The hexosemonophosphate oxidative pathway in alloxan diabetes

During an investigation of the hormonal control of glycolytic and nonglycolytic pathways of carbohydrate metabolism, a striking decrease in the levels of enzymes of the hexosemonophosphate (HMP) oxidative pathway was observed in rat liver in experimental diabetes.

Following preliminary starvation for 48 hours, young adult male rats (180 g) were rendered diabetic by the subcutaneous injection of alloxan[1] and killed 10 days after the injection. The control and experimental groups of animals were pair fed. Glucose-6-phosphate (G-6-P) and 6-phosphogluconate (6-PG) dehydrogenase activities of liver were determined as described previously by following the rate of reduction of TPN spectrophotometrically[2]. Preliminary results, shown in Table I, indicate that the levels of activity of both dehydrogenases are significantly reduced in diabetes.

TABLE I

INFLUENCE OF ALLOXAN DIABETES ON THE ACTIVITY OF RAT LIVER GLUCOSE-6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES

	Control rats	*Alloxan diabetic rats*	*P*
Number of animals	6	6	
Average daily sugar excretion	—	5.9 g	
Units* enzyme/g liver			
G-6-P dehydrogenase pH 7.6	149 ± 9	58 ± 6	< 0.001
6-PG dehydrogenase pH 9.0	187 ± 13	82 ± 4	< 0.001
6-PG dehydrogenase pH 7.6	111 ± 4	40 ± 4	< 0.001
Total liver enzyme/100 g body wt			
G-6-P dehydrogenase pH 7.6	636 ± 62	263 ± 36	< 0.001
6-PG dehydrogenase pH 9.0	794 ± 77	370 ± 33	< 0.001
6-PG dehydrogenase pH 7.6	469 ± 28	181 ± 20	< 0.001

* A unit of enzyme activity is defined as the quantity of enzyme which reduced 0.01 μmole TPN/min at 20° C.

This investigation is being extended to include an evaluation of the relative significance of the glycolytic and hexosemonophosphate oxidative pathways in normal and diabetic liver slices by the use of G-1-^{14}C and G-6-^{14}C [3].

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[1] E. H. KASS AND B. A. WAISBREN, *Proc. Soc. Exp. Biol. Med.*, 60 (1945) 303.
[2] G. E. GLOCK AND P. MCLEAN, *Biochem. J.*, 55 (1953) 400.
[3] B. BLOOM AND DE WITT STETTEN, Jr., *J. Am. Chem. Soc.*, 75 (1953) 5446.

Received December 24th, 1954

The denaturation of desoxypentose nucleic acid

A number of observations[1–5] have shown that agents which generally produce only physical changes alter the structure of native, biologically active DNA in a manner analogous to that occurring in the denaturation of proteins. An earlier investigation[6] of the effect of acid had shown that the highly extended DNA particle contracted substantially as the pH was lowered to 2.6 but was unchanged in molecular weight. We wish to report here that a similar situation exists in regard to the effect of heat: moreover, some observations we have made on the characteristics of the denaturation process and the denaturated product seem to be relevant to the problem of the structure of DNA and the validity of the proposal[7] that there are numerous interruptions in the polynucleotide strands.

The intrinsic viscosity (at 25°) extrapolated to zero gradient, $[\eta]$, was measured as a function of the temperature to which the DNA solution had been heated for one hour. In the most stable DNA preparations the $[\eta]$ maintained its room temperature value of 72 until 85° C. At higher temperatures $[\eta]$ decreased rapidly reaching a limiting value of 4.3 near 100°. In the presence of 8 *M* urea the pattern of viscosity decay was the same except that it was shifted to temperatures about 17° lower. The energy of activation in both cases was 93 kcal. We interpret this as the energy

required to break a sequence of hydrogen bonds (in the WATSON-CRICK[8] model) of sufficient length that the original structure will not reform.

