

resembled one another in moderating effect, but differed considerably from control dialysate or amphetamine<sup>9,10</sup>.

**Zusammenfassung.** Die Wirkung des Schlafdialysates des Kaninchens auf das Gehirn eines normalen Empfängers kennzeichnet sich durch Erregbarkeitszunahme des mediozentralen somnogenen thalamischen Feldes (Amplitudenzunahme der im Cortex ausgelösten Potentiale). Eine ähnliche Wirkung übt Chlorpromazin als zentraldämpfendes Pharmakon und Substanz P aus,

während zentrale Stimulantien wie Amphetamin eine umgekehrte Wirkung entfalten.

N. SCHNEIDERMAN<sup>11</sup>, M. MONNIER<sup>12</sup>,  
and F. LEMBECK

*Physiologisches Institut der Universität Basel  
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<sup>11</sup> Present address: Department of Psychology, University of Miami (Florida USA).

<sup>12</sup> Requests for reprints should be sent to Prof. M. MONNIER, Physiologisches Institut, Vesalgasse 1, Basel (Switzerland).

## Mucopolysaccharides in Experimental Hepatic Fibrosis

Chronic administration of hepatotoxic agents induces hepatic sclerosis in the rat, which is generally well established after 100 days. At the beginning of this pathologic change one can see the accumulation of an extracellular material, which gives histochemically a positive periodic acid-Schiff reaction and displays metachromasia after staining with toluidine blue. The mucopolysaccharide nature of this substance is now generally accepted according to the findings of POPPER et al.<sup>1</sup> The possible role of this material in hepatic fibrogenesis led us to study the biochemical modifications of mucopolysaccharide constituents during chronic carbon tetrachloride intoxication in rat.

14 Wistar male rats (300–400 g) received subcutaneous carbon tetrachloride (0.15 ml in 0.5 ml mineral oil) twice weekly over a period of 3 months. The animals were allowed a regular diet. At the end of the experiment the 14 experimental and 5 control animals were killed, the liver immediately homogenized at 0°C, delipidized<sup>2</sup> and dried. The following quantitative determinations were then performed: hydroxyproline<sup>3</sup>; total hexosamines<sup>4</sup>; extraction of<sup>5</sup> and determination of hexuronic acids<sup>6</sup>; and total protein bound sulphates<sup>7</sup>. Our results are summarized in the Table.

The following points may be emphasized: (1) At the end of the 3-month period of intoxication, micronodular fibrosis was macroscopically visible and the accumulation of collagen in the fibrotic livers was marked. This was demonstrated by a fivefold increase in the hydroxyproline content of damaged livers as compared to the normal ones. (2) The total hexosamine content was increased by 79% and protein bound sulphates by 94% of the normal values. The differences between these averages and those of the controls are very significant. (3) In contrast with these obvious changes, the concentration of hexuronic acids in the pathologic livers was practically the same as that in the normal livers. The high value of the correlation coefficient (Figure) between the hexosamine concentration and the protein bound sulphates in the pathologic livers is evidence that the hexosamine-rich material is sulphated.

<sup>1</sup> H. POPPER, F. SCHAFFNER, H. HUTTERER, N. PARONETTO, and F. BARKA, *Ann. New York Acad. Sci.* **86**, 1075 (1960).

<sup>2</sup> W. C. SCHNEIDER, *J. biol. Chem.* **161**, 293 (1945).

<sup>3</sup> H. J. KIVIRIKKO and F. LIESMAA, *Scand. J. clin. lab. Invest.* **2**, 128 (1959).

<sup>4</sup> N. F. BOAS, *J. biol. Chem.* **204**, 553 (1953).

<sup>5</sup> A. J. BOLLET, *J. clin. Invest.* **37**, 858 (1958).

<sup>6</sup> T. BITTER and H. M. MUIR, *Anal. Biochem.* **4**, 330 (1962).

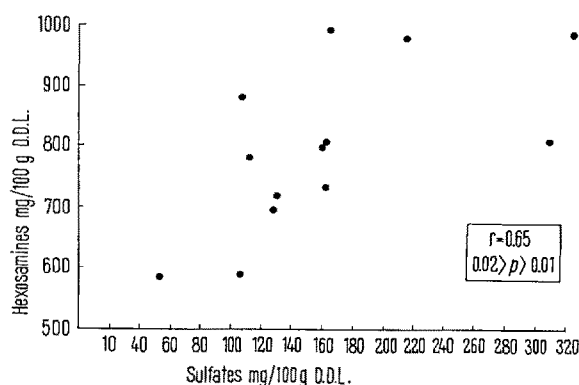
<sup>7</sup> K. S. DODGSON, *Biochem. J.* **78**, 312 (1961).

Mean values and standard deviations of hydroxyproline, total hexosamine, total hexuronic acid and protein-bound sulphate concentration in dried de-fatted liver of normal and CCl<sub>4</sub>-treated rats

	Hydroxyproline mg/100 g	Total hexosamines mg/100 g	Total hexuronic acids mg/100 g	Protein-bound sulphates mg/100 g
Normal (5)	108.04 ± 14.29	441.66 ± 34.40	144.33 ± 18.52	72.32 ± 12.97
CCl <sub>4</sub> -treated (14)	510.51 ± 142.04	794.89 ± 105.38	136.14 ± 28.57	164.80 ± 54.75
<i>t'</i>	4.77	5.93	0.015	2.64
<i>p</i>	≤ 0.001	≤ 0.001	> 0.90	0.02 > <i>p</i> > 0.01

*t'*: significance test. *p*: significance level.

