wave produced by ten times greater quantities of MEHL's mucoprotein or the "cancer substance". The "cancer substance" and MEHL's mucoprotein show practically identical electrophoretic patterns.

1

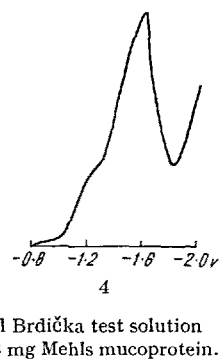
5 ml Brdička test solution
Sensitivity × 200.

2

5 ml Brdička test solution
+ 0·1 mg ovomucoid
Sensitivity × 200.

3

5 ml Brdička test solution
+ 1 mg cancer substance
Sensitivity × 200.



4

5 ml Brdička test solution
+ 0·8 mg Mehls mucoprotein.
Sensitivity × 200.

It has thus been demonstrated that the substance responsible for the BRDIČKA reaction is a mucoprotein very similar if not identical with MEHL's mucoprotein, which is a component of normal blood. This suggests that BRDIČKA's reaction indicates an increase in the level of normal mucoproteins in the blood and not the presence of a new substance which is absent in the blood of healthy subjects.

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Zusammenfassung

Es wird gezeigt, dass die Brdičkasche Filtratreaktion durch eine Erhöhung der Konzentration des normalerweise im Serum vorkommenden Mucoproteins verursacht wird. Dieses Mucoprotein enthält Cystein, so dass keine Modifikation der ursprünglichen Interpretierung der Reaktion durch BRDIČKA erforderlich scheint.

Acid-soluble Nucleotide Derivatives in Cell Nuclei Isolated in a Non-aqueous Medium

In recent studies on the composition of cell nuclei isolated by non-aqueous procedures (KAY, SMELLIE, HUMPHREY, and DAVIDSON[1]) it has been shown that considerable amounts of material are removed from such nuclei when they are extracted with dilute citric acid. This extractable material includes some of the nuclear protein and a considerable proportion of the nuclear ribonucleic acid (RNA) together with other acid-soluble substances including simple nucleotides. The presence of acid soluble nucleotides and related nucleosides and free bases is of considerable interest inasmuch as they are likely to be connected in some way with the metabolic role of the nucleus. In this brief note some observations on these components of cell nuclei are recorded.

Samples of nuclei prepared from a variety of tissues according to the method described by KAY *et al.*[1] were extracted in the cold with 0·7 *N* perchloric acid in the proportion of 5 ml/100 mg nuclei. The mixture was centrifuged and the residue was washed twice with 2 ml cold 0·7 *N* perchloric acid. The residues were reserved for estimation of RNA and DNA by the modification of the SCHMIDT-THANNHAUSER method described by SMELLIE, HUMPHREY, KAY, and DAVIDSON[2]. The combined extracts were treated with butanol to remove nucleosides and free bases leaving the nucleotides and inosine in the aqueous phase according to the method of GOLDWASSER[3]. The butanol extracts were taken to dryness and digested with 12 *N* perchloric acid at 100° to yield the individual purine and pyrimidine bases. The aqueous phase containing the nucleotides and inosine was also evaporated to dryness and digested with perchloric acid. Portions of the digest were applied to WHATMAN 3MM paper and the bases separated by two dimensional chromatography, using descending isopropanol: HCl (WYATT[4]) followed by ascending butanol: ammonia (MAC NUTT[5]). Appropriate blanks were also run. The bases were located in ultraviolet light and were eluted with 0·1 *N* HCl for all except guanine which required 1·6 *N* HCl. From readings of the optical density at appropriate wavelengths in the ultraviolet the amounts of each base were calculated and were then related to the DNA-phosphorus present in the original extracted sample. The values were thus obtained as μg base per mg DNA-P.

In some samples the total ultraviolet absorption of the perchloric acid extract was measured. For this estimation the perchloric acid extracts were diluted so that 1 ml was derived from an amount of nuclei containing 10 μg DNA-P. Absorption readings in the ultraviolet at 260 mμ were then made in the Beckman spectrophotometer, using appropriate perchloric acid blanks.

The values for the total ultraviolet absorption at 260 mμ of perchloric extracts of rabbit nuclei are shown in Table I. Since the figures quoted are related to the same amount of DNA-P it may be assumed that the various extracts are derived from comparable numbers of nuclei. The values show a wide scatter but the general pattern which emerges is that the nuclei from adult

[1] E. R. M. KAY, R. M. S. SMELLIE, G. F. HUMPHREY, and J. N. DAVIDSON, Biochem. J. (1955), in the press.
[2] R. M. S. SMELLIE, G. F. HUMPHREY, E. R. M. KAY, and J. N. DAVIDSON, Biochem. J. *60*, 177 (1955).
[3] E. GOLDWASSER, Biochim. biophys. Acta *13*, 341 (1954).
[4] G. R. WYATT, Biochem. J. *48*, 584 (1951).
[5] W. S. MACNUTT, Biochem. J. *50*, 384 (1952).

Total ultraviolet absorption at 260 mμ of perchloric acid extracts of non-aqueous nuclei isolated from various rabbit tissues
1 ml extract is derived from an amount of nuclei containing 10 μg DNA-P.

