OBSERVATIONS ON THE DETERIORATION OF GLUCOSE-^{14}C

NOME BAKER*, A. P. GIBBONS, AND REGINALD A. SHIPLEY

Radioisotope Service, Veterans Administration Hospital, and the Departments of Biochemistry and Medicine, School of Medicine, Western Reserve University, Cleveland, Ohio (U.S.A.)

Recently our attention became focused upon the decomposition of ^{14}C-labeled glucose resulting from possible self-irradiation or from sterilization at high temperatures. A review of the literature showed that despite the extensive use of glucose-^{14}C as a tracer in biochemical studies, very little work has been published relating to the stability of glucose-^{14}C during autoclaving or during storage. Although certain preparations of high specific activity glucose-^{14}C have been observed to turn red upon storage in the dry state**, polyalcoholic compounds are not considered to be particularly susceptible to decomposition by self-irradiation[1].

In the preparation of glucose-^{14}C from plant and animal polysaccharides and in the sterilization of glucose for parenteral administration, high temperatures are often employed. During a metabolic study in this laboratory, glucose-^{14}C in the concentration of 12 mg/ml distilled water had been autoclaved for 20 min at 120° and stored frozen for two years with negligible deterioration***. Subsequently, however, a more dilute solution of glucose-^{14}C (approx. 0.14 mg/ml) had been observed to undergo extensive decomposition after similar treatment. Aside from the difference in concentration of glucose in the heat-resistant and heat-labile solutions, another known variable was the specific activity of the original samples. The glucose-^{14}C of the more dilute solution had a specific activity 70 times that of the labeled sugar in the more concentrated solution.

The present studies are concerned with the stability of glucose-^{14}C when stored in frozen solution or in the dry state, and when subjected to autoclaving in solutions of varying carrier and pH.

EXPERIMENTAL

Uniformly-labeled glucose-^{14}C was purchased from California Foundation for Biochemical Research. We are indebted to the latter foundation for additional gifts of the compound and for data regarding its commercial preparation. The glucose-^{14}C had been prepared by photosynthesis, using Canna leaves. Purification had been accomplished by paper chromatography, using first a phenol–water system, then a butanol–acetic acid system and finally again a phenol–water system.

Three different batches of the radioactive sugar were used in compiling the present data. The first two were received within one week after being prepared. One of these was reddish-brown when received (Prep. I) but was described by the manufacturer as being better than 99.5% pure,

* Present address: Radioisotope Service, Veterans Administration Center, Los Angeles 25, Calif. (U.S.A.).

** M. VOLK, Personal communication.

*** Unpublished observations using paper chromatography.

References p. 586.

radiochemically. The other two batches were white, having been subjected to an additional purification over a Duolite A-4 anion-exchange resin. One of these (Prep. II) was a purification of Prep. I; the other (Prep. III) was purchased a year later and no data from the manufacturer were obtained regarding the minimum degree of radiochemical purity.

Chromatograms were prepared by placing approximately 10 μl glucose-^{14}C solution on 1 inch strips of Whatman No. 1 paper. The strips were developed in butanol–glacial acetic acid–H_2O (50:11:25) by descending chromatography and dried at room temperature. Radioautographs of the air-dried paper strips were prepared using no-screen X-ray film. These were exposed for from 3 to 60 days, depending upon the level of activity on the chromatogram. In order to allow a more meaningful comparison of different radioautographs, the exposure index (E.I.) was calculated in each case; E.I. = c/s $\times$ days exposure $\times$ 10^{-2}. The R_F of glucose averaged 0.23. Counts of active spots were made with a thin-window Geiger tube and compared with counts made of the original spot at the origin before development. Both sides of the paper were counted in every case.

