Diabetologia 6, 412–413 (1970)

Animal Experiments Concerning the Disturbed Wound-Healing in Diabetes Mellitus*

H. Wagner, G. Junge-Hülsing, H. Otto, J. Adler and W. H. Hauss

Medical Clinic of the University of Münster (Director: Prof. Dr. W.H. Hauss)

Received: July 15, 1969

Summary. A series of experiments with alloxan-diabetic and control rats was done in order to investigate growth of cotton-pellet granulomas and incorporation rate of ^{35}S-sulphate into sulphated mucopolysaccharides of the granulation tissue. — It could be demonstrated that in the alloxan-diabetic animals there was not only markedly less formation of granulation tissue, but a pronounced blocking of synthesis of sulphated mucopolysaccharides in the granulation tissue as well. — These findings are assumed to be caused by the lack of insulin in alloxan diabetes, and to be related to the delayed wound-healing in patients with badly controlled diabetes mellitus.

Expériences sur l'animal concernant la guérison perturbée des blessures dans la diabète sucré

Résumé. Une série d'expériences a été effectuée avec des rats diabétiques par l'alloxane et des rats témoins afin d'étudier la croissance de granulomes de pellets de coton et la vitesse d'incorporation du ^{35}S-sulfate dans des mucopolysaccharides sulfatés du tissu de granulation. — Il a pu être démontré que chez les animaux diabétiques par l'alloxane, il y avait non seulement une formation nettement diminuée de tissu de granulation, mais aussi un blocage prononcé de la synthèse des mucopolysaccharides sulfatés dans le tissu de granulation. — On suppose que ces résultats sont causés par le manque d'insuline dans le diabète alloxanique et qu'ils sont en relation avec la guérison retardée des blessures chez les sujets ayant un diabète sucré mal équilibré.

Tierexperimenteller Beitrag zur gestörten Wundheilung bei Diabetes mellitus

Zusammenfassung. Es wurde im Tierexperiment an gesunden und alloxandiabetischen Ratten das Wachstum und der Sulfomucopolysaccharidstoffwechsel des Granulationsgewebes von Cotton-pellet-Granulomen überprüft. — Die Untersuchungen haben ergeben, daß bei alloxandiabetischen Tieren eine deutliche Verminderung der Granulationsgewebsbildung sowie eine ausgeprägte Hemmung des Mesenchymstoffwechsels in neugebildeten Granulationsgewebe im Vergleich zu normalen Kontrolltieren bestehen. — Die Befunde werden auf den bei Alloxandiabetes nachweisbaren Insulinmangel zurückgeführt und lassen den Schluß zu, daß die verminderte Wundheilung bei Patienten mit dekompensiertem Diabetes mellitus auf einer Störung des Mesenchymstoffwechsel beruht.

Key-words: Diabetes mellitus, metabolism of sulphated mucopolysaccharides, granulation tissue, wound-healing.

In earlier experiments with alloxan-diabetic rats we were able to demonstrate not only a decrease of synthesis, but above all an excessively delayed turnover-rate of sulphated mucopolysaccharides of ground substance [4, 1, 8]. These changes account for the gradual accumulation of ground substance in the transit-zones of various organs in diabetes mellitus, which can be shown by histochemical techniques and by electron microscopy as well [2, 3, 7].

In the following article we report our results of further experiments on growth and mesenchymal metabolism of granulation tissue in alloxan-diabetic rats. This series of experiments was done with respect to the clinical experience where patients with badly controlled diabetes mellitus often show a decreased resistance against infections, and a delayed wound-healing.

Methods

The experiments were carried out in 40 male alloxan-diabetic rats (Wistar II), weighing 150 g to 180 g, and in 40 healthy rats of the same age and weight. To induce diabetes mellitus a large sample of rats were injected subcutaneously with alloxan, 200 mg/kg, as a freshly prepared 5%-solution. From this sample of rats the 40 animals in which the alloxan diabetes was most pronounced in weekly checks of blood glucose (hexokinase method) were chosen for the experiment. The blood glucose levels in these animals were constantly higher than 300 mg/100 ml (ranging from 310 to 650 mg/100 ml). The diabetes remained untreated. After a period of 6 weeks from the beginning of alloxan diabetes, cotton pellets were implanted [6]. The 1st, 4th, 7th and 14th day after implantation a group of 10 diabetic and 10 control animals was killed. We determined the wet weight of the prepared cotton-pellet granulomas, and investigated the rate of incorporation of ^{35}S-sulphate into the sulphated mucopolysaccharides (SMPS) of the connective tissue of the granulomas [5].

The data given in Table 1 and Figs. 1 and 2 for wet weight and specific activity are mean values calculated from 10 animals.

Fig. 1 shows the weight curves of the cotton-pellet granulomas. In healthy animals the formation of granulation tissue was most pronounced on the 4th day after implantation. Thereafter wet weights decreased with increasing collagenization. In alloxan-diabetic rats, however, the formation of granulation tissue, derived from the wet weight, was markedly less on all days of investigation.

* This work was supported by the Landesversicherungsanstalt Westfalen, the Bergbauberufsgenossenschaft Bochum and the Deutschen Forschungsgemeinschaft.