Tissue	DNA-P % dry wt	RNA-P % dry wt	Optical density (mean and range)	
Appendix	1·01	0·48	0·469 (0·294–0·644)	(12)*
Bone marrow	0·67	0·30	0·580 (0·440–0·720)	(5)
Intestinal mucosa	0·64	0·45	0·673 (0·493–0·853)	(7)
Lung	0·45	0·27	0·818 (0·551–1·085)	(11)
Thymus	1·68	0·35	0·277 (0·183–0·371)	(7)
Embryo liver	0·38	0·37	1·031 (0·512–1·550)	(3)

* Figures in brackets show the number of extracts examined.

tissues may be arranged in the following order of decreasing content of acid soluble ultraviolet absorbing material:–Lung, Intestinal mucosa, Bone marrow, Appendix, Thymus.

In embryo liver nuclei the mean value is higher than for any of the adult tissues but again the scatter is very great. High values were also found in two preparations of non-aqueous nuclei from the fowl GRCH 16 tumour.

The nucleotide bases found in the acid extracts included adenine which was most abundant, hypoxanthine, guanine, uracil and very small amounts of cytosine. In the nucleoside and free base fraction the bases were less plentiful than in the nucleotide fraction. Adenine and hypoxanthine were most abundant; guanine and uracil were present in smaller amounts and cytosine was found only in traces.

The scatter of values in different preparations was very great but the tissues could be arranged in the following order of decreasing base content:

Nucleotide bases:

(a) *Adenine.* Intestinal mucosa, bone marrow, appendix = lung, thymus.
(b) *Hypoxanthine.* Intestinal mucosa = lung, bone marrow, appendix, thymus.
(c) *Guanine.* Intestinal mucosa, bone marrow = lung, appendix, thymus.
(d) *Uracil.* Intestinal mucosa = bone marrow = appendix = lung, thymus.
(e) *Cytosine.* No important differences–all values low.

Non-nucleotide bases:

(a) *Adenine.* No particular trend as individual differences were too great.
(b) *Hypoxanthine.* Intestinal mucosa = lung, bone marrow = appendix, thymus.
(c) *Guanine.* No important differences–all values low.
(d) *Uracil.* No important differences–all values low.
(e) *Cytosine.* Present only in traces.

Results from two preparations of GRCH 16 tumour nuclei suggest that these nuclei are rich in adenine nucleotides but the most surprising feature of this material is the very high content of non-nucleotide hypoxanthine. This may be due in part to autolytic changes occurring either before removal from the animal or during the processing of the tissue.

Since mono-, di- and tri-nucleotides of adenine, guanine, cytosine and uracil are known to be present generally in living cells (SACKS, LUTWAK, and HURLEY[1]; SCHMITZ, POTTER, HURLBERT, and WHITE[2]; SCHMITZ, HURLBERT, and POTTER[1]; SMITH and MILLS[2]), it is not surprising to find evidence of the occurrence of these bases and hypoxanthine as nucleotides, nucleosides and free bases in acid extracts of non-aqueous nuclei. Nucleotides of these bases have also been observed by EDMUNDS and LEPAGE[3] in nuclei prepared in aqueous sucrose homogenates of liver and of Flexner Jobling carcinoma.

It is interesting however to find that in adult rabbit tissues there is no evidence of any preferential accumulation of such materials in those tissues in which cell division is active and the turnover of DNA is high (SMELLIE *et al.*[4]).

This work is being continued on a larger scale with material labelled with ^{14}C.

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Zusammenfassung

Zellkerne, die aus Kaninchengewebe mittels eines nichtwässrigen Verfahrens bereitet werden, enthalten in ihren säurelöslichen Bestandteilen Nukleotide von Adenin, Hypoxanthin, Guanin, Uracil und (in sehr geringen Mengen) von Cytosin. Diese Stoffe wurden in Lunge, Darm-Mucosa, Knochenmark, Appendix und Thymus in abnehmender Konzentration vorgefunden.

[1] J. SACKS, L. LUTWAK, and P. D. HURLEY, J. Amer. chem. Soc. *76*, 424 (1954).
[2] H. SCHMITZ, V. R. POTTER, R. B. HURLBERT, and D. M. WHITE, Cancer Res. *14*, 66 (1954).
[3] M. P. EDMONDS and S. A. LEPAGE, Cancer Res. *15*, 93 (1955).
[4] R. M. S. SMELLIE, G. F. HUMPHREY, E. R. M. KAY, and J. N. DAVIDSON, Biochem. J. *60*, 177 (1955).
[5] British Empire Cancer Campaign Research Fellow.

Über das Cu^{2+}-Bindungsvermögen einiger Aminosäuren und Peptide

Metallionen und biologische Wirkung[1]

Wir haben in einer früher erschienenen Arbeit[2] gezeigt, wie das Cu^{2+}-Bindungsvermögen von biologischen Systemen durch das Vorhandensein von gelösten basischen Partikeln bestimmt wird. Die Menge der gefunde-

[1] 37. Mitteilung; 36. Mitteilung: R. GALL und H. ERLENMEYER, Helv. chim. Acta *38*, 1421 (1955).
[2] S. FALLAB und H. ERLENMEYER, Exper. *11*, 174 (1955).