Methode und die Proteinbestimmung nach Angaben von LOWRY et al.[13].

Das Ergebnis der Untersuchungen ist in der Figur dargestellt. Es treten rhythmische Aktivitätsänderungen auf. Der erste Aktivitätsgipfel wird ca. 10 min nach der Befruchtung, ein weiterer während der Anaphase der ersten Furchungsteilung erreicht. In zwei Versuchen zeichnete sich ausserdem eine gesteigerte Aktivität im Metaphasestadium ab. Es bestehen jedoch bei verschiedenen Eigelegen deutliche Unterschiede im Aktivitätsverlauf der TdR-Phosphorylierung, die wir auf einen unterschiedlichen Reife- bzw. Überreifezustand der Eier im Ovar zurückführen möchten.

Die rhythmischen Aktivitätsschwankungen zeigen, dass die d-Nucleotidreserve der Eier eine gewisse Periodizität der Phosphorylierung von TdR in Abhängigkeit vom Zellzyklus nicht zu unterdrücken vermag. Ein ähnliches Ergebnis wurde auch an Kröteneiern (*Bufo bufo*) gewonnen.

Aus radioautographischen Untersuchungen an *Echinarachinus parma*[14] und aus biochemischen Analysen an *Strongylocentrotus purpuratus*[15] geht hervor, dass die erste DNS-Synthesephase ca. 30 min nach der Befruchtung noch vor dem Verschmelzen der Pronuclei beginnt, die zweite und die folgenden Synthesephasen während der Telo- und frühen Interphase der Furchungsteilungen einsetzen. Die Gipfel des beobachteten Aktivitätsverlaufes der TdR-Phosphorylierung liegen – wie auch bei den bisher bekannten Fällen – jeweils vor Beginn der zugehörigen Synthesephasen von DNS.

Summary. Although the eggs of sea urchins contain a large quantity of d-nucleotides, the activity of TdR-kinase shows rhythmical variations during mitosis. The activity increased before the beginning of DNA-synthesis.

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[14] E. B. SIMMEL und A. D. KARNOFSKY, J. Cell Biol. *10*, 59 (1961).
[15] R. T. HINEGARDNER, B. RAO und D. E. FELDMAN, Exp. Cell Res. *36*, 53 (1964).
[13] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR und R. J. RANDALL, J. biol. Chem. *193*, 256 (1951).

The Distribution of Bilirubin in Rat Liver Fractions

The development of a method to measure bilirubin in liver[1] has enabled a study of the distribution of bilirubin in liver after intravenous infusion of the pigment to be made. The method has also enabled a study of the amount of bilirubin in the tissues of jaundiced GUNN[2] rats to be made. These concentrations have previously been determined using [^{14}C]-bilirubin[3].

Bilirubin was dissolved in aqueous sodium bicarbonate-sodium chloride[4] solution and infused intravenously for 30 min into anaesthetized rats. Sufficient bilirubin was infused to produce the serum level of bilirubin found in the GUNN rats investigated (8–12 mg/100). After 30 min the animals were killed, the livers perfused with warm saline and then fractionated into nuclear, mitochondrial, microsomal and cell sap fractions[5]. Livers from male and female jaundiced GUNN rats were fractionated in a similar manner. Bilirubin was determined in the fractions from each set of experiments after resuspending the particulate material in phosphate-citric acid buffer (pH 2.2)[6]. GUNN rat tissues were homogenized in phosphate-citric acid buffer (pH 2.2) and bilirubin determined. Serum bilirubin levels were determined by the method of MALLOY and EVELYN[7].

The amount of bilirubin in the tissues of male and female jaundiced GUNN rats was determined as shown in the Table. The livers of 14 male and female jaundiced GUNN rats were fractionated and the bilirubin determined in the subcellular fractions (Figure 1). Almost identical means were obtained for the male and female GUNN rats. Figure 2 shows the amount of bilirubin in the subcellular fractions of Wistar rat liver infused with bilirubin for 30 min.

The amounts of bilirubin found in GUNN rat tissues is similar to that found using [^{14}C]-bilirubin[3]. Brain tissue contains only small amounts of bilirubin when determined by both methods. Jaundiced GUNN rats are deficient in UDP-transglucuronylase (uridine diphosphate glucuronate glucuronyl transferase, acceptor unspecific EC 2.4.1.17) using bilirubin as a substrate. The distribution of bilirubin in the subcellular fractions of the liver shows that there is little bilirubin in the microsomal fraction, where the enzyme is situated; most of the bilirubin is in the cell sap. In the infusion experiments using Wistar rats, more is found in the microsomal fraction and less in the cell sap.

Bilirubin concentrations in organs of jaundiced GUNN rats

Tissue	Bilirubin μg/g Male Range	Male Mean	Female Range	Female Mean
Liver	52–76	62 ± 7 (7)	59–68	62 ± 2 (6)
Spleen	23–54	36 ± 13 (7)	28–62	47 ± 17 (4)
Kidney	28–45	39 ± 7 (7)	37–58	49 ± 7 (6)
Ileum	13–19	17 ± 3 (7)	11–27	15 ± 7 (5)
Lung	34–62	41 ± 10 (7)	14–63	46 ± 13 (6)
Brain	6–18	11.5 ± 4 (7)	3–18	12 ± 5 (6)

Mean values are ± I.S.D. Number of animals in parentheses.