A completely denatured, stable product is produced either by exposure to pH 2.6 (with or without 8 *M* urea) followed by dialysis to neutral pH or by heating the neutral saline solution to 100° C for 15 minutes provided the DNA concentration was below 0.02%. This product was found to have the same molecular weight as the native DNA (7.7 million for the sample reported on here). Its sedimentation constant (s^0_{20}) is 30 in contrast to 21 for the native DNA.

The question then arises as to whether or not the observed changes in s^0 and $[\eta]$ are consistent with an unchanged molecular weight. The changes which these quantities undergo upon denaturation are of course mutually dependent but their quantitative interrelationship can only be computed in terms of models. Hence the prerequisite for a strict comparison between theory and experiment is the precise identification of the native and denatured DNA with a tractable model. Since this last step has not been carried out, all that can be done at present is to see if reasonably appropriate models are approximately compatible with the observed data. Two models merit consideration: (1) The free-draining coil for which it is required that

$$\frac{s^0\,[\eta]}{\overline{r^2}} = \frac{(1 - \bar{v}\,\varrho)}{3600\,\eta_0}$$

where v is the partial specific volume (taken as 0.55), ϱ is the density and η_0 the viscosity of the solvent, and $\overline{r^2}$ is the mean square end-to-end length obtained from light scattering; (2) the unsolvated ellipsoid of high axial ratio for which

$$\frac{s^\circ\,[\eta]^{1/3}}{M^{2/3}} = 3.5\cdot 10^6\,\frac{(1 - \bar{v}\,\varrho)}{\eta_0\,N}$$

The values of the right sides of these two equations (theoretical) are listed below together with the values obtained by substituting the experimentally determined quantities in the left sides.

	$(\overline{r^2})^{1/2}$	*Free-draining coil*	*Unsolvated ellipsoid*
Theoretical		$1.25\cdot 10^{-2}$	$2.62\cdot 10^{-16}$
Native DNA	7100 A	3.0	2.25
Denatured DNA	2400	2.1	1.2

Although the differences among the entries in each column are somewhat greater than experimental error, they are not excessive when account is taken of the discrepancy (extent unknown) between the model and the DNA molecule as well as of the effects of polydispersity. Consequently the constancy of the molecular weight upon denaturation cannot be called into question by the changes observed with s^0 and $[\eta]$. Indeed the molecular weight does not enter into consideration with the free-draining coil model at all: this emphasizes the arbitrariness involved in deducing changes in molecular weight from s^0 and $[\eta]$ when large configurational changes occur simultaneously.

By using the same heat treatment that we have employed, DEKKER AND SCHACHMAN[7] obtained a product with values of s^0 and $[\eta]$ approximately four times lower than ours. Using the ellipsoidal model these results were interpreted as evidence for up to a 100-fold decrease in molecular weight. For the reasons mentioned above a quantitative interpretation of this data in terms of molecular weight decrease is not valid, but there is little doubt that a substantial decrease occurred upon denaturation of their material. Instead of interpreting their results as evidence for an interrupted structure for DNA, it would appear, in view of our observations, more reasonable to conclude that chain scission had been introduced in their sample either by enzymatic attack during the isolation of the DNA or by chemical degradation during the denaturation. The behavior of the sample we have studied gives no evidence of an interrupted structure when the denaturation is carried out by means that do not give rise to a superimposed, continuous degradation.

The other major evidence offered for the interrupted structure is the titration of groups in the pH range of 5 to 6[9]. Since the binding of protons in the region is eliminated when salt is added[10], we believe that the binding in the absence of salt is simply the result of spreading the titration range of the $-NH_2$ groups. This is well known as the field effect in the titration of polyelectrolytes.

Finally, we note the claim of ALEXANDER AND STACEY[11] to have observed a halving of the molecular weight of DNA by 4 *M* urea and their interpretation of this as the separation of the two strands of the WATSON-CRICK model. It is clear that we have made no such observation even with considerably more vigorous treatment. Indeed, it would appear that the molecular weight could not be determined under the conditions ALEXANDER AND STACEY describe (light scattering in 4 *M* urea) since the prerequisites for interpreting scattering from a three component system such as this are not experimentally available[12]. It is our conclusion that the rate at which urea (8 *M* or less) attacks

the native DNA structure is imperceptible at room temperature. Moreover, when the structure is attacked at higher temperatures or lower pH, it does not appear that conditions have yet been found which will simultaneously break all the hydrogen bonds (or other interchain bonds) thereby permitting separation of the undegraded polynucleotide strands.