RESULTS

A. Stability of high specific activity glucose-^{14}C during storage in frozen aqueous solution

A solution of high specific activity glucose-^{14}C (Prep. II) (20 μC, 0.14 mg/ml distilled H_2O) was chromatographed two days after arrival in the laboratory (Fig. 1A). Some of this solution was divided into separate tubes and stored frozen in the deep freeze. In Fig. 1B, C and D are shown radioautographs of solutions which were chromatographed 0.5, 2 and 8 months, respectively, after the date of preparation of the glucose-^{14}C solution. After 2 months of storage, there was evidence of the formation of a minute amount of a new radioactive compound having an R_F slightly larger than glucose. There was slight, if any, further deterioration after 6 months of additional storage. The non-glucose spot was estimated to have no more than 1% of the activity in the glucose spot. No significant destruction of Prep. I was found after 8 months of storage in frozen solution.

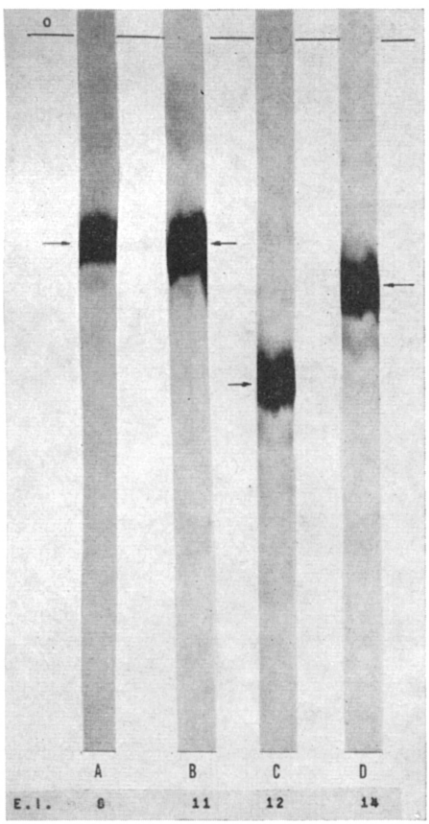

Fig. 1. Autoradiograms of high specific activity glucose-^{14}C after storage in frozen aqueous solution. The radioactive sugar was stored for 2 weeks (B), 2 months (C), and 8 months (D). The control (A) was never frozen and was chromatographed immediately after being dissolved. The origin (O) is at the top; chromatography was descending using a butanol–acetic acid–water system. The glucose spot is indicated by the arrow. The exposure index (E.I.) is defined in the text.

References p. 586.

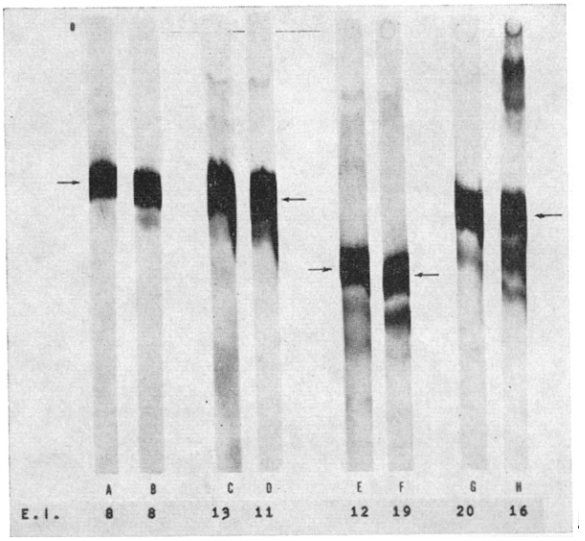

Fig. 2. Autoradiograms of high specific activity glucose-^{14}C after storage, dry. A and B were prepared two days after the labeled glucose was received. The others were stored for two weeks (C, D), eight weeks (E, F), and eight months (G, H). In the cases of A, C, E, and G only, the glucose-^{14}C had been purified by passage over an anion-exchange resin. See Fig. 1 for additional details of the chromatographic procedure, etc.

