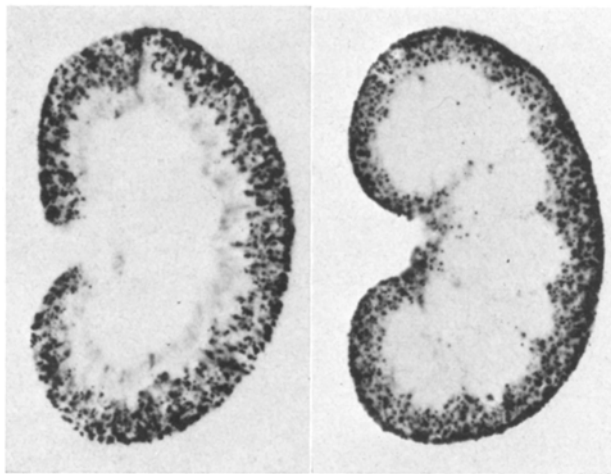
regional flow rates. Absolute flow values to any organ of the systemic circulation can be calculated from regional isotope concentration, cardiac output and the amount of indicator injected.

The same principle has been applied to assess the distribution of blood flow within single organs[6]. Particles labelled with β-emitting isotopes are used. Their distribution is visualized by autoradiography; quantitative data are obtained by densitometry of the autoradiograph.

According to this technique, blood flow through the renal cortex was studied in adult mongrel dogs anesthetized by pentobarbitone (40 mg/kg body wt.) given i.v. A suspension of 131-J labelled macroaggregated albumin particles of 5–50 μ diameter was slowly infused into the left ventricle through a vinyl catheter. 4 min after the injection the animals were sacrificed, both kidneys removed and frozen rapidly. Sections about 2–3 mm thick were exposed to an Agfa-Gevaert Graphic Film for 12 to 24 h. The concentration of the labelled material in the different areas which could be distinguished in the autoradiographs was determined quantitatively by measurements of the optical density. Calibration of the optical density readings in units of isotope concentration were obtained from a curve relating the blackening of gelatine standards to their known concentration of 131-J.

Typical autoradiographs for the dog kidney are shown in Figures 1a and b. Perfusion of the renal cortex is not uniform and considerable variations in blood flow distribution can be observed. 3 layers could be differentiated: a small, normally highly perfused superficial area, a midcortical area and a third zone in the juxtamedullary portion of the cortex. A similar pattern is observed in the kidney of rabbits and cats.



Regional distribution of blood flow in the renal cortex.

Zusammenfassung. Es wird ein autoradiographisches Verfahren zur Bestimmung der regionalen Durchblutungsverteilung in differenziert strukturierten Organen beschrieben. Mit dieser neuen Methode wird die Durchblutung der Nierenrinde untersucht. Es wird nachgewiesen, dass die Durchblutungs- bzw. Widerstandsverteilung innerhalb der Rinde nicht einheitlich ist, sondern dass sich drei verschieden durchblutete, anatomisch voneinander abgrenzbare Teile unterscheiden lassen.

H. Flohr

Physiologisches Institut der Universität Bonn und Institut für Klinische Strahlenkunde der Universität, 6500 Mainz (Germany), 26 February 1969.

[6] H. Flohr, Proc. Int. Congr. physiol. Sci., Washington DC 1968, vol. VII, 413, 138.

Limitations in the Quantitative Separation of DNA Samples by Density Gradient Centrifugation

The measurement of nuclear uptake of exogenous DNA by cells was recently reported[1]. The calculation of uptake involved the estimation of small amounts of radioactive donor BU-DNA present in the host cell non-radioactive DNA after the 2 materials had been separated by CsCl centrifugation. Controls for these experiments consisted of a simple mixture of the 2 types of DNA. It has usually been assumed that the separation of DNA molecules by CsCl density-gradient centrifugation is complete under the conditions normally employed, namely 65–70 h at 30,000 rpm and 25 °C. Some further experiments will be reported which suggest that this may not always be the case.

