latter value, however, should be regarded with caution in view of the fact that such an estimation of the molecular weight presupposes that all the peptide chains are in the open form with their amino end-groups free to react with fluorodinitrobenzene; also the actual estimation itself may involve a relatively high degree of inaccuracy.

Fibrino-peptide thus appears to consist primarily of acidic and neutral amino acids, indicating that the part of the fibrinogen molecule from which the peptide is derived is richer in these than the rest of the protein. Fibrino-peptide was found to have an isoelectric point in the region of pH 3.3[9], and it would therefore represent at physiological pH highly charged negative centres in the fibrinogen molecule. During the enzymic phase of the clotting reaction[4]

$$\text{fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin} + \text{fibrino-peptide},$$

the release of the peptide would remove these strongly charged centres and would thereby help the particles to approach one other more easily in the process of clot formation.

We wish to thank Professor W. T. ASTBURY, F.R.S. for his interest and encouragement throughout our investigations.

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Received September 25th, 1952

UNRELIABILITY OF THE DATA HITHERTO REPORTED ON THE DESOXYPENTOSENUCLEIC ACID CONTENT OF CELL NUCLEI DETERMINED BY MICROSPECTROPHOTOMETRY

by

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Recently efforts have been centred on the microspectrophotometric measurements of the DNA content of individual nuclei for various biological materials. Meanwhile, serious discrepancies have emerged among the results published from different laboratories, especially about the constancy *vs.* variability of the DNA content of individual diploid nuclei from the same species and sometimes even from the same type of cells[1,2,3,4]. Although such discrepancies may partially be due to factors involved in the FEULGEN reaction, in preparation of the material, or in reduction of the measured values[5,6], the most important source of error seems to be due to the use of photometric equipments[7,8,9] the optical system of which does not eliminate the little known "SCHWARZSCHILD-VILLIGER effect" (S–V effect), which has been foreseen theoretically and which proved experimentally to cause considerable error in the measurement of transmittance[10]. Especially with objects of such a high ab-

sorption as will frequently be encountered in cytochemical work, the magnitude of this error in transmittance may amount to 10–200 times the true value[10].

It will now readily be understood that the data of microspectrophotometry hitherto reported are subject to considerable inaccuracy. Indeed, we have been deeply impressed by finding that, while values of extinction of Feulgen stained nuclei reported in the literature are usually less than unity, a larger part of the values obtained by ourselves on similar material with an instrument free from "S–V effect"[11] are higher than 1, and not infrequently even higher than 2. This means that values of transmittance obtained by other investigators are generally more than, but those of our measurements are mostly less than, 10%. As is shown in a previous report[10], the magnitude of the flare light caused by the "S–V effect", which acts additively in the measurement of transmittance, is expected theoretically to amount to about 13.5% in the usual optical system, so that the noted discrepancy may well be explained by considering the term of "S–V effect". In this connection it is to be mentioned that the magnitude of the increment in transmittance caused by "S–V effect" is an "instrument-factor", and it is thus impossible to correct the value of transmittance measured by instruments without consideration of "S–V effect".

The following experiment has now been made in order to show how misleading the results of the measurement are when the "S–V effect" is not eliminated. Two series of measurements were made on the same assemblage of rat liver cell nuclei, fixed in 50% formalin and stained with the Feulgen reaction, by focussing a small image of the light source at the centre of spherical nuclei, as described previously[11]. For the diameter of this image values of 9 and 1 μ were taken, of which the former does, and the latter does not, involve "S–V effect", although the effect is still not very large in the former case. In general, "S–V effect" gave rise to a significant lowering of values for DNA contents of individual nuclei as expressed by r^2E [11]. Furthermore, the introduction of this effect shifted most of the nuclei of Class II with the medium DNA content (assumed as tetraploid)[1], which stained heavily and gave low values of transmittance (10–15%), into Class I with the lowest DNA content (assumed as diploid), and augmented the variance of the r^2E value of the latter group. However, one nucleus more lightly stained retained its position in Class II even with "S–V effect". Its transmittance was, in reality, as high as 22.3%. Such results are the natural consequence of the fact that the "S–V effect" causes much less error in measuring objects with higher transmittance.

