tion of the results may depend on other information concerning the shape and dimensions of the molecule.

Summary.—The thickness of a unimolecular layer of horse heart metmyoglobin molecules adsorbed onto barium stearate was calculated to be 62 A. by means of the B.E.T. equation for multimolecular adsorption.

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A COMPARISON OF THE CONTENT OF DESOXYRIBOSENUCLEIC ACID (DNA) IN ISOLATED ANIMAL NUCLEI BY CYTOCHEMICAL AND CHEMICAL METHODS

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In 1948, Boivin, Vendrely and Vendrely[1, 2] presented evidence that the amount of desoxyribosenucleic acid (DNA) in nuclei of different tissues was constant for the same animal and twice that of the sperm. These authors suggested that such a constant relationship applies to all diploid animal cells and might be an expression of the genetical equipment of the cells.

While some recent work[3–6] lends support to this idea, other studies stand in contradiction. In 1949, Mirsky and Ris[6] reported analyses on the content of DNA in nuclei of various tissues of *mammals* which showed a quite different relationship to the sperm from that reported by Boivin, Vendrely and Vendrely. According to Mirsky and Ris, in calf thymus, calf lymph nodes, beef kidney and beef liver, the amount of DNA in diploid nuclei in relation to the amount of DNA in the sperm varied from 2.5 to 3:1, in contrast to the 2:1 ratio found for the same nuclei by Boivin, Vendrely and Vendrely.

In 1950, Pasteels and Lison,[7] working on the rat, reported that the DNA in nuclei of diploid liver and pancreas cells showed a relationship to the sperm which was only 1.3–1.5:1, which is again a significant departure from the ratio found by the French workers.

Considering that Mirsky and Ris found values of DNA for *mammalian* diploid nuclei which are much too high while Pasteels and Lison found values which are much too low in relation to the sperm, such deviations, if confirmed, would, of course, be a strong argument against the concept of Boivin, Vendrely and Vendrely that the amount of DNA in all diploid somatic nuclei of animals is exactly twice that of the sperm.

A reinvestigation of this particular problem therefore seemed essential and it was decided to make a determination of the DNA on the same preparations of isolated animal nuclei by two independent, quite different methods: the chemical method, in which the amount of DNA per nucleus is computed from a large number of nuclei subjected to biochemical analysis, and the cytochemical method, which allows a direct estimation of DNA in a single nucleus through photometric measurements. In the present paper we report the results on the amount of DNA in nuclei of these two methods when applied to various tissues of beef and rat.

Material and Methods.—Liver, kidney, spleen and sperms were obtained from freshly killed beef and rats, the nuclei isolated and the amount of DNA determined chemically as previously described by Vendrely and Vendrely.[2] For the cytochemical analysis, the same suspension of isolated nuclei was used for smears, which were fixed in Carnoy and 50% Formalin and on which the Feulgen reaction was carried out. The DNA was estimated by photometric measurements with an apparatus similar to that of Pollister and Moses,[8] and by the method as described by Schrader and Leuchtenberger.[4] The cytochemical analysis was also done on sections of the same organs fixed in Carnoy and 50% Formalin.

Results.—In the following table the results of the cytochemical and chemical analysis of DNA in isolated nuclei of different tissues of beef and rat are presented. It should be pointed out that the amount of DNA per nucleus found by the cytochemical analysis is a mean value expressed in arbitrary units (as previously described),[4] while the amount of DNA per nucleus by the chemical analysis is given in absolute amounts in 10^{-6} γ.

If we consider first the cytochemical analysis of the DNA of different tissues of the beef, it is evident that the liver, spleen and kidney contain approximately the same amount of DNA per nucleus and twice that of the sperm. The proportion is also maintained if we look at the data obtained by the chemical analysis on the same suspension of nuclei. If we now examine the cytochemical analysis of the DNA in the tissues of the rat, it is evident that in the liver the nuclei fall into three classes with values of DNA per nucleus of 3.3, 6.6 and 12.1 (arbitrary units), respectively.

Furthermore, these values represent nearly exact multiples of each other. The picture is quite different if we consider the amount of DNA of the nuclei of the kidney of the rat. Here only a mean value of 3.3 per nucleus was observed, which corresponds to the lowest class of DNA found in liver nuclei.