[1] T. HARGREAVES, Clinica chim. Acta *11*, 278 (1965).
[2] C. H. GUNN, J. Hered. *29*, 137 (1938).
[3] R. SCHMID and L. HAMMAKER, J. clin. Invest. *42*, 1720 (1962).
[4] K. WEINBREN and B. H. BILLING, Br. J. exp. Path. *37*, 199 (1956).
[5] W. C. SCHNEIDER and G. H. HOGEBOOM, J. biol. Chem. *183*, 123 (1950).
[6] T. C. MCILVAINE, J. biol. Chem. *49*, 183 (1921).
[7] H. T. MALLOY and K. A. EVELYN, J. biol. Chem. *117*, 481 (1937).

HERWEGH, VAN ROY and DE GROOTE[8] added sodium bilirubinate to rat liver homogenate at 4 °C and found that most of the bilirubin was present in the microsomes and cell sap fraction. If human serum or albumin was used as the suspending vehicle for bilirubin then most of the bilirubin was found in the cell sap fraction, the distribution being similar to that observed here in the GUNN rat. The distribution of bilirubin in liver in vitro depended on the suspending medium in which bilirubin is added. HERWEGH et al.[8] interpreted their results as showing that albumin and serum competed for bilirubin with the subcellular fractions and so prevented the uptake of bilirubin by the fractions. In the in vivo experiments bilirubin is presented to the liver attached to rat serum proteins, and we would expect that the bilirubin could be found mainly in the cell sap because of this binding to serum proteins.

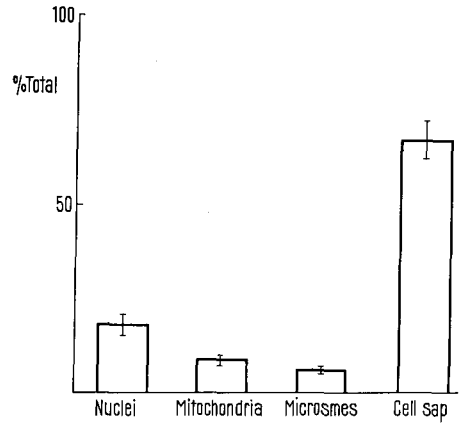

Fig. 1. Bilirubin content of the liver of jaundiced male and female GUNN rats. Results are means of 14 animals ± I.S.D.

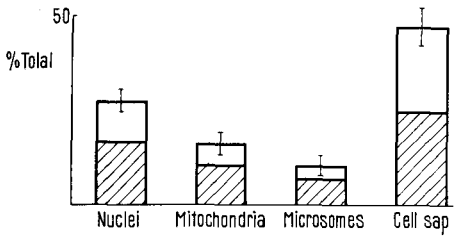

Fig. 2. Bilirubin content of fractions from rat liver after bilirubin infusion. Results are means of 5 experiments ± I.S.D. $\overline{/7/}$, Direct reacting bilirubin.

Zusammenfassung. Die Bilirubinmengen in den Geweben ikterischer GUNN-Ratten werden zusammen mit den in den subzellulären Anterilen vorliegenden Bilirubinmengen ikterischer GUNN-Rattenlebern bestimmt. Die Bilirubinbestimmung erfolgte nach i.v. Infusion von Bilirubin in die Rattenleber: es findet sich vorwiegend im Leberzellensaft der ikterischen GUNN-Ratte, verteilt sich jedoch nach Bilirubininfusion gleichmässig über alle Leberanteile. Die teils bekannten, teils bestätigten Unterschiede können durch Plasma-Eiweiss-Bindung des Bilirubins erklärt werden.

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January 31, 1966.

[8] K. HERWEGH, F. VAN ROY, and J. DE GROOTE, Tijdschr. Gastroent. *7b*, 184 (1964).

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Amperometric Study on Lanthanum Polytungstates

The formation and composition of lanthanum polytungstates obtained by the interaction of lanthanum nitrate and different alkali tungstates have been investigated by means of amperometric titrations between the reactants at different pH values in aqueous-ethanolic media. The sharp breaks obtained provide cogent evidence for the formation of $La_2O_3 \cdot 3WO_3$ and $La_2O_3 \cdot 7WO_3$ in the pH ranges 5.5–6.5 and 4.2–5.5 respectively. Amperometric titrations provide a simple and rapid method of determination of La^{3+} ions.

An outstanding characteristic of tungstates is their strong tendency to form condensed complex ions in solution[1], and from these polytungstates crystallize depending upon the pH, which plays an important role in the aggregation process. The formation of polytungstates is usually attributed to combination with corresponding acids, as if they were separate chemical individuals. This led the earlier workers to believe that similar salts of heavy metals may be precipitated as a result of metathesis[2]. BRITTON and GERMAN[3], however, contended that heavy metal tungstates are not precipitated by interaction of metal salts and alkali tungstates.

In the literature, there are few references to the study of La^{3+}-tungstates and most workers had based their conclusions on analytical results. HITCHCOCK[4] obtained La^{3+}-tungstate by adding $LaCl_3$ to Na_2WO_4. ROGERS and SMITH[5] prepared ammonium La-tungstate having the composition $2(NH_4)_2O \cdot La_2O_3 \cdot 16WO_3 \cdot 16H_2O$. HOGBOM[6] reported the formation of $Na_8La_2(WO_4)_7$ by dissolving

[1] G. JANDER and H. JAHR, Z. anorg. allg. Chem. *194*, 383 (1930).
[2] F. ABEGG, *Handbuch der Anorganischen Chemie* (Leipzig 1921), p. 577, 636.
[3] H. T. S. BRITTON and W. L. GERMAN, J. chem. Soc. Lond. *1429* (1931).
[4] F. R. M. HITCHCOCK, J. Am. chem. Soc. *17*, 483 (1898).
[5] A. ROGERS and E. F. SMITH, J. Am. chem. Soc. *26*, 1774 (1904).
[6] A. J. HOGBOM, Bull. Soc. chim., Paris *42*, 2 (1884).