by the pharmaceutical industry. Many of the substances used in the formulation of medicinals are polymorphic and frequently exhibit melting points, solubilities, pharmacological activities, and other important properties which differ markedly from each other; as a result, the conversion of one form to another upon aging presents an important problem, as it can often curtail the shelf life of products.

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Notes

Chemical Effect of High Level Gamma Irradiation on Blood Glucose *in Vitro*

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Furan-2,5-dicarboxylic acid is one of the decomposition products of blood glucose subjected to high energy gamma irradiation.

THE EFFECT of ionizing radiation on materials of biological interest is important to our understanding of radiation hygiene. Pioneering studies have been made on the changes produced in amino acids, proteins, carbohydrates, and other biochemicals upon bombardment with nuclear radiation.

Changes in aqueous solutions of biochemicals upon irradiation are complex. Due to free radical formation, a large quantity of hydrogen is formed upon irradiation of water containing traces of halides. Also, dissolved oxygen in irradiated water will react with (H) radicals present to form the free radical (HO_2). Therefore, oxidizable or reducible solutes present in an irradiated aqueous medium may be readily oxidized or reduced.

Because of its importance to living organisms, we have been interested in the effect of ionizing radiation on blood glucose.

It is possible that the effect of irradiation of blood glucose and other blood constituents may provide an insight into the mechanism of radiation sickness. Since blood acts as a transportation agent for the body and as a solvent for so many complex compounds, it could be the source or carrier of toxic decomposition products resulting from irradiation.

Radiation effects on blood glucose have been noted both *in vitro* and *in vivo* (1–4). Irradiation of both dogs and rats has resulted in the development of hypoglycemia.

One could expect that the effects of ionizing radiation on blood might produce several by-products from blood glucose since blood would yield many other ions and radicals besides those of water upon irradiation. These other substances may alter the process of decomposition or react quickly with the products.

Previous research by us indicated that furan-2,5-dicarboxylic acid might be one of the irradiation decomposition products of glucose solutions. The objective of this study, therefore, was to show whether furan-2,5-dicarboxylic acid is produced upon irradiation of blood.

EXPERIMENTAL

Irradiation of Blood Plasma.—Rabbit blood plasma was chosen as the medium in which the effects of gamma radiation on blood glucose would be studied. Heparinized blood was centrifuged, the plasma decanted, and kept under refrigeration. Some hemolysis occurred.

A total of 9.01 mg. of radioactive glucose randomly-labeled with carbon^{14} was added to 8 ml. of the blood plasma contained in a small beaker. The specific activity of the radioglucose was 1.0 mc. per mmole, and a total activity of 50 μc. was present. After solution was evident, the blood plasma was poured into a half-ounce prescription bottle, and the beaker was rinsed twice with approximately 4-ml. portions of blood plasma, the rinsing combined with the original solution in the bottle.

For irradiation, the bottle containing the labeled blood plasma was fastened to a pegboard constructed in such a manner that the cobalt^{60} irradiation source could be positioned at a known distance by remote control. The source was placed exactly 3 inches from the bottle.

The Co^{60} source had a gamma ray activity of 103.6 curies. At a distance of 3 inches the blood plasma received an approximate dose of 23,760 roentgens per hour or a total of approximately 1,200,000 roentgens in the 50.5 hours of irradiation.

Radiochemical Analysis by the Carrier Technique.—A 100-mg. portion of synthesized furan-2,5-dicarboxylic acid was added to the irradiated plasma. The carrier furan-2,5-dicarboxylic acid was then solated from the irradiated system by the following process.

Received May 29, 1961, from the Division of Pharmacy, South Dakota State College, Brookings.
Accepted for publication July 19, 1961.
Abstracted in part from a dissertation presented to the Graduate Division of South Dakota State College by Linda Kay Rames in partial fulfillment of the requirements for the Master of Science degree, August 1960.
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The irradiated blood plasma was heated until a brownish-gray, dry mass was produced and, after cooling, 100 ml. of 50% sulfuric acid was added to the residue. After heating at 180° for 1 hour, the resulting mixture was cooled and diluted with distilled water, filtered, and the carbonaceous material on the filter washed with two portions of a 1:1 ethanol-water solution.

