intensity of the chemiluminescence increases even more [2]. With our present photomultipier assembly it was not possible to obtain a time dependency curve of this reaction.

In order to elucidate this catalytic effect of irradiated glucose on the decomposition of hydrogen peroxide, and organic peroxides and hydroperoxides, we tested some compounds which possibly arise by the action of ionizing radiation on glucose [1]. The relative luminescence intensities of the non-irradiated substances investigated in the alkaline hydrogen peroxide system were: glucose 0, glyceraldehyde 100, dihydroxyacetone 7, arabinose 0, glucuronic acid 1. From these data it can be tentatively concluded that glyceraldehyde may possibly be one of the substances responsible for the luminescence of irradiated glucose. This hypothesis is strengthened by the fact that non-irradiated glyceraldehyde exhibits a strong luminescence in aqueous alkaline solution without added peroxide.

Infrared spectroscopy showed that the irradiation induced carboxyl groups already in the solid glucose, while carbonyl groups are not formed until the irradiated glucose reacts with water.

By means of electron spin resonance, it has been shown that long-lived free radicals are induced by X-rays in different substances and in living material [3-5 and quoted literature]. It has been strongly indicated — that at least part of the biological radiation effects are produced *via* free radicals. A portion of the radiation damage in dry plant seeds is latent and can be modified during storage by, *e. g.*, temperature and oxygen. However, when the water content of the seeds rises, *e. g.* during germination, the damage becomes manifest, (*cf.* discussion) [7].

The observations described in this communication may give a method by which we hope to be able to elucidate the role played by water and oxygen in radiobiological processes. The phenomena studied in this communication are also relevant in the discussion concerning the radiation preservation of foodstuffs and the possible mutagenicity of these. It is worthwhile in this connection to mention that mutations have been observed when biologic material — barley and wheat seeds — is treated with the aqueous solutions of irradiated substances, *e. g.* glucose, glycine [8], agar [9].

The investigations are being continued on a quantitative and qualitative basis, including emission spectroscopy during and after irradiation with ionizing radiation.

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Use of Butyl Acetate in Determination of Sialic Acid

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Determination of sialic acid can be performed with several reactions: the Bial [1,2], direct Ehrlich [3], tryptophane-perchloric acid [4], diphenylamine [5,6] and sulphuric acid — acetic acid [7] reactions. Orcinol used in Bial's reaction has been replaced by resorcinol [8]. In all orcinol and resorcinol reactions presented in the literature the colour is extracted by amyl alcohol. This solvent extracts very effectively the developed pigments, but its disadvantages are the long procedure of purification, the necessity of centrifugation after extraction, and especially its ability to extract also the false pigments produced by other carbohydrates. Ion exchange resins and dichromatic readings are used to eliminate

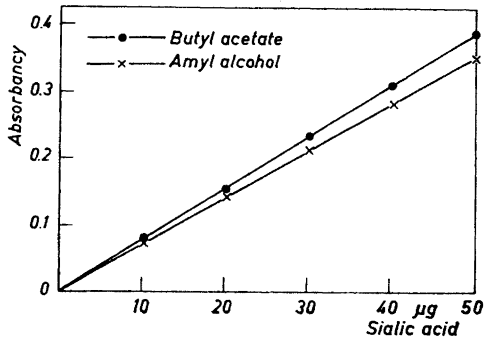

Fig. 1. Standard curves of N-acetylsialic acid read at 580 mμ against the blank. — ● — ● — Extracted with 4 ml of butyl acetate. × — × — × Extracted with 4 ml of amyl alcohol.

this last mentioned error [2,3,9]. The purpose of this work was to find an extracting solvent possessing as little of the mentioned disadvantages as possible.

The determination of sialic acid was performed by the resorcinol reaction [8], using as standard N-acetylsialic acid.* Of the many tested organic solvents, only butyl acetate, in addition to amyl alcohol, was found to be suitable. When used in 100 % concentration its ability to extract was negligible, but after addition of butyl alcohol this capacity was increased. For this reason *Butylium aceticum 85 percent normal ("Merck")* was chosen for use as such. Some minutes after extraction this solvent separates as crystal-clear layer without centrifugation also when proteins are present. The corresponding determinations were performed with both amyl alcohol and butyl acetate, using 4 ml of each solvent. The photoelectric readings were made with the Beckman Spectrophotometer model B in 10 mm cells.

It is seen from Fig. 1 that butyl acetate extracts increasing amounts of N-acetylsialic acid correctly. The two curves deviate because amyl alcohol dissolves more of the

* The N-acetylsialic acid was kindly presented to us by Dr. Lars Svennerholm, M. D., Gothenburg, Sweden.

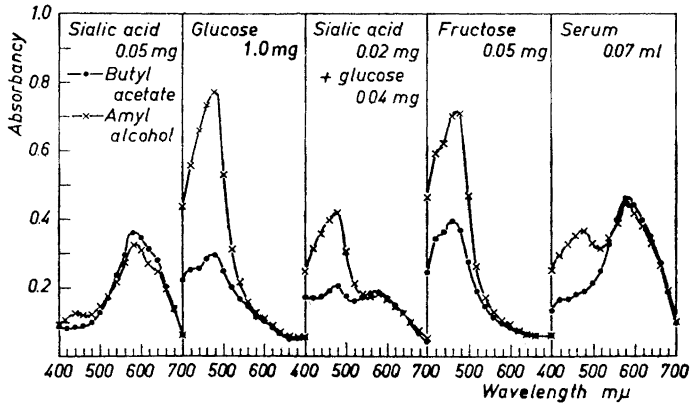

Fig 2. Absorption curves of N-acetylsialic acid, glucose, mixture of glucose and N-acetylsialic acid, fructose and serum without precipitation of proteins. Readings against reagent blank.

water layer, resulting in lower extinctions. It was observed that when the final volume in the reaction increased from 2 to 8 ml and the extraction was performed with 4 ml in each case, the absorbancy was somewhat smaller in greater volumes when butyl acetate was used. With amyl alcohol it remained constant. Thus butyl acetate seems to have a somewhat lower extracting capacity. This is, however, sufficient if the volume used in the reaction is 2—4 ml.

The shapes of the absorption curves in Fig. 2 are quite similar for both solvents, the maximum being at 580 mμ. At shorter wavelengths there is a slight difference, however. Examples of absorption spectra produced by carbohydrates (Fig. 2) are those of fructose and glucose. These substances have their maxima around 480 mμ. As is seen from the figure, butyl acetate has a remarkably lower peak at this point. Thus butyl acetate does not extract all the disturbing pigments, which is apparent already macroscopically from the strikingly yellow colour of the water layer after extraction if false pigments are present. Also at 580 mμ the absorbancy of glucose is smaller in the butyl acetate extraction. One mg of glucose corresponds to about 15 μg of sialic acid when butyl acetate is used, and to 21 μg with amyl alcohol.

From the absorption curves for a mixture of glucose and sialic acid and for serum rich in disturbing substances it is seen that butyl acetate leaves most of the disturbing pigments in the water layer.

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