manifestations of arousal occurred during and after the infusion as well, they cannot be attributed to some rebound effect.

Control infusions of dialysing fluid also show a gradual decrease of the cortical delta activity. This becomes statistically significant after 10 min. After the infusion, a further small decrease is still detectable for 20 min.

The comparison of the effect of histamine solution and control fluid reveals a much steeper decrease during the infusion of histamine. The difference is significant from the 8th min throughout the experiment.

The visceral effects of histamine infusion are the following under our experimental conditions: (1) Slight transitory decrease of blood pressure, but not significant when compared to the control experiment. (2) Increase of pulse rate during the infusion, significant after 10 min; after the infusion, the pulse rate gradually slows down. (3) The respiratory rate has a tendency to rise, but chiefly after the infusion.

These findings suggest that histamine has a waking action independant of the slight unsignificant visceral alterations and that it could play a role in the regulation of wakefulness[11].

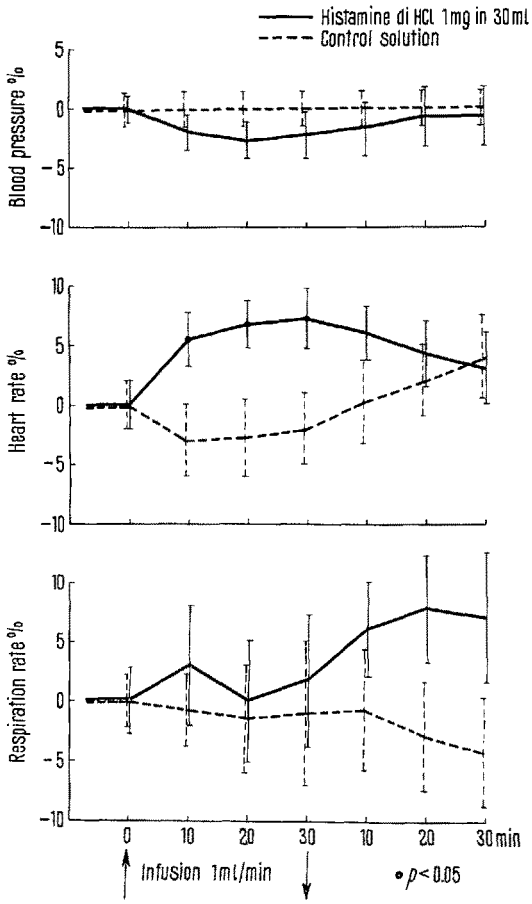

Fig. 2. Variation of blood pressure, heart rate and respiration rate in % during and after i.v. injection of Histamine solution or control solution in the rabbit (mean values of 8 rabbits in each case).

Zusammenfassung. Die i.v. Infusion von 1 mg Histamin-2HCl in 30 ml bei einer konstanten Strömung von 1 ml/min während 30 min führt zu einer statistisch signifikanten Abnahme der spontanen kortikalen Delta-Aktivität beim Kaninchen. Dieser elektrographische Weck-Parameter, der gleichzeitig mit einer Desynchronisierung des EEG einhergeht, ist bedeutend grösser bei Kaninchen, die Histamin erhalten haben, als bei Kontrolltieren. Die Weckreaktion dauert nach Ende der Infusion weitere 30 min an. Gleichzeitig bewirkt Histamin ein geringfügiges, nicht signifikantes Absinken des arteriellen Blutdruckes, eine Zunahme der Herzfrequenz sowie ein leichtes Ansteigen der Atmungsfrequenz. Aus diesen Befunden geht hervor, dass Histamin bei unwichtigen visceralen Auswirkungen bei der Regulierung des Wach-Schlaf-Zustandes eine Rolle spielen könnte.

M. MONNIER, M. FALLERT, and I. C. BHATTACHARYA

Physiological Institute, Basel (Switzerland), October 28, 1966.

[11] We are very much indebted to Dr. P. SCHMID for his help in the statistical work, to Miss Y. LANZ for her technical assistance, and to the Direction of Hoffmann-La Roche & Co. AG for the financial support.