B. Deterioration of high specific activity glucose-^{14}C during storage, dry

Ten-μl aliquots of the solution described above (in Section A) and of a similar solution made from Prep. I were delivered to a spot plate, and allowed to evaporate to dryness at room temperature. The spot plate was placed in a covered box and stored at room temperature in a cabinet. Sterile techniques were not used. After storage for various lengths of time (2 weeks to 8 months), the dried glucose-^{14}C was dissolved and chromatographed. Based upon actual counts of radioactivity on the chromatogram, Preps. I and II seemed to have undergone similar degrees of deterioration. A maximum of 85 % of the glucose was recovered at the glucose spot after 8 months of storage in both samples. However, the radioautographs (Fig. 2) showed marked differences between Preps. I and II. Some deterioration was noted as early as one week following storage of the dried material in both preparations. After 8 months of storage, the destruction of Prep. I was extensive (Fig. 2H). Prep. II, after storage for the same period of time showed slight, but unquestionable deterioration (Fig. 2G). Apparently something was present in the less highly purified material (Prep. I) which accelerated decomposition during storage, "dry". Since breakdown products of each sample (Preps. I and II) seem to have identical R_F's, some of the same contaminant which hastened destruction of Prep. I may have been active in Prep. II even after passage through the ion-exchange resin.

C. Decomposition of high-specific activity glucose-^{14}C during autoclaving in distilled water

Aliquots of Preps. I and II similar to those used in A and B above were placed in individual tubes (Virtis screw-cap, alkali-free culture tubes) and autoclaved for 20 min at 15 lb pressure, 120°. The results of these experiments are shown in Fig. 3. Marked destruction was observed in both solutions; however, that seen in Prep. I is much greater than that of the ion-exchange-treated glucose-^{14}C (compare Fig. 3D with Fig. 3B). Although small amounts of radioactive material were found to be spread over the length of the chromatogram after autoclaving, the major breakdown

References p. 586.

products are similar in nature to those observed in Fig. 2H. This suggests that the material which promotes deterioration of glucose-^{14}C during "dry" storage also accelerates glucose destruction during autoclaving, and that the rate of the destruction process itself is increased by heat.

Quantitative estimation of the percent destruction of glucose due to autoclaving was attempted by the direct counting of radioactivity on the paper strip. In the case of Prep. I, only 19 % of the total activity applied to the paper was recovered at the glucose spot. In three other similarly treated samples of Prep. I which had been stored frozen for 2 weeks, 2 months and 8 months, respectively, the corresponding recoveries were 30 %, 19 % and 38 %. The recoveries of glucose-^{14}C estimated for autoclaved samples of Prep. II were 96 %, 61 %, 53 % and 68 % after storage in the deep freeze for 0, 0.5, 2 and 8 months, respectively; the value of 96 % seem to be erroneously high according to the autoradiogram (Fig. 3B).

Because it had been observed previously that a more concentrated solution with lower specific activity could be autoclaved with almost no noticeable deterioration,

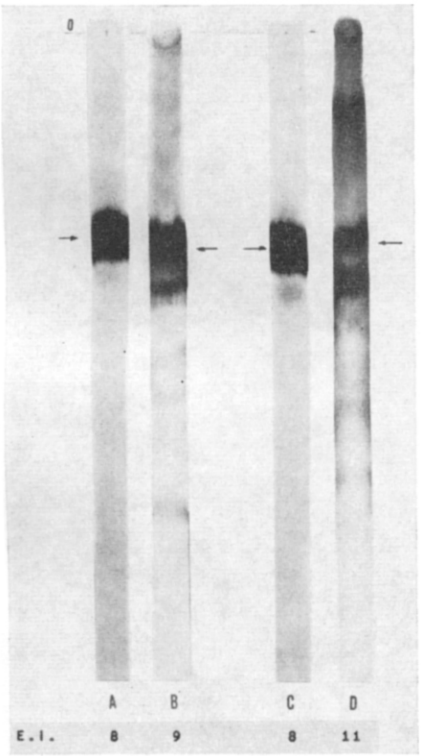

Fig. 3. Autoradiograms of solutions of high specific activity glucose-^{14}C before and after autoclaving. A and C, not autoclaved. B and D autoclaved. The radioactive glucose had been purified by passage over an anion exchange resin in A and B (Prep. II, but not in C and D (Prep. I). See Fig. 1 for additional details of the chromatographic procedure, etc.