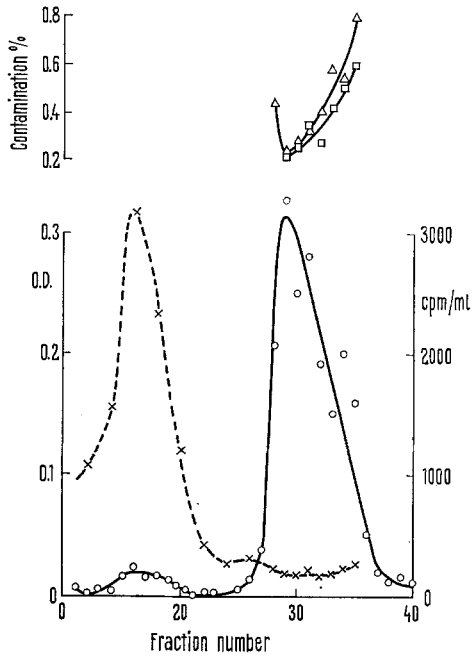
Nucleic acid samples were isolated from exponentially growing lymphoma cells in a manner previously described[1]. 3 samples were employed for these experiments, namely an unlabelled unsubstituted ('light') DNA, and the 2 samples which possessed both ^{14}C activity and also 50% replacement of thymine by 5-bromouracil ('heavy') DNA. In one of these latter specimens BU-^{14}C-DNA, the ^{14}C activity was present in adenine, guanine and thymine moieties by virtue of radioactive formate labelling. This sample was given a prior CsCl centrifugation, and only the 'heavier' material was used for further experiments. This was mainly DNA in which both strands were BU-substituted, and no DNA should be present which had escaped bromouracil substitution (a complication in the earlier experiments). The second substituted sample (^{14}C-BU-DNA) was labelled with 2-^{14}C-BUdR only (Schwarz Bioresearch Inc.) during the last 24 h of exponential growth. The presence of radioactivity in bases other than bromouracil was less than 1% as determined by thin-layer chromatography[2].

Mixtures of unsubstituted, non-radioactive DNA and either BU-^{14}C-DNA or (^{14}C-BU)-DNA were subjected to CsCl centrifugation. Calculations were made of the degree of contamination of light material by heavy, over a series of fractions in each run, in which corresponding tubes for mixture and control were compared. Results are expressed in the Table, and the last 2 experiments are plotted in the Figure.

From this Table it is apparent that the lack of resolution of the light and heavy DNA molecules can be appreciable. From the Figure it is also apparent that the contamination is not maximal with the light DNA peak, but is displaced even further to the lighter side. The

[1] A. B. Robins and D. M. Taylor, Nature *217*, 1228 (1968).
[2] P. S. Bond, J. Chromat. *34*, 554 (1968).
[3] I should like to thank Miss P. S. Bond for expert assistance, and Dr. D. M. Taylor for helpful discussion.

contamination is not decreased under conditions where separation should be more efficient, namely at lower degrees of loading of the CsCl with DNA. Nor is it decreased when the proportion of 'heavy' DNA is decreased. Both these latter facts suggest that an association between the molecules of DNA may be occurring which is not resolved on CsCl density gradient centrifugation. The observation may be of importance under certain experimental conditions when quantitative separations are required.



Lower curves: Optical density and ^{14}C-activity profiles for run 3 of the Table. —○—, O.D. at 260 nm; ---×---, cpm/ml of diluted fractions. Upper curves: Contamination of 'light' DNA by 'heavy' DNA (µg/100 µg). —△—, run 3 of the Table; —□—, run 4 of the Table.

Contamination of 'light' DNA peak by radioactive 'heavy' DNA

Run	% 'heavy' DNA added	Loading of CsCl (µg DNA/ml)	Contamination (µg 'heavy'/ 100 µg total)
1	10.5	55	0.1–1.0
2	4.5	65	0.1–0.3
3	2.0	42	0.2–0.8
4	2.0	21	0.2–0.6

In runs 1 and 2, the 'heavy' DNA had been fractionated as described above (BU-^{14}C-DNA). In runs 3 and 4, 'heavy' DNA carried the ^{14}C marker on the bromouracil only (^{14}C-BU-DNA).