Table 1

	Control rats Mean value ± SEM	Alloxan-diabetic rats Mean value ± SEM	*P*
Wet weight of the granulomas in mg			
1st day	23.83 ± 4.23	19.00 ± 1.46	0.15
4th day	88.00 ± 13.71	28.42 ± 3.81	0.0005
7th day	75.50 ± 8.72	27.00 ± 6.19	0.0025
14th day	31.88 ± 4.72	23.00 ± 2.07	0.10
Specific activity in ^{35}S-sulphate cmp/100 µg sulphated mucopolysaccharides			
1st day	2758.40 ± 206.95	905.00 ± 354.00	0.005
4th day	15688.40 ± 1752.13	3910.90 ± 659.59	0.0005
7th day	16317.16 ± 1273.22	5810.00 ± 1017.51	0.0005
14th day	9776.66 ± 1292.29	5069.48 ± 530.48	0.0025

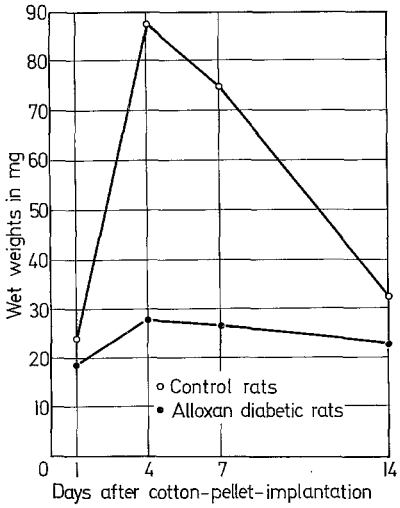

Fig. 1. Wet weights of granulation tissue of cotton-pellet granulomas

The significant difference between alloxan-diabetic and control rats is also to be seen when the rate of incorporation of ^{35}S-sulphate in SMPS of granulation tissue is investigated (Fig. 2). The rate of incorporation in alloxan-diabetic rats, expressed as ^{35}S-sulphate cpm/100 µg sulphated MPS of connective tissue, was reduced to 30% of that in controls on the days of most pronounced granulation (4th and 7th day).

These findings are evidence that in alloxan-diabetic rats the rate of reproduction of cells, i. e. the tendency to proliferation, was diminished. Moreover, the metabolism of the mesenchymal cells in the granuloma was decreased, as measured by the rate of incorporation of ^{35}S-sulphate in SMPS. Probably these changes were caused by the lack of insulin in alloxan diabetes. The results of our investigation support the hypothesis that the decreased resistance against infection and the delayed wound-healing in patients with poorly controlled diabetes mellitus is caused by a disturbance in mesenchymal metabolism in diabetes.

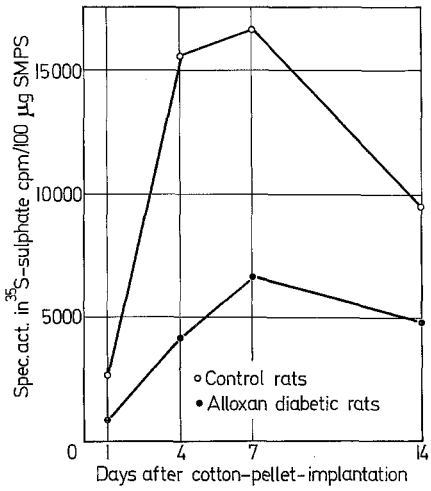

Fig. 2. Mesenchymal metabolism of granulation tissue of cotton-pellet granulomas (Incorporation of ^{35}S-sulphate into SMPS of connective tissue)

References

1. Hauss, W.H., Junge-Hülsing, G., Otto, H.: Untersuchungen über den Stoffwechsel der Mucopolysaccharide bei alloxandiabetischen Ratten. In: Diabetische Angiopathie, S. 229–231. Berlin: Akademie-Verlag 1964.
2. — — Themann, H.: Zur Pathogenese von Durchblutungsstörungen. Med. Welt **19**, 7–21 (1967).
3. — — Gerlach, U.: Die unspezifische Mesenchymreaktion. Stuttgart: G. Thieme 1968.
4. — — Kuckulies, I., Otto, H., Rawytsch, J., Wagner, H.: Verlängerte biologische Halbwertszeit der sulfatierten Mucopolysaccharide bei Alloxandiabetes als Ursache der diabetischen Mesenchymstoffwechselstörung. Z. klin. Chem. Biochem. **6**, 241–244 (1968).
5. Junge-Hülsing, G.: Untersuchungen zur Pathophysiologie des Bindegewebes. Heidelberg: A. Hüthig 1965.
6. Meier, R., Schuler, W., Desaulles, P.: Zur Frage des Mechanismus der Bindegewebshemmung durch Cortisone. Experientia **6**, 469–472 (1959).
7. Otto, H., Themann, H., Wagner, H.: Qualitative und quantitative elektronenmikroskopische Untersuchungen an Hautkapillaren jugendlicher Diabetiker. Klin. Wschr. **45**, 299–307 (1967).
8. Schiller, S., Dorfmann, A.: The Metabolism of Mucopolysaccharides. In: Animals, IV. The Influence of Insulin. J. biol. Chem. **227**, 625–632 (1957).

Dr. H. Wagner
Med. Universitätsklinik und Poliklinik
D-44 Münster
Westring 3