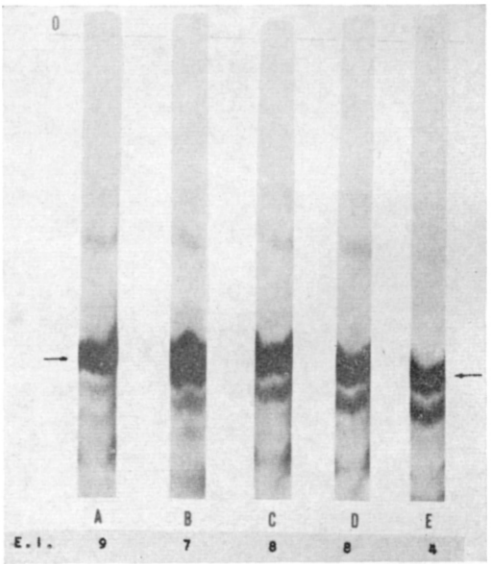

Fig. 4. Autoradiograms of solutions of glucose-^{14}C whose specific activity and concentration were varied during autoclaving. Prep. III (anion-exchange-treated) was used in each case. A, not autoclaved. B, C, D and E had 10, 4, 2, and 0.8 mg of non-radioactive glucose added, respectively, per ml of solution. See Fig. 1 for additional details of the chromatographic procedure, etc.

References p. 586.

a controlled experiment was carried out to determine the relationship between glucose concentration and decomposition during autoclaving. Prep. III was used to prepare a solution containing approximately 20 μC, 0.14 mg/ml distilled water. Although Prep. III was supposed to be of purity comparable to that of Prep. II, several radioactive contaminants were found in the former when it was examined chromatographically immediately after being dissolved in water (Figs. 4A and 5A). The chromatographic pattern of the contaminants appears to be similar to that noted in Figs. 2H and 3D. Approximately 10% of the radioactivity was present in compounds other than glucose-^{14}C. This degree of contamination in Prep. III interfered with the quantitative estimation of the recovery of glucose in the experimental samples. Carrier, non-radioactive glucose ranging in amount from 1.6 to 20 mg glucose was added to four tubes, each of which contained approximately 0.2 μC of the dissolved Prep. III. The volumes were then adjusted to 2.0 ml and the tubes autoclaved under the usual conditions. In Fig. 4 the results of this experiment are shown. As the total concentration of glucose decreased, and the specific activity increased, the extent of glucose-^{14}C breakdown increased. Separate experiments (constant specific activities with varying concentrations of glucose) have shown that the extent of destruction during autoclaving is a function of total glucose concentration rather than a function of specific activity of the glucose-^{14}C.

D. Modifications in the destruction of glucose-^{14}C during autoclaving in the presence of dilute acids under aerobic and anaerobic conditions

In order to determine whether the deterioration of glucose-^{14}C was affected by acidification or withdrawal of oxygen, an experiment was carried out in which approximately 0.2 μC of Prep. III was dissolved in distilled water, dilute H_2SO_4 or dilute HCl. The experimental details and results of the experiment are shown in Fig. 5. Apparently the destruction of glucose during autoclaving in distilled water cannot be prevented by means of anaerobic conditions, but it may be almost, if not completely, inhibited by the presence of 0.5 *N* HCl. When 0.5 *N* H_2SO_4 was used, deterioration was not prevented; moreover, the pattern of breakdown products differed considerably from that found after autoclaving in distilled water. Interestingly, in a N_2 atmosphere, H_2SO_4 appears to produce a different "breakdown pattern" than it does in air. Most of the contaminants present in the control (nonautoclaved) glucose-^{14}C (Fig. 5A) were destroyed by the HCl treatment; however, two new products having greater R_F values were formed. The average ^{14}C recoveries, uncorrected for contamination in Prep. III, at the glucose spot were as follows: non-autoclaved control, 86%; autoclaved in water, 55% (B–D); autoclaved in 0.5 *N* HCl, 89% (E–G); autoclaved in 0.5 *N* H_2SO_4, 44% (H–J). Estimated recoveries for samples that were open to the autoclave, sealed in air, or in nitrogen were similar. The lowest recovery (26%) occurred in H_2SO_4 with a nitrogen gas phase.