The Hexose Content of the α-Chains of Bone Collagen

Hexoses and substances which react as hexoses have been found in the collagens of a wide variety of tissues[1–4]. However, it has not been possible to analyze the highly purified single-chain α-components of collagen, because of the leaching out of anthrone-reacting substances from the CM-cellulose resin used to chromatographically separate the α-chains. The recent development of methods for the separation of the 3 α-chains of bone collagen by filtration through Bio-gel P-300 resin (Bio-Rad Laboratories, Richmond, California, USA) and free flow electrophoresis[5] has provided us with an opportunity to analyze the purified single α-chains of a collagen, which have not been subjected to CM-cellulose chromatography.

Bone gelatin was prepared from powdered 12–14-week-old chicken metatarsal bones by methods previously described[6]. The third and fourth LiCl and KSCN extracts were used since these were found to contain no detectable

[1] W. GRASSMANN, H. HÖRMANN, and R. HAFTER, Hoppe-Seyler's Z. physiol. Chem. *307*, 87 (1957).

[2] J. GROSS, B. DUMSHA, and N. GLAZER, Biochim. biophys. Acta *30*, 293 (1958).

[3] H. HÖRMANN, Leder *13*, 179 (1962).

[4] O. BLUMENFELD, P. PAZ, P. M. GALLOP, and S. SEIFTER, J. biol. Chem. *238*, 3835 (1963).

[5] C. J. FRANÇOIS and M. J. GLIMCHER, in *Symposium International sur la Biochemie et la Physiologie du Tissu Conjonctif*, 1965 (Ed. P. COMTE; Société Ormeco et Imprimerie du Sud-Est, Lyon, France).

[6] M. J. GLIMCHER and E. P. KATZ, J. Ultrastruct. Res. *12*, 705 (1965).

2-deoxy sugars[7]. After the separation of the mixed α-components from the higher molecular weight β- and γ-polymers by filtration through Bio-gel P-300 resin[8], the α_1-, α_2-, and α_3-chains were isolated by free flow electrophoresis in 6*M* urea at pH 5.25 at 4 °C[5], using the apparatus described by HANNIG[8]. The homogeneity of the α-fractions was assayed by polyacrylamide gel electrophoresis[9] at 10 °C in 6*M* urea[10], CM-cellulose chromatography[11], and by amino acid analyses.

Aliquot portions of the 3 α-chains which had been desalted by filtration through Bio-gel P-10 resin were reacted with anthrone[12] and the absorption spectrum measured between 460 nm and 700 nm. The hexose content was computed on the basis of the extinction coefficient measured at 625 nm using glucose as a standard. A sample containing all the reagents, but no protein, and one containing protein and sulphuric acid but no anthrone reagent, gave no reaction measured at 625 nm.

The hexose content of the 3 α-chains, expressed as moles hexose/1000 amino acid residues, calculated by analyses of known volume-aliquots of gelatin for both hexoses and amino acids, and by analyses of hexose and amino acids on known weights, is 3.9 for α_1-, 2.5 for α_2-, and 4.3 for α_3-chains. The moles of hexose/collagen macromol computed on the basis of a molecular weight of 320,000 and an average residue weight of 92[4] is approximately 12 moles of hexose/collagen mol. The absorption spectrum of the 3 α-chains after reaction with anthrone gave a single symmetrical peak with a maximum at 625 nm, similar to glucose, and showed no other peaks. Furthermore, no 2-deoxy sugars[7] and no hexosamines could be detected colorimetrically[13], and no hexosamine peaks were seen in the chromatographs of large samples obtained on the automatic amino acid analyzer. It seems likely, therefore, that the anthrone-reacting substances were hexoses.

The content of hexoses has been calculated using glucose as a standard, whereas both glucose and galactose, which have different extinction coefficients after reaction with anthrone, have been identified in ichthyocol[4]. Therefore a direct comparison between the values reported in the present study and those obtained by BLUMENFELD et al.[4] for ichthyocol is not entirely valid, since the distribution of hexoses may be different for different tissues. Nonetheless, based on the anthrone reaction, the hexose content of ichthyocol/collagen macromol (~9 moles hexose/mol collagen)[4] and of chicken-bone collagen (~12 moles hexose/collagen macromol) are similar. Moreover, from the present study it may be concluded that hexoses are an integral component of the single-stranded α-chains of at least one collagen[14].