We wish to thank Dr. NORMAN SIMMONS (University of California, Los Angeles) for the samples used in this work and for the support given by the Public Health Service Grant C-2170.

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1 S. ZAMENHOFF, H. ALEXANDER AND G. LEIDY, *J. Exptl. Med.*, 98 (1954) 373.
2 G. GOLDSTEIN AND K. G. STERN, *J. Polymer Sci.*, 5 (1950) 687.
3 R. THOMAS, *Biochim. Biophys. Acta*, 14 (1954) 231.
4 B. E. CONWAY AND J. A. V. BUTLER, *J. Chem. Soc.*, (1952) 3075.
5 D. O. JORDAN, *Progress in Biophysics*, Academic Press, (1951), p. 51.
6 M. E. REICHMANN, B. H. BUNCE AND P. DOTY, *J. Polymer Sci.*, 10 (1953) 109.
7 C. A. DEKKER AND H. K. SCHACHMAN, *Proc. Natl. Acad. Sci.*, 40 (1954) 894.
8 F. H. C. CRICK AND J. D. WATSON, *Proc. Roy. Soc. (London)*, A223 (1954) 80.
9 W. A. LEE AND A. R. PEACOCKE, *J. Chem. Soc.*, (1951) 3361.
10 W. A. LEE AND A. R. PEACOCKE, *Research*, Suppl., 6 (1953) 155.
11 K. A. STACEY AND P. ALEXANDER, *Trans. Faraday Soc.*, 50 (1954) 303.
12 W. H. STOCKMAYER, *J. Chem. Phys.*, 18 (1950) 58.

Received January 5th, 1955

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BOOK REVIEW

Histochemical Methods, WALTHER LIPP, Oldenburg Verlag, Munich, 1954. Six issues annually, 24 pages each. D.M. 30.— (for six issues).

The title of this collected work is wider in scope than is in keeping with the content of these three issues. Frequently in the literature the chemical analysis of cell fragments obtained by differential centrifugation and the ultra micro-analysis of very small tissue areas or large cells (Linderstrøm-Lang) are both considered as belonging to Histochemistry or Cytochemistry. In order to differentiate between these two fields Gomori gave the name *microscopic histochemistry* to the field dealt with in this work. A generally recognized nomenclature will perhaps be necessary fairly soon in order to avoid misunderstandings. (In the German literature Voss suggests the use of *Topochemie* instead of *Histochemie*.)

Dr. Lipp's collection consists of single leaflets, already perforated for insertion, which are received in six yearly issues and which the reader can assemble in the prescribed manner. The three issues which have appeared contain leaflets for various parts of the complete collected work. In this way the volume can be continually added to when all the earlier literature has been incorporated. This original method fits in well with the way in which, in general, the research worker assembles and keeps his own literature.

In the first three issues one finds, amongst other things, critical reports on Schiff's Reagent for aldehyde groups, on methods of detection of arginine and sulph hydryl groups, and on impregnation of radioactive tissue sections with silver salts.

The present issues give a comprehensive survey of international literature and the reviewer found many references valuable for histochemical research.

Whether this collected work, as is stated in the Introduction, will help the research worker who is unfamiliar with Chemistry, but occasionally makes use of histochemical methods, to achieve good results, will be seen from experience. In this connection the reviewer would like to emphasize that the development of Histology in the direction of Histochemistry not only means that procedures for empirical colouring methods with a morphological objective are replaced by histochemical procedures, but also that one accordingly tends to interpret the histochemical findings within the bounds of general biochemical research.

If this objective is not put too much into the background, one can expect an invasion of the literature with descriptions of results obtained with the help of histochemical methods in all kinds of tissues and cells, and also the promotion of the development of general Biochemistry.

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