Résumé. Des mélanges de l'ADN («léger») et de l'ADN-^{14}C-BU («lourd») ont étés centrifugés dans le chloride de caesium pendant 65 h à 60,000 *g*. Le résidu de l'ADN «lourd» dans l'ADN «léger» fut de l'ordre de 0.1 à 1%. La grandeur du résidu n'a pas correspondu à la proportion de l'ADN «lourd», ni à la concentration totale de l'ADN dans le chloride de caesium.

A. B. ROBINS

Biophysics Department, Institute of Cancer Research, Sutton (Surrey, England), 7 February 1969.

Proteinzuwachs und Glukoseaufnahme isolierter Myokardzellen von Hühnchenembryo und Ratte in der Primärkultur

Die Aufklärung molekularbiologischer Mechanismen auch an Körperzellen höherer Organismen erfordert in zunehmendem Masse den Einsatz geeigneter Zellpopulationen in Primärkultur. Insbesondere für die Charakterisierung des transmembranen Transportmechanismus kommt solchen Versuchsmodellen eine Bedeutung zu, da sie den Extrazellularraum leicht zu kontrollieren gestatten. Im Rahmen unserer Untersuchungen über den Wirkungsmechanismus des Insulins prüften wir unter diesem Aspekt die Eignung von Myokardzellen, die den Vorteil eines sichtbaren Funktionskriteriums, der autorhythmischen Kontraktion, besitzen, und berichten über einige basale Parameter, die das allgemeine Verhalten von Primärkulturen aus dem Myokard des Hühnchenembryos und der neugeborenen Ratte umschreiben.

Die Präparation der Zellen erfolgte aus 10 Tage alten Hühnchenembryonen bzw. maximal 48 h alten Ratten mittels fraktionierter Trypsinierung der mechanisch zerkleinerten Herzen in Anlehnung an CAVANAUGH[1], HALLE[2] und HARARY[3]; methodische Einzelheiten siehe[4]. Die Kultur der Myokardzellen des Hühnchenembryos (Ch) wurde aus 2 ml Zellsuspension (halbsynthetisches Nährmedium M 12), die im Mittel 500 000 Zellen enthielt, angelegt, der Anteil der toten Zellen (Trypanblau) betrug 5–15%. Nach 22 h Inkubation (37 °C) war ein geschlossener Zellrasen mit einem Anteil von 10–20% pulsierender Zellen entstanden. Die Kultur der Myokardzellen der neugeborenen Ratte (R) wurde in wenig modifizierter Weise angelegt, ein geschlossener Zellrasen mit einem Anteil von 80–90% pulsierender Zellen lag nach 40–42 h vor.

Nach dieser Zeit erfolgte der erste Wechsel des Mediums und damit der eigentliche Beginn der Versuche, in denen über mehrere 24-Stunden-Perioden das Gesamtprotein der Zellen (nach [5] mit Lowry-Lösung) sowie der Glukosegehalt des Mediums (Glukoseoxydase-Peroxydase nach [6]) ermittelt wurden.

Für den Proteinzuwachs der Kulturen (Startprotein um 200 µg) fanden wir ein unterschiedliches zeitpropor-

[1] M. W. CAVANAUGH, J. exp. Zool. *128*, 573 (1955).
[2] W. HALLE, Acta biol. med. german. *10*, 387 (1963).
[3] I. HARARY und B. FARLEY, Exp. Cell. Res. *29*, 451 (1963).
[4] B. ZIEGLER, H. G. LIPPMANN, R. MEHLING und E. JUTZI, Acta biol. med. german., in Vorbereitung.
[5] J. RERABEK, in *Leitfaden der Gewebezüchtung* (Gustav-Fischer-Verlag, Jena 1960), p. 206.
[6] P. KÖHLER, Z. ges. inn. Med. *17*, 674 (1962).