The pH of the solutions of Prep. III which were autoclaved in the absence of acid (Figs. 4A–E and 5B–D) was determined before and after autoclaving. Before autoclaving the glucose solutions tended to have a lower pH (mean 6.1; range, 5.4–6.7) than did distilled water (6.6). After autoclaving, the pH rose (mean, 6.3; range 5.4–7.1). There was no apparent correlation between deterioration of glucose-^{14}C and pH within this range.

References p. 586.

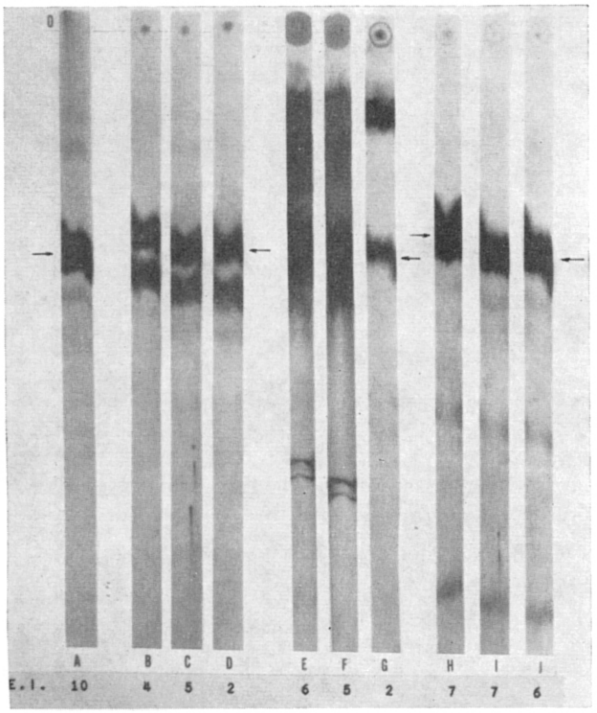

Fig. 5. Autoradiograms of solutions of glucose-^{14}C autoclaved in the presence of dilute acids under aerobic and anaerobic conditions. A, not autoclaved; B–D, distilled water; E–G, 0.5 *N* H_2SO_4; H–J, 0.5 *N* HCl. B, E, H, flame-sealed prior to autoclaving with air as the gas phase; C, F, I, autoclaved with a needle in the rubber cap of the vial; D, G, J, autoclaved in a flame-sealed vial with nitrogen (5 min flushing) as the gas phase. The volume of the autoclaved solution in each case was 2 ml. Excess sulphate (E–G) was removed with $BaCl_2$. Volumes were reduced by lyophilization, the residues dissolved in a total volume of 75 μl H_2O and applied to the chromatography paper in 5 μl aliquots. See Fig. 1 for additional details of the chromatographic procedure, etc.

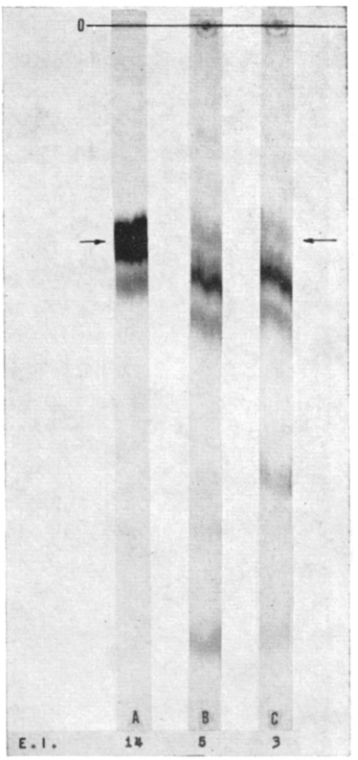

Fig. 6. Autoradiogram of autoclaved glucose-^{14}C after yeast fermentation. All three samples were autoclaved. A, not treated with yeast; B, C, treated with yeast in the presence (C) and absence (B) of 1 mg added glucose carrier. See Fig. 1 for additional details of the chromatographic procedure, etc.