These results suggest that the accumulated mucopolysaccharide-like material is hexosamine-rich and sulphated, but devoid of uronic acids. SHETLAR et al.<sup>8</sup> have noted the relative lack of hexuronic acids in wound tissue from rabbits and dogs. The accumulation of sulphates in hepatic tissue following toxic injury is in accord with the findings of JUNGE HÜLSING and HAUSS<sup>9</sup> and PATRICK et al.<sup>10</sup>, in which S<sup>35</sup> incorporation by mucopolysaccharides was increased in rabbits and rats following liver injury. However, the former authors assumed an increased hepatic accumulation of chondroitin sulphates and did not specifically measure the hepatic uronic acid content.



Relationship between total hexosamine and protein-bound sulphate concentrations in fibrotic livers. (Dried defatted tissue.)  $r$  = correlation coefficient.  $p$  = significance level.

As our data were obtained on whole tissue, they do not allow conclusions regarding the intracellular location and chemical structure of the substance or substances. However, they strongly suggest that the sulphated hexosamine-rich material accumulated during liver fibrosis might possess a structure similar to the glycosaminoglycans described in different types of normal connective tissues<sup>11</sup>.

The isolation and chemical determination of the substance is now under way.

**Résumé.** L'intoxication chronique au tétrachlorure de carbone produit dans le tissu hépatique du rat une forte augmentation des hexosamines et des sulfates liés sans modifier la teneur en acides uroniques. Ces modifications sont parallèles au processus de fibrillogénèse. Ces données indiquent l'accumulation pathologique d'un matériel de type glycosaminoglycan sulfaté.

EDITH MIHAESCO\* and C. MIHAESCO

\*Centre de recherches isotopiques de l'Institut national de la Santé et de la Recherche Médicale, Hôpital Beaujon, Clichy (Seine, France), June 21, 1965.

<sup>8</sup> M. R. SHETLAR, E. LACEFIELD, B. WHITE, and A. J. SCHILLING, *Proc. Soc. exp. Biol. Med.* 100, 501 (1959).

<sup>9</sup> G. JUNGE HÜLSING and W. HAUSS, *Struktur und Stoffwechsel des Bindegewebes* (Eds. W. H. HAUSS and H. LOOSE; G. Thieme Verlag, Stuttgart 1960), p. 83.

<sup>10</sup> R. S. PATRICK and J. S. KENNEDY, *J. Path. Bact.* 88, 549 (1964).

<sup>11</sup> L. ROBERT and Z. DISCHE, *Biochem. biophys. Res. Comm.* 10, 209 (1963).

## Unit Responses of the Lateral Geniculate Body to Light Flashes during Wakefulness and Synchronized Sleep

The existence of a reticular control upon the responsiveness of cells in the lateral geniculate body (LGB) is well established<sup>1,2</sup>. The enhancement during reticular<sup>1,2</sup> or natural<sup>3</sup> arousal of the geniculate responses evoked by electric shocks to the chiasm or optic nerve shows that the influence is facilitatory in nature. It is surprising, however, that the responses evoked by light flashes actually decrease during, or soon after, mesencephalic reticular stimulation<sup>1</sup>. The hypothesis<sup>1</sup> of an occlusion at the level of the geniculate, between reticular and retinal inputs, cannot easily account for this discrepancy.

This work is an attempt to re-examine the problem by a study of the evoked responses of single LGB units.

Experiments were performed on cats with the brain stem sectioned immediately rostral to the exit of the 5th nerve (midpontine pretrigeminal preparation<sup>3</sup>). This preparation characteristically shows EEG and behavioural patterns of wakefulness with some episodes of synchronized sleep. The pupils were paralysed with atropine. Spikes were recorded from the dorsal part of LGB with steel microelectrodes. In order to control the effect of eye movements during the waking state, some experiments were performed on curarized cats. The LGB cell firing rate

was measured by a Beckman counter. A Sylvania 1160 glow tube was used to apply light flashes 300 msec in duration, the start of the flash being synchronized with the counter to facilitate detection of changes in rate. The response to the light pulse was calculated with respect to the average taken during 6 sec before the light pulse. EEG activity was continuously recorded by screws implanted in the skull.

The results can be summarized as follows: (1) During sleep the LGB cell activity occurs in bursts of high frequency (300–500/sec), while during wakefulness it shows random patterns. This confirms previous findings in the intact animal<sup>4</sup>. The average spontaneous frequency is also generally higher during wakefulness. A study of the standard deviation of the frequency in 1 sec compared to the average frequency shows a strong increase (up to 3–4 times) during sleep. (2) The firing rate of LGB units in

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<sup>3</sup> C. BATINI, G. MORUZZI, M. PALESTINI, G. F. ROSSI, and A. ZANCHETTI, *Arch. ital. Biol.* 97, 1 (1959).

<sup>4</sup> D. H. HUBEL, *J. Physiol., Lond.* 150, 91 (1960).