Résumé. Les 3 chaînes α du collagène de l'os de poulet ont été isolées en combinant les techniques de filtration sur gel et d'électrophorèse en veine libre. Des substances donnant une réaction d'anthrone positive, avec spectre d'absorption caractéristique des hexoses, ont été mises en évidence dans ces 3 chaînes. En utilisant le glucose comme standard, la détermination des hexoses fournit une valeur de 12 moles d'hexoses par molécule de collagène.

C. J. FRANÇOIS and M. J. GLIMCHER

Department of Orthopedic Surgery, Harvard Medical School and the Massachusetts General Hospital, Boston (Massachusetts, USA), August 2, 1966.

[7] W. SCHNEIDER, in *Methods in Enzymology*, (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press Inc., New York 1957), vol. 3, p. 680.
[8] K. HANNIG, Z. analyt. Chem. *181*, 244 (1961).
[9] Y. NAGAI, J. GROSS, and K. A. PIEZ, Ann. N.Y. Acad. Sci. *121*, 494 (1964).
[10] C. J. FRANÇOIS and M. J. GLIMCHER, Biochim. Biophys. Acta *97*, 2 (1965).
[11] K. A. PIEZ, E. A. EIGNER, and M. S. LEWIS, Biochemistry, N.Y. *2*, 58 (1963).
[12] T. A. SCOTT JR. and E. H. MELVIN, Analyt. Chem. *215*, 555 (1955).
[13] J. W. ROSEVEOR and E. L. SMITH, J. biol. Chem. *236*, 425 (1961).
[14] We thank Miss DIANE BRICKLEY and Mrs. CYLA SILBER for their technical assistance. This work was supported by grants from the U.S. Public Health Service, National Institutes of Health (AM-06375) and the John A. Hartford Foundation Inc. C. J. FRANÇOIS is a Fellow of the Helen Hay Whitney Foundation, on leave of absence from the Institut Interuniversitaire des Sciences Nucléaires de Belgique.

Einfluss des Licht-Dunkel-Wechsels auf den O_2-Umsatz der Rattenleber

In einer früheren Arbeit haben KIKUCHI und WEIHE[1] über eine Tagesperiodik des Sauerstoffverbrauchs der Leber von Sprague-Dawley-Ratten berichtet, die in bezug auf die möglichen tageszeitlichen Schwankungen der verschiedenen Funktionen der Leber und der Wärmeproduktion wichtig ist. Jetzt wurde die Tagesperiodik des O_2-Umsatzes der Leber bei Wistar Ratten bei normalem und umgekehrtem Kunsttag untersucht.

Material und Methoden. Männliche Wistar-Ratten wurden verwendet, die beim Töten zur Untersuchung 60–70 Tage alt waren. Die Tiere wurden in einer künstlichen Klimakammer, Raumtemperatur 24 ± 0,25 °C, RF 60 ± 3%, gehalten. Sie erhielten Wasser und Standardfutter (CE-2, Japan CLEA Co.) ad libitum. Bei normalem Kunsttag war die Lichtzeit von 07.00 bis 19.00 h und bei umgekehrtem Kunsttag von 19.00 bis 07.00 h. Die Lichtintensität über dem Käfig war 50–60 Lux. Bei jedem Versuch wurden die Tiere um 07.30 h, 14.00 h und 17.00 h im leichten Ätherrausch durch Ausbluten aus der Bauchaorta getötet. Schneiden der Leber, Schnittdicke ca. 0,5 mm, war nach DEUTSCH[2]; Messung des O_2-Verbrauchs nach der Warburg-Methode; Medium: Krebs-Ringer-Phosphatlösung, pH 7,3; Gasphase: 100% O_2; Wasserbadtemperatur: 37,5 °C. Das Glykogen der Leber wurde nach der Anthron-Methode[3] und Stickstoffgehalt der Leber nach Mikro-Kjeldahl bestimmt.

Zu jedem Versuchszeitpunkt wurden 8–12 Tiere untersucht. Zur Qo_2-Messung wurden von jeder Leber Doppelbestimmungen vorgenommen.

[1] M. KIKUCHI und W. H. WEIHE, Experientia *20*, 706 (1964).
[2] W. DEUTSCH, J. Physiol. *87*, 56P (1936).
[3] J. VAN DER VIES, Biochem. J. *57*, 410 (1954).