E. The non-fermentable nature of the products of glucose deterioration

Two of the major products which are known to be formed from glucose in the presence of alkali are mannose and fructose[2]. Both of these hexoses are yeast-fermentable. In order to determine whether the products formed after the autoclaving of glucose-^{14}C were likewise yeast-fermentable, solutions of Prep. III (approximately 0.4 μC in 2 ml distilled H_2O) were autoclaved and incubated with a yeast preparation for 0.5 h in the presence (Fig. 6C) or absence (Fig. 6B) of 1 mg added glucose carrier. The results of these experiments may be seen in Fig. 6 in which autographs of the autoclaved, fermented solutions are compared with that of an autoclaved solution which was not yeast-treated (Fig. 6A). The major product formed after autoclaving (R_F slightly greater than glucose) appears to be non-fermentable. This is similar to the observations of DE WHALLEY *et al.*[3], who found that the products of heat destruction of fructose are non-fermentable.

References p. 586.

DISCUSSION

When high-specific-activity glucose-^{14}C is stored in a "dry" form, it may be subject to destruction by self-irradiation, bacterial degradation, or chemical decomposition due to the presence of a contaminant in the preparation. Although the first two of these possibilities cannot be ruled out from the present experiment, the presence of a material in some preparations of glucose-^{14}C which hastens the destruction of the sugar is strongly suggested. The destructive action of this substance may be prevented almost completely by storage in frozen solution. The contaminant may be removed, at least partially, by a single passage over a Duolite A-4 anion exchange column. Whether or not it may be removed entirely by repeated passage over such a column has not been established in the present investigation.

The deterioration of glucose-^{14}C during autoclaving seems to be related to the process of deterioration which was observed during "dry" storage: less deterioration took place in the material which was passed over an anion-exchange column; the products of decomposition traveled to the same positions on the chromatograms. A contaminating anion could combine with hydrogen ions from the weakly acidic glucose molecule. Such a loss of hydrogen ions from glucose might precede the intramolecular rearrangements and cleavages which take place in the presence of alkali. This explanation has been offered previously to explain the deterioration of glucose in the presence of acetate, succinate, and formate[4,5]. CIANCI AND PANNAIN[6] have reported a similar phenomenon in which the thermal destruction of non-radioactive glucose in the presence of a phosphate buffer was increased in direct proportion to the buffer capacity of the solution. In apparent contrast to the present findings, these same authors reported a greater destruction of glucose during autoclaving in the presence of high concentrations of glucose (80 mg/ml distilled water) than in low concentrations (5 mg/ml). However, this is not necessarily inconsistent with the present findings since an increased rate of *total* glucose destruction could have been masked by the diluent effect of added non-radioactive glucose. Theoretically, the total glucose destruction may be calculated from the product of (the fraction of the glucose-^{14}C destroyed) $\times$ (the total amount of glucose initially present). The estimates of % glucose-^{14}C destroyed were not considered sufficiently accurate to be used in such a calculation.

The reason(s) for the observed protective effect of carrier glucose or dilute HCl against destruction of glucose-^{14}C during autoclaving is not certain. However, at least two hypotheses may be suggested. One explanation is based upon the special property of HCl to combine with free radicals[7]. The latter are known to be formed as a result of either heating or irradiating organic compounds. Once formed, they may catalyze the further deterioration of the organic molecule[8]. By reacting with the free radicals formed, HCl may prevent the catalysis of glucose-^{14}C deterioration. If free radicals catalyze the destruction of glucose noted here, carrier glucose would serve to protect the radioactive sugar by competing with the labeled molecules for the free radicals. The second explanation is based upon the acidic properties of both glucose and HCl. They could substitute for the labeled glucose as hydrogen donors. The failure of dilute H_2SO_4 to afford protection may be reconciled with the latter hypothesis by noting that H_2SO_4 itself has special oxidative properties. This could account for the unique pattern of destruction observed when this acid was present during the autoclaving of

References p. 586.

glucose-^{14}C. OST AND BRODTKORB[9] have reported the breakdown of dextrose in the presence of dilute sulfuric acid and heat. Levulinic acid, formic acid and humic substances were formed. These authors also noted that destruction during autoclaving with or without acid, was considerably more extensive at 145° than at 120°.