Another example may be taken from measurements on nuclei of liver cells and of the first spermatocytes (leptoten stage) of a young rat, fixation and staining being similar to those in the preceding experiment. As is seen from Table I, the value without "S–V effect", *i.e.* the true value, for DNA content of the first spermatocyte nuclei was much higher than that of the liver nuclei, whereas with "S–V effect" both gave values nearly equal to each other. The more marked decrease in the value of the spermatocyte nuclei is caused by a very low transmittance (1–2%) due to their remarkably intense staining.

TABLE I

COMPARISON OF MICROSPECTROPHOTOMETRIC MEASUREMENTS OF NUCLEAR DNA CONTENT CONDUCTED ON FEULGEN-STAINED NUCLEI OF LIVER CELLS AND THE FIRST SPERMATOCYTES OF A RAT WITH AND WITHOUT "SCHWARZSCHILD-VILLIGER EFFECT"

	With S–V effect		*Without S–V effect*	
	No. of nuclei measured	*Mean of relative DNA content (r^2E) and standard deviation*	*No. of nuclei measured*	*Mean of relative DNA content (r^2E) and staudard devision*
Liver	28	5.48 ± 0.85	25	6.05 ± 0.69
First spermatocyte	10	4.98 ± 0.76	10	10.14 ± 0.67

As evidenced by the above examples, there is a serious doubt about the reliability of the data heretofore published, whether or not they are favorable to either of the views respecting the constancy or variability of DNA content of the individual nuclei, as long as they were obtained, through microspectrophotometry without considering elimination of the "S–V effect". This statement, then, leads us inevitably to a suspicion that all the conclusions of biological importance drawn from such data are once more open to question.

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Received May 5th, 1952

ELECTRON MICROSCOPY OF ACTOMYOSIN

by

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The macroscopic appearance of the contractile muscle proteins depends on pH, ionic strength, and ATP content. It is possible to show that the electron microscopic appearance of these proteins depends also on the pH and on the presence or absence of ATP if in the buffered system the salt concentration is kept at such a low level that the effect of drying is minimized.

No pH effect was found with F-actin under the conditions of these experiments. Actin solutions reveal indefinitely long, freely distributed, uniform threads at all pH values between 5.5 and 8.5. Myosin and actomyosin have a very similar appearance in the pH range of 5.5 to about 7: fibrous aggregates at pH 5.5 and aggregates embedded in an amorphous film at pH 7. Between pH 7.3 and 8 myosin appears as an amorphous film in contrast to actomyosin which reveals actin-like threads embedded in an amorphous matrix.

In the presence of ATP and Mg, actomyosin forms a contracted plug at low pH [1]. This plug consists of fibrous aggregates, in some respects similar to myosin or ATP-free actomyosin at the corresponding pH. However, a difference is evident in that many, relatively short, actin-like threads can be seen fraying out at the end of the fibrous aggregates. It seems likely that they are the remnants of a continuous network within the intact plug which became disrupted during preparation.

In the presence of ATP and Mg, actomyosin forms a clear solution at around neutral pH [1]. The electron micrographs show a continuous amorphous protein film like that observed in myosin solutions at a somewhat higher pH. In addition, indefinitely long, actin-like threads are uniformly distributed throughout the fields resembling F-actin prepared alone. This is in contrast to ATP-free actomyosin at the same pH where shorter threads are concentrated in the cloud-like areas of amorphous myosin. After the ATP has been completely hydrolyzed, the actomyosin forms a fine, non-contractile precipitate. Its microscopic appearance now coincides with that of ATP-free actomyosin.

At alkaline pH actomyosin forms a firm, transparent gel after much of the ATP has been hydrolyzed[1]. Microscopically a network is seen which consists of strands made up of loosely associated, indefinitely long, actin-like threads embedded in an amorphous matrix. It appears that the contracted plug and the gel are similar in having a framework of actin-like threads, but differ in that the myosin-like matrix forms a fibrous aggregate around this network at the acid reaction, and forms a characteristically amorphous matrix around the network at high pH.

It was not possible to characterize actomyosin units as such morphologically. The structures in general consist of actin-like threads embedded in a myosin-like matrix which corresponds to the myosin alone at the respective pH. The presence of ATP accentuates the appearance of actin-like threads. The pictures obtained of the clear solution in the presence of ATP and Mg at near neutral pH and physiological salt concentration appear to demonstrate the dissociation of actomyosin.

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Received September 29th, 1952

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