The filtrate was heated to remove some of the ethanol in which the furan-2,5-dicarboxylic acid is very soluble. When the volume was reduced to 100 ml., heating was stopped and the solution cooled. The acid filtrate was then successively extracted in a continuous liquid-liquid extractor with 75-ml. portions of ether. A total of 525 ml. was used for the extraction. After evaporation of the ether extract, the residue was dissolved in 50 ml. of hot alcohol, filtered, and evaporated down to dryness in a tared counting planchet.

A radioassay of the residue in the planchet was performed using a windowless gas counter. The weight of the residue assayed was 58.7 mg. and the activity was 89,456 c.p.m., corresponding to a specific activity of 1,524 c.p.m. per mg.

The planchet was then placed in a beaker and the residue dissolved in hot alcohol, activated charcoal was added and the material boiled, filtered hot, evaporated, and radioassayed in the same manner as the first sample.

The white crystalline residue weighed 11 mg. with an activity of 14,424 c.p.m., corresponding to a specific activity of 1,311 c.p.m. per mg.

The residue was purified a third time with a final sample weight of 10.7 mg. with an activity of 14,775 c.p.m. corresponding to a specific activity of 1,381 c.p.m. per mg.

A distilled water solution of the final residue showed ultraviolet absorption characteristics similar to that of irradiated glucose solutions and synthesized furan-2,5-dicarboxylic acid.

DISCUSSION

Though furan-2,5-dicarboxylic acid was isolated as a metabolic product by Flashentrager, *et al.* (5), as early as 1937, its physiological action and biochemical transformation have not been extensively studied.

Research (6, 7) has indicated that the formation of furan-2,5-dicarboxylic acid in a biological system is dependent on the presence of glucuronic acid or galacturonic acid rather than that of glucose. This indicates that the first step in the metabolic formation of furan-2,5-dicarboxylic acid from glucose would require oxidation to glucuronic acid or rearrangement and oxidation to galacturonic acid.

The formation of radicals from the ionization of water provides a medium in which these oxidative processes are probable.

The formation of radicals during the irradiation of blood would be enhanced by the presence of halide ions. Of course, the complexity of blood would lead to the formation of ions and other radicals besides (H) and (OH).

Evidence indicates that the second step in the formation of furan-2,5-dicarboxylic acid would be formation of ketohexose by further oxidation or rearrangement of glucuronic acid (7).

Ring closure of the ketohexose would yield a heterocyclic ring containing oxygen with substituents on carbons 2 and 5. Dehydration of this structure by removal of two hydrogen and two hydroxyl groups would yield the furan ring. Evidence has already been produced proving that oxidation of the substituents on carbons 2 and 5 of the furan ring occurs readily (7).

Under the conditions of our experiment it is apparent that furan-2,5-dicarboxylic acid is a decomposition product of blood glucose upon irradiation of blood plasma *in vitro*.

The presence of radioactivity in the sample of furan-2,5-dicarboxylic acid indicates beyond a doubt that this product resulted from a transformation of the radioglucose added to the blood plasma.

The difference in activity of the purified and unpurified samples was probably due to large quantities of degradative fragments of radioglucose and possibly other organic substances formed by the action of the concentrated sulfuric acid. Since other radioactive compounds can be formed, it is essential that the furan-2,5-dicarboxylic acid isolated be purified to a constant specific activity in order to state that the activity is due mainly to radioactive furan-2,5-dicarboxylic acid. It can be seen from a comparison of the specific activities of the second and third isolations (1,311 c.p.m./mg. *vs.* 1,381 c.p.m./mg.) that purification of the furan-2,5-dicarboxylic acid was adequate for our conclusions. In addition, the ultraviolet absorption characteristics of the isolated product agreed with those of synthesized furan-2,5-dicarboxylic acid.

CONCLUSIONS

1. A study of a chemical effect of high level gamma irradiation on blood glucose *in vitro* using the radioactive tracer technique has been made.
2. Furan-2,5-dicarboxylic acid was shown to be one of the decomposition products formed from high level gamma irradiation of blood glucose *in vitro*.
3. A further study of the effect of irradiation of blood on blood glucose with radioactive glucose would be feasible to prove the identity of other possible decomposition products.

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