The protective action of dilute HCl and the destructive action of dilute H_2SO_4 during the autoclaving of solutions of glucose is of considerable practical interest in the hydrolysis of polysaccharides. In comparing the yield of glucose from glycogen during hydrolysis at 100° in 5 *N* H_2SO_4 *versus* 1 *N* HCl, several authors have concluded that the former was preferable[10,11]. This phenomenon should be reinvestigated in the light of the present findings.

Another application of the present findings is concerned with the sterilization of solutions of glucose-^{14}C for parenteral administration and for purposes of bacteria-free storage. Although the optimium conditions for sterilization have not been established in the present work, certain precautions may be suggested if glucose-^{14}C solutions are subjected to the high temperature associated with autoclaving. Solutions of glucose-^{14}C should be autoclaved in the presence of a low concentration of hydrochloric acid and, if possible, in the presence of carrier glucose. In addition, passage of the labeled sugar over an anion exchange column prior to autoclaving is desirable.

ACKNOWLEDGEMENT

We wish to thank Dr. B. M. TOLBERT of the Radiation Laboratory, University of California, Berkeley, for his helpful criticisms of the manuscript.

SUMMARY

1. The decomposition of several preparations of glucose-^{14}C was studied under the following conditions: frozen storage, dry storage, and autoclaving (aerobically, anaerobically, in water, 0.5 *N* HCl and 0.5 *N* H_2SO_4).

2. Glucose-^{14}C of high specific activity underwent extensive decomposition during storage in the dry state and during autoclaving unless either 0.5 *N* HCl or sufficient carrier glucose was present during the autoclaving process.

3. Destruction seemed to be dependent, in part at least, upon the presence of an unidentified, non-radioactive, anionic contaminant of the glucose-^{14}C.

REFERENCES

1 B. M. TOLBERT AND R. M. LEMMON, *Radiation Research*, 3 (1955) 52.
2 C. A. LOBRY DE BRUYN AND W. ALBERDA VAN EKENSTEIN, *Rec. trav. chim.*, 14 (1895) 203.
3 H. C. S. DE WHALLEY, N. ALBON AND D. GROSS, *Analyst*, 76 (1951) 287.
4 P. A. ASHMARIN AND A. D. BRAUN, *Byull. Eksptl. Biol. Med.*, 4 (1937) 374; cited in *Chem. Abstr.*, 33 (1939) 7279.
5 P. A. ASHMARIN AND A. D. BRAUN, *Arch. sci. biol. (U.S.S.R.)*, 42 (1936) 61; cited in *Chem. Abstr.*, 31 (1937) 8513.
6 DI V. CIANCI AND L. PANNAIN, *Soc. Ital. Biol. Sperm.*, 7 (1932) 1442.
7 A. O. ALLEN, C. J. HOCHANADEL, J. A. GHORMLEY AND T. W. DAVIS, *J. Phys. Chem.*, 56 (1952) 575.
8 W. A. WATERS, *The Chemistry of Free Radicals*, Clarendon Press, Oxford, 1946, pp. 10 and 132.
9 H. OST AND T. H. BRODTKORB, *Chem. Ztg.*, 35 (1911) 1125.
10 M. SAYHUN, *J. Biol. Chem.*, 103 (1933) 203.
11 B. SJÖGREN, T. NORDENSKJÖD, H. HOLMGREN AND J. MÖLLERSTROM, *Arch. ges. Physiol.*, 240 (1938) 427.

Received November 2nd, 1957

References p. 591.