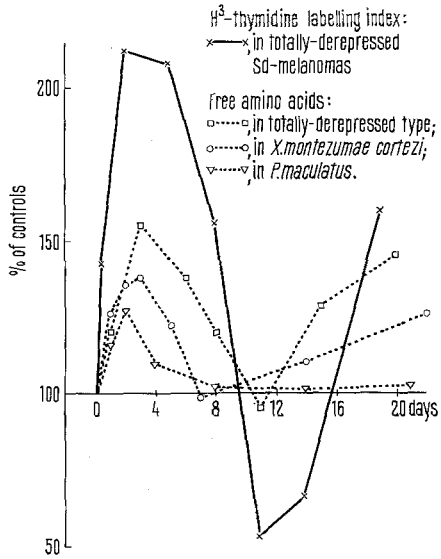
tion in technical systems which are on the threshold of stability[4]. In the Figure, one can see the fluctuations of total free amino acids of *Platypoecilus maculatus* (Mexico)[5], *Xiphophorus montezumae cortezi*[6] and the melanoma-bearing hybrids of *Platypoecilus maculatus* (Mexico), *Xiphophorus helleri* (Rio Papaloapan)[6] in the course of 3 weeks[7]. These curves resemble each other. The tumour-bearing hybrids show the greatest fluctuations in amino acid content.

In order to determine the stimulating effect of the amino acids, we have measured the DNA replication in the melanomas several times during the amino acid fluctuations by pulse labelling (injection of $H^3$-thymidine into the tumours), preparing autoradiographs and establishing the labelling index (labelled nuclei per total number of nuclei). The results of these investigations[8] are shown also in the Figure. The fact that this curve resembles those of fluctuations in amino acid content, suggests that DNA replication in melanomas, which in principle is already found to be stimulated by amino acids[1], depends upon the respective level of these substances present in the fish. It is also significant that the fluctuations of the labelling index curve are much greater than those of the amino acids. This confirms our earlier findings that the effect of amino acids upon DNA replication is stimulative[1, 4, 9, 10].




Fluctuations of free amino acid-concentration and $H^3$-thymidine labelling index in fish after transference from fresh water into diluted seawater (2.5‰). Total free amino acids: TCA-extraction of musculature; determination through ion exchange chromatography (Beckmann Unichrom Amino Acid Analyser). The standard error of the mean in the totally derepressed type was ± 2.3%, in *X. montezumae cortezi* ± 1.7%, and in *P. maculatus* ± 4.3%.
$H^3$-thymidine labelling index: Injection of 3 µl $H^3$-thymidine-containing isotonic solution (thymidine-methyl-$H^3$, New England Nuclear Corp., Boston (Mass., USA); specific activity 16.5 µC/m*M*; concentration 5 µC/ml) per melanoma. Incubation for 2 h. For details on the production of the autoradiographs see reference[1]. The controls were found to vary within ± 15% of the mean.

*Zusammenfassung.* Nach abrupter Überführung von Zahnkarpfen aus Süsswasser in verdünntes Meerwasser schwingt sich der Pool der sogenannten freien Aminosäuren von einem ursprünglichen auf einen neuen erhöhten stationären Zustand ein. Ein ganz entsprechendes Zeitverhalten hat der $H^3$-Thymidin-Markierungsindex (Anzahl markierter Kerne/Gesamtkernzahl) in Melanomen von Bastarden, deren Farbzellen genetisch total dereprimiert sind.

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[10] This research has been supported by a grant from Deutsche Forschungsgemeinschaft and Stiftung Volkswagenwerk.

## PRO EXPERIMENTIS

### Regional Distribution of Blood Flow in the Renal Cortex

Renal cortex normally appears to be perfused homogeneously. Under appropriate experimental conditions, however, a different vascular reactivity in distinct regions can be demonstrated[1-3]. These observations may be essential in understanding normal kidney function and drug action[2, 4].

The introduction of the particle distribution method of measuring regional blood flow[5] has facilitated the investigation of the problem in the experimental animal. Details of the technique have been published elsewhere. In brief, inert radioactively labelled particles, which are larger in diameter than the capillaries, are injected into the systemic circulation; provided the indicator is completely mixed in the blood which leaves the left ventricle and is completely extracted in one pass through the capillary bed, its distribution represents the pattern of

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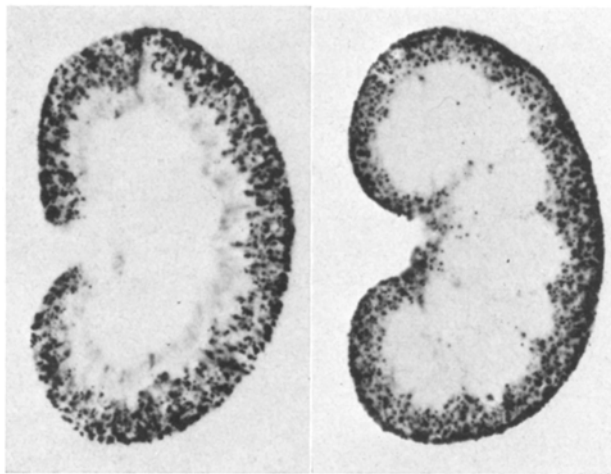
regional flow rates. Absolute flow values to any organ of the systemic circulation can be calculated from regional isotope concentration, cardiac output and the amount of indicator injected.

The same principle has been applied to assess the distribution of blood flow within single organs[6]. Particles labelled with $\beta$-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 $\mu$ 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.

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## 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

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