TECHNICAL PAPERS

Total White Cell Counts of Peripheral and Heart Blood of the Rat

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Little attention has been given to the possible difference in white cell distribution in the circulatory system. Law and Heston (*6*) found a marked difference in the total white cell count of heart and peripheral blood in albino mice. Since these workers employed nembutal anesthesia before making the cardiac puncture (*7*) and since nembutal has been shown to produce hemodilution in experimental animals (*1–3, 5*), there remained the question of whether the low white count of blood taken from the heart was due to the anesthesia or was a normal condition in the unanesthetized animal.

Blood samples were taken from the periphery and heart of 10 adult male albino rats. The peripheral blood sample was obtained by clipping the tip of the tail and using the free-flowing blood. The heart blood was taken by cardiac puncture immediately after stunning the rat with a blow on the head. Total white cell counts were made on the blood sample. In order to ascertain any possible alteration in blood concentration as a result of the head blow, determinations were also made on the red cell numbers

TABLE 1

(Average of 10 animals)

	Peripheral	Heart
White cell numbers/mm³	23,810	6,425
Red cell numbers/mm³	8,967,000	8,790,000
Specific gravity of blood	1.0529	1.0526

and the specific gravity of the blood. The blood cell counts were made by use of standard dilution pipettes, diluting fluids, and hemocytometers. The specific gravity of the blood was determined by the copper sulfate method of Phillips, *et al.* (*4*). The results are summarized in Table 1.

It is clear from the data in Table 1 that, while the red cell numbers and density of the blood taken from the tail and heart are of the same value, there is a marked difference in the white cell count. This shows that the blood from the two sources is of uniform concentration and that the low white count of the heart blood is not due to hemodilution. While it may be true that the extremely low leucocyte count obtained by Law and Heston (*6*) on the heart blood of mice was due in part to the anesthesia employed, there can now be no doubt as to the qualitative validity of their findings.

If this marked difference in the numbers of leucocytes in the peripheral and heart blood of mice and rats is true for mammals in general, researchers should take care that they compare blood samples from the same source only. It is suggested that there may be a significant clinical difference in the leucocyte count of blood taken from patients by venipuncture and that taken by pricking the finger or ear.

The high white cell numbers in the peripheral blood as contrasted with the very low numbers in the heart blood is probably concerned with the immunity functions of the leucocyte.

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Ultraviolet Light-Absorption of Alkali-treated Solutions of Carbohydrates

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The production of light-absorbing substances by the action of alkali on carbohydrates has long been recognized. A study of the ultraviolet absorption spectra of some carbohydrates has been made by Gabryelski and Marchlewski (*1*).

We found that similar absorption was produced when starch or starch fractions were dispersed in alkali and heated. Since amylose gave much more absorption than amylopectin, it was hoped that we had found an "end-group" determination which would be a direct function

[1] The authors are indebted to several members of the Starch and Dextrose Division of this laboratory and especially to Robert J. Dimler, who supplied many of the materials necessary to this work and whose advice was indispensable.

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of molecular weight. Later knowledge indicates, however, that under our present experimental conditions we have found a method similar to that of Schoch (*6*) for alkali lability but involving a more sensitive means of detection. The alkali lability method is a measure of the amount of alkali neutralized by acids formed in the reaction.

If there is a direct correlation between the amount of absorption produced in the alkali reaction and the molecular weights of the dextrose polymers, certain conditions must be fulfilled. These are: first, that the same absorbing group is produced in all cases; second, that the reaction occurs at an existing reducing end-group or at an end-group produced by the action of alkali; and third,