In the chemical analysis, all the size classes of nuclei in the liver are, of course, thrown together and the results of such lumping is expressed by the fact that the amount of DNA per liver nucleus is considerably higher than that of the kidney.

Discussion of the Results.—In evaluating the data it should be pointed out that the cytochemical values given in the table for the amount of DNA per nucleus of a tissue actually represent a mean of a number of individual DNA values. While this mean amount is constant and the

TABLE 1

COMPARISON OF THE AMOUNT OF DESOXYRIBOSENUCLEIC ACID (DNA) IN ISOLATED NUCLEI OF DIFFERENT TISSUES OF BEEF AND RAT BY CHEMICAL AND CYTOCHEMICAL ANALYSIS

		CYTOCHEMICAL ANALYSIS		
TYPE OF ANIMAL	TYPE OF TISSUE	NO. OF NUCLEI MEASURED	MEAN AMOUNT OF DNA PER NUCLEUS (ARBITRARY UNITS)	CHEMICAL ANALYSIS, AMOUNT OF DNA PER NUCLEUS (10^{-6} γ)
Beef	Liver	30	3.3 ± 0.11	6.8
Beef	Spleen	23	3.0 ± 0.08	7.0
Beef	Kidney	26	3.3 ± 0.10	6.4
Beef	Sperm	25	1.6 ± 0.03	3.3[a]
Rat	Liver	15	3.3 ± 0.08	
Rat	Liver	21	6.6 ± 0.24	8.2
Rat	Liver	7	12.1 ± 0.50	
Rat	Kidney	38	3.3 ± 0.06	5.5

[a] This value is taken from previous analyses by Vendrely and Vendrely.[3]

NOTE: The amount of DNA determined by cytochemical analysis is given in arbitrary units.

same for diploid nuclei of different tissues for the same animal there is a variation from nucleus to nucleus within each tissue, sometimes as high as 50%. For example, the beef spleen which gave an average amount of DNA of 3 per nucleus, similar to the average amount for beef kidney and beef liver, showed individual nuclei with values of DNA ranging from 2.7 to 4.0. Whether such differences are due to errors inherent in the cytochemical photometric method as previously discussed,[9] or are due to biological variations in the amount of DNA among individual nuclei of a tissue cannot be decided at the present time.

If we compare now the results obtained by the cytochemical and the chemical analyses of DNA in the nuclei of different tissues of the beef, it is evident that by both analyses the diploid nuclei of the somatic tissues show twice the amount of DNA of the sperm. Therefore, the unavoidable

conclusion follows that constancy of the DNA in nuclei of the beef, as first reported by Boivin, Vendrely and Vendrely in 1948, holds true, even when individual nuclei are analyzed for their content of DNA (as done by the cytochemical studies), instead of analyzing a large mass of nuclei and computing the value for one nucleus. These results, therefore, do not confirm the measurements of DNA of *mammalian* nuclei as reported by Mirsky and Ris.[6] According to the last-named authors, the higher values of DNA in the beef liver (which represent three times the value of the sperm—namely, 8.4 to 2.82) cannot be explained by the presence of polyploid nuclei, for they state: "Polyploidy is not the explanation of the high values found for beef liver, for in counting liver nuclei the double sized nuclei were counted separately and the necessary correction was made."

In view of the fact that the results which we obtained in the present study by both cytochemical and chemical analyses are in complete agreement with the findings previously obtained on beef by Vendrely and Vendrely, the discordant findings of Mirsky and Ris remain unexplained. It might be of interest to state that in the cytochemical analysis of DNA of the nuclei of the beef liver only two nuclei among thirty-two examined showed amounts of DNA which were twice that of the values reported. A similar relationship holds true for spleen and kidney. It should be pointed out, however, that occasionally nuclei were found which had a considerably lower value than 3.3, especially if nuclei of very small size with a pycnotic appearance were measured. Whether these are degenerating, dying nuclei so familiar to every pathologist, or whether the lower value is due to errors in the photometric measurements cannot be decided at this moment with certainty. We are inclined to believe that even in the normal organs there are always a small number of degenerating nuclei present which gradually lose their DNA as demonstrated for pycnotic nuclei by Leuchtenberger.[9]

Turning now to data on rat nuclei, the finding of three distinct classes with multiple values of DNA, 3.3, 6.6 and 12.1 respectively in the analysis of isolated liver nuclei is in good agreement with the three classes of DNA values found by Ris and Mirsky[10] in sectioned material of rat liver. A similar agreement between amounts of DNA found by the cytochemical analysis in *isolated nuclei* and tissue *sections* has been found also in our own studies.

