describe phase of test substances (Test III):

ff	free flowing	fl	flakes
powd	powder	subl	sublimate
cryst	crystals	liq	liquid
granl	granules	polym	polymer

TABLE II

LABORATORY DATA FOR TESTS IV, VI, VII, VIII AND IX

Substance and Quality	Code No.	Test IV. Particles in Solution				Test VI. Differential Refractometry						Test VII. X-ray Diffraction Analysis			Test VIII. Melting Point Determination, °C				Test IX. Barium Carbonate Precipitation			Code No.
		g/ml H_2O	Control	Heated	Result	Control & Heated, Same Conc. % Conc.	Δd	Two Controls, 1% Diff. in Conc. % Conc.	% Conc.	Δd'	Result	Control	Heated	Result	Control	Heated	Mixture	Result	Control	Heated	Result	
AMINO ACIDS																						
L-α-Alanine, N.R.C.	2	0.2500/3	clear	clear	P	7.70	18	7.70	7.61	231	P											2
L-Arginine hydrochloride, N.R.C.	3	1.500/3	clear	clear	P	33.33	82	33.33	33.0	668	P											3
L-Aspartic acid, N.R.C.	5	0.0575/30	clear	clear	P	0.19	10	0.19	0.188	61	P											5
L-Glutamic acid hydrochloride, C.P.	8	0.3750/3	clear	clear	P	11.12	15	11.12	11.00	239	P											8
Glycine, N.R.C.	10	0.7500/3	clear	clouded	F	20.00	1773	20.00	19.8	1335	F											10
L-Histidine, N.R.C.	11	0.6000/30	clear	clear	P	1.96	25	1.96	1.94	65	P											11
4-Hydroxy-L-proline, N.R.C.	12	0.7500/3	clear	clear	P	20.00	31	20.00	1.98	443	P											12
L-Isoleucine, N.R.C. (allo free)	13	0.3250/30	clear	clear	P	1.07	91	1.07	1.06	34	F											13
L-Leucine (methionine free)	14	0.0130/30	clear	clear	P	0.0433	14	0.0433	0.0429	43	P											14
L-Lysine hydrochloride, N.R.C.	15	1.5000/3	clear	clear	P	33.33	149	33.33	33.00	379	P											15
L-Methionine, N.R.C.	16	0.1000/3	clear	clear	P	3.23	20	3.23	3.20	33	P											16
L-Phenylalanine, N.R.C.	17	0.4500/30	clear	clear	P	1.48	15	1.48	1.46	88	P											17
L-Threonine, N.R.C. (allo free)	20	0.1500/3	clear	clear	P	4.76	41	4.76	4.71	67	P											20
L-Tryptophan, N.R.C.	21	0.1500/30	clear	clear	P	0.498	11	0.498	0.493	11	(F)											21
L-Tyrosine, N.R.C.	22	0.0100/30	clear	clear	P	0.0333	21	0.0333	0.0330	43	P											22
L-Valine, N.R.C.	23	0.1250/3	clear	clear	P	4.0	47	4.0	3.96	61	P											23
ALCOHOLS AND POLYOLS																						
Dulcitol (D-galactitol), N.R.C.	43														186-188	186-188	186-188	P				43
D-Mannitol, N.R.C.	46														165-167	165-167	165-167	P				46
VITAMINS																						
Biotin, crystalline	52														229-230	229-230	229-230	P				52
INORGANIC SALTS																						
Ammonium phosphate, dibasic, crystal, reagent, A.C.S.	83											25%	25%	P								83
Potassium bicarbonate, crystal, reagent, A.C.S.	87	1.0/3	clear	clear	P														clear	clear	P	87
Sodium phosphate, dibasic, reagent, A.C.S.	88											100%	100%	P								88
MISCELLANEOUS																						
Succinic acid, crystal, reagent	94														187-189	187-189	187-189	P				94
Sodium formate, crystal, reagent	98	1.0/2	clear	clear	P														clear	clear	P	98