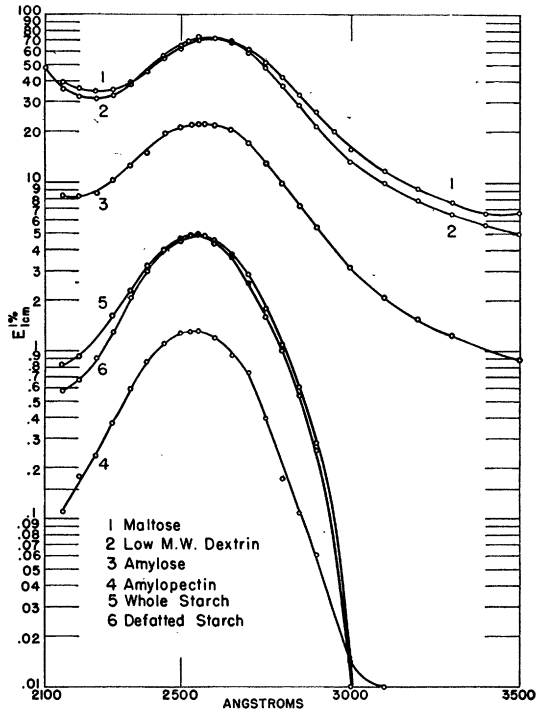

FIG. 1. Values of $E^{1\%}_{1\,cm}$ vs. wave length for starch, starch fractions and simpler carbohydrates.

that the reaction per molecule continues to the same extent in a given length of time. We have found that the first and second of these requirements are satisfied, but not the third.

A series of experiments was performed using starch and starch fractions of known history and low nitrogen content. These materials were a whole starch, a defatted starch, amylose, and amylopectin, all prepared in this laboratory from Iowa 939 hybrid corn. The starch was fractionated by the method of Schoch (*4*). For comparative purposes a number of other compounds were studied. These included maltose, a dextrin of 7 dextrose residues, cellulose, sorbitol, maltobionic acid, and Schardinger α-dextrin. Samples were heated for various lengths of time in the manner described below. Compounds which contained reducing end-groups reacted in alkaline dispersion to produce one or more compounds with a strong absorption band at 2,820 A. When these alkaline solutions were exposed to air, the absorption decreased rapidly. However, if the solutions were acidified, the peak of the absorption band was shifted to 2,550 A, and the absorption did not diminish when the solutions were exposed to air. To avoid reaction with oxygen, a vacuum technique was devised for the reaction with alkali, and the measurements were made on acidified solutions. Typical absorption curves at the end of a 4-hr reaction period are shown in Fig. 1. The semilog plot of absorption coefficient against wave length shows that

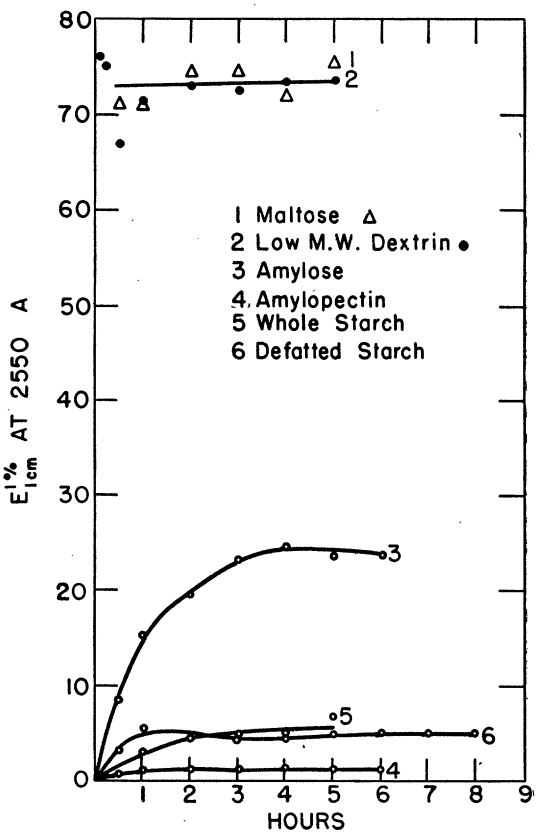

FIG. 2. Variation in values of $E^{1\%}_{1\,cm}$ at 2,550 A with reaction time.

the curves of the lower molecular weight compounds are reasonably parallel. This suggests that the same absorbing group is present in the reaction products. The lack of parallelism between the low-molecular-weight compounds and the higher polymers may be attributed to differences in light scattering between heated samples and unheated blanks. Thus, the first requirements stated earlier is satisfied.

As we have shown, compounds with reducing end-groups undergo reaction to produce the characteristic absorption. A number of compounds without such end-groups—sorbitol, maltobionic acid, and Schardinger α-dextrin, which is a cyclic polymer of dextrose—do not

produce the absorption. A reducing end-group is necessary to the reaction, and the second requirement is met.