Pasteels and Lison[7] also report three classes of DNA values in the rat liver nuclei in sections but each of these classes has a value which is about 30% too low, if compared with the results in other organs. While kidney, for instance, has one class of DNA with a value of 227, the liver has the three size classes—156, 347, 772. Hence the measurements in the kidney find no equivalent in the liver nuclei where the minimum value is significantly lower, while the two higher values stand in no multiple relation to the single class represented by the kidney nuclei.

We cannot confirm these results because, although we found also only one class of DNA in kidney in contrast to the three classes in the liver as did Pasteels and Lison, the amount of DNA in the nuclei of the kidney of the rat is approximately the same as the lowest amount of DNA in the liver nuclei.

If we now compare the results obtained by our cytochemical analysis of DNA in nuclei of rat liver and rat kidney with the chemical analysis on the same material, it is evident that the higher value of DNA in the liver nuclei—$8.2 \times 10^{-6}\gamma$ as compared with the lower value of $5.5 \times 10^{-6}\gamma$ for the nuclei of the kidney—is an expression of the polyploidy in the liver. We feel that these studies demonstrate rather well the importance of the simultaneous application of the cytochemical and chemical method for the analysis of DNA on the same material. Only by the cytochemical method was it possible to recognize in the rat liver three different types of nuclei which were characterized by three different amounts of DNA (multiples of each other). In contrast the result of the chemical method represents an average value of three types of nuclei with different amounts of DNA and is, therefore, not the true quantity of DNA of the individual nuclei in the liver. This does not by any means lessen the importance of the chemical analysis of DNA in diploid nuclei, particularly if we keep in mind that if the chemist presents a value of DNA for a nucleus it is based on much larger samples of nuclei (billions), while the cytochemist—even if he were able to study 1000 nuclei of one tissue—always analyzes only at comparatively small population of nuclei in respect to the whole tissue. There seems to be no doubt as to the desirability of employing both methods simultaneously on the same material, especially in cases involving polyploidy or in tissues which show pathological changes.

Summary and Conclusions.—DNA was determined in the same preparation of isolated animal nuclei by a cytochemical and a chemical method. Contrary to the findings of Mirsky and Ris, we found by both methods that diploid nuclei of various tissues of beef contain the same amount of DNA and twice that of the sperm. The constancy in the amount of DNA was also observed in the diploid class of liver and kidney nuclei of the rat in contrast to the findings of Pasteels and Lison.

This analysis, based on two different and independent methods, thus gives firm support to the concept of Boivin, Vendrely and Vendrely, that mammalian diploid nuclei of various tissues contain a constant and characteristic amount of DNA and that this amount is double that which is carried by the sperm.[11]

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STRUCTURES IN E. COLI RESEMBLING CHROMONEMATA

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Although bodies having the characteristics of nuclei have been described in studies of E. coli with the light microscope and with the electron microscope, the internal structure of the nuclei or of the chromosomes has not been observed hitherto.[1–4] The observations reported here show the presence of structures resembling chromonemata, and chemical analyses of the particulate fraction in which the apparent nuclear bodies are concentrated fulfill expectations from previous studies.[3, 4]

Bacteria in the early log phase of growth were suspended in 5% citric acid and immediately subjected to sonic vibration (9 kc.)[5] for 45 minutes and then subjected to differential centrifugation. A heavy black pigmented fraction was obtained at 900 g. and a light unpigmented one at 3600 g. Very little additional solid material was collected at 55,000 g.[6] The average size of the heavy particles was 0.96 *t* 0.002 μ × 0.63 *t* 0.002 μ, which was approximately the size of the Feulgen-positive bodies of the unbroken cells.

The heavy fraction was suspended in distilled water and a drop of the suspension on a collodion film supported by wire mesh was frozen on a block of solid CO_2. The frozen material was then evacuated and shadowed with chromium. When examined in the electron microscope many of the particles showed rather regular peripheral lobulations, suggesting the presence of a banded or helical structure. In many instances, helical structures whose coils corresponded with the peripheral lobulations could be observed (figure 1). Most of the particles observed gave some indication of the presence of helices although these structures appeared much