A sample of amylopectin was treated and after acidification was precipitated by the addition of ethanol. The absorbing compound remained in the supernatant liquid and thus did not appear when the solid was redispersed in alkali. When this alkaline dispersion of recovered amylopectin was heated for a second time, no absorption developed. When the same experiment was carried out on amylose, however, as much absorption was produced by the second treatment as by the first. It is evident, therefore, that the reaction is stopped by a structural difference, possibly a branch point, in the case of amylopectin. The amount of destruction resulting from the alkali treatment has been estimated by following the changes produced in the optical activity of the various materials at the end of 4 hrs. In the case of maltose and the low-molecular-weight dextrin, the destruction was complete, since no activity remained after the reaction. Amylose, however, retained 72% of its activity, and amylopectin, 99%. Consequently, the third requirement is not satisfied, at least under the present experimental conditions.

Although no quantitative relation between the amount of absorption and molecular weight has been established, it is possible that other conditions or accurate knowledge of the rate at which dextrose residues are destroyed could lead to such a relationship.

The variation in the rate of formation of the absorbing compound is illustrated in Fig. 2. It will be noticed that with maltose and the dextrin, maximum absorption was reached in a few minutes, while nearly 3 hrs were required for amylose. Because all the curves had leveled off after 4 hrs reaction time, this period was chosen for making comparisons.

TABLE 1

Material	$E^{1\%}_{1\ cm}$ at 4 hrs	Alkali number, ml 0.1 N NaOH/gm dry sample
Low-molecular-weight dextrin	73.5	78
Maltose	72.0	78
Amylose	24.3	22
Amylopectin	1.3	5
Whole starch	4.9	9
Defatted starch	4.8	8

It is interesting that a fortuitous agreement exists between values of $E^{1\%}_{1\ cm}$ and alkali lability numbers, as shown in Table 1.

If the values of $E^{1\%}_{1\ cm}$ given in the table for amylose and amylopectin are used to estimate the relative molecular sizes of these compounds, amylopectin is about 19 times larger than amylose. Actually, because the amylopectin reaction is stopped at an early stage, the ratio should be less. The literature (*2, 3, 5*) gives both larger and smaller ratios. The composition of the whole starch, calculated from the absorption values, is 84% amylopectin and 16% amylose. In several preparations, 17–20% purified amylose has been isolated from this starch, although the iodine sorption of the starch is equivalent to an amylose content of about 26%.

We intend to continue the study of the reaction of alkali with the several dextrose polymers.

The experimental procedure follows:

The sample (100 mg) for alkali treatment was placed in arm A of the reaction vessel shown in Fig. 3. The

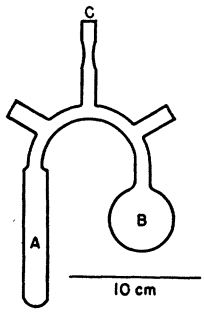

FIG. 3. Tube used in the reaction with alkali.

vessel was sealed by tube C to a stopcock leading to a vacuum line. Ten ml of 0.5 N sodium hydroxide solution was pipetted into arm B. The side arms of A and B were then sealed, and the solution in B was frozen by means of a solid carbon dioxide-alcohol bath. The system was next evacuated. (It is, of course, necessary to open the stopcock leading to the vacuum system very gently to avoid transferring a sample of starch or starch derivative out of the reaction vessel.) When the system had been pumped to a reasonably low, fixed gas pressure (about 10^{-5} mm Hg), the stopcock connecting the vacuum line and the reaction vessel was closed. To release the dissolved air, the sodium hydroxide solution was allowed to melt, was then refrozen, and the system was again evacuated to a fixed gas pressure of 10^{-6} mm Hg. After the vessel had been sealed off at the constriction in C and the solution had melted, the sample was dispersed by shaking the apparatus. The reaction was carried out by immersing the entire reaction vessel in a steam bath for the required time. The apparatus was then cooled, opened, and 2 cc of 12% hydrochloric acid was added immediately, after which the solution was transferred to a volumetric flask. When the proper dilution was reached, the ultraviolet absorption spectrum was measured against an unheated blank of the same material at the same concentration. The spectra were measured with a Beckman spectrophotometer.

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