greater metabolic breakdown or in a faster excretion of the *l*-epimer remains to be clarified.

Summary. Nutritional muscular dystrophy in the rabbit can be cured by *l*-alpha-tocopheryl acetate administered either orally or intravenously. Based on duration of survival after a single 50 mg oral dose the average estimated daily requirement was 2.0 mg per kg of body weight for 3 rabbits that received *d*-alpha-tocopheryl acetate and 9.5 for 2 rabbits that received *l*-alpha-tocopheryl acetate. After intravenous treatment with the same dose of these compounds, using 2 rabbits in each group, the estimated requirements were 1.2 and 8.0 for the *d*- and *l*-epimers, respectively. Liver and serum concentrations of tocopherol decreased more rapidly after treatment with *l*-alpha-tocopheryl acetate than after treatment with the same amounts of *d*-alpha-tocopheryl acetate. These observations suggest that the biological inferiority of *l*-alpha-tocopherol is due, at least in part, to a faster rate of loss from the body.

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Autologous Skin Grafts in the Rat. Uptake of ^{35}S-sulfate.* (30237)

A. MARCKMANN (Introduced by G. Asboe-Hansen)
The Connective Tissue Research Laboratory, Department of Dermatology, University of Copenhagen, Rigshospital, Copenhagen, Denmark

In a previous report(1) the results of analyses on mucopolysaccharides and collagen in rat skin autografts during the healing process were described. Five days after surgery a reduction in content of acid mucopolysaccharides in the grafts was found. The following period was characterized by an increased content of acid mucopolysaccharides. Fifty-four and 103 days after surgery the content did not differ significantly from that of intact, symmetrical skin. The present study aimed at elucidating the incorporation of ^{35}S-sulfate into sulfomucopolysaccharides in full-thickness skin autografts of rats.

Material and methods. Among 67 young male Wistar rats of an inbred strain (State Serum Institute, Copenhagen) weighing 179 ± 3.2 g, seven served as controls, while 60 had full-thickness skin autografts. From the

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right side of the body a circular piece of skin was excised and freed of subcutaneous tissue. The graft was turned 180 degrees and repositioned by suture. A detailed description of the surgical procedures has been given(2). Grafting as well as changing and removal of dressings were performed while the animal was anesthetized with ether. The dressings were definitely removed 10 or 11 days after surgery together with the sutures.

Radioisotope study. Carrier-free ^{35}S-sulfate in aqueous solution with an activity of 10 mC per ml was obtained from the Radiochemical Centre, Amersham. Suitable volumes of 0.04% sodium sulfate were added to a final activity corresponding to 1 mC per ml. The animals were killed by ether at intervals from 3 to 103 days after surgery. Forty-eight hours before sacrifice each animal received 0.2 ml of radioactive solution intraperitoneally. Grafts and symmetrical skin were removed and prepared as earlier described(1). In non-wounded animals symmetrical skin samples were removed and freed of subcutaneous tissue also. This group served to check the reliability of the procedures and technic.

Minced samples were lyophilized for 48 hours over phosphopentoxide at a pressure of 2 mm Hg. Defatting was carried out by mechanical shaking for one hour in acetone, one hour in ether and another hour in acetone. Finally, the samples were lyophilized.

The uptake of ^{35}S-sulfate was determined according to the procedure described by Moltke(3) with minor modifications. Adding 1.1 ml of a 12.5% solution of sodium sulfate (Na_2SO_4, 10 H_2O) (w/w), 30-50 mg of dried defatted tissue were digested in round Pyrex flasks with 15 ml of fuming nitric acid for 48 hours in a sandbath at 220°C. The white residue was dissolved in 10 ml of redistilled water to which 2 drops of concentrated hydrochloric acid were added, and sulfate was precipitated under boiling as barium sulfate during slow addition of 1.5 ml of a 25% solution of barium chloride. After cooling, the precipitate was suspended in 50 ml of redistilled water and allowed to sediment. During slow suction of the suspension through filter paper (Whatman 542) in a Tracerlab precipitation apparatus E-8B, the precipitate was retained and subsequently dried in warm air. The weights of the dry precipitates were all close to 100 mg, corresponding to infinite layer thickness. The samples were placed in Philips automatic sample exchanger (PW 4001) and their radioactivity measured with a Geiger-Müller tube. At least 4000 impulses were registered, corresponding to a statistical counting error of less than 2%. The radioactivity is given in counts/100 sec/100 mg of dry, defatted tissue after correction for background activity, physical decay of the isotope and for variations in body weight of the animals.

Results. The results appear in Table I, together with the ratio between radioactivity of grafts and symmetrical, uninjured skin. One animal with failure of the graft 8 days after surgery was excluded. So was one animal 28 days after grafting, as radioactivity of both graft and skin was as low as about 10% of the mean values of this group. Finally, one sample from an animal 103 days after surgery was lost. The mean body weights of the animals at sacrifice, expressed as per cent of weight at operation, are also given in Table 1.

The data on radioactivity were submitted to statistical analysis according to Student's "t" test. The difference between the uptake of $^{35}SO_4$ in symmetrical skin samples from non-operated animals (in Table I appearing at time 0 after surgery) was not significant, thus proving the reliability of the procedures. Three and five days after surgery the uptake into grafts was significantly lower than in symmetrical skin. Eight days after surgery the reverse was found, and still 54 days after grafting the uptake into grafts was higher than that in symmetrical skin. In 103-day-old grafts, however, the difference was not significant. The ratio between the radioactivities of grafts and symmetrical skin clearly reflects these differences. Uptake of $^{35}SO_4$ into "uninjured" skin from operated animals showed a general tendency to be lower than the uptake into skin of non-operated animals ($p<0.05$). The tendency was most pronounced in the period immediately after surgery. The uptake in uninjured skin of ani-

TABLE I. Uptake of ^{35}S-sulfate into Skin Autografts and Control Skin of Rats, and Radioactivity Ratio of Autograft to Skin.

Days after surgery	No. of animals	^{35}S-sulfate uptake		Ratio graft to skin	Body weight at sacrifice
		Graft	Symm. skin		
0	7	1058 ± 59‡	1005 ± 61	1.06 ± .04	100.0
3	7	277 ± 25*	396 ± 29	.72 ± .06	91.1
5	7	378 ± 55*	721 ± 75	.54 ± .07	103.8
8	6	875 ± 131*	558 ± 70	1.58 ± .13	92.3
12	7	965 ± 78†	533 ± 43	1.83 ± .14	97.2
16	6	1160 ± 89†	634 ± 37	1.83 ± .10	122.5
21	6	1250 ± 67†	784 ± 37	1.59 ± .03	135.0
28	5	1503 ± 243†	871 ± 129	1.70 ± .09	137.6
54	7	1290 ± 127†	766 ± 42	1.69 ± .15	163.7
103	6	837 ± 127‡	695 ± 90	1.10 ± .09	233.7

Figures represent mean and s.e. of mean values.
Radioactivity is given in counts/100 sec/100 mg dry fat free tissue.
Values of the grafts are significantly different from the values of symmetrical skin at 5% (*) and 1% (†) level of probability. (‡) indicates no significant difference.
Body weight of animals in each group represents mean weight at sacrifice in per cent of weight at operation.

mals with 28-day-old grafts, however, did not differ significantly from the uptake in skin of non-operated animals, but 54 and 103 days after surgery, the uptake into uninjured skin of grafted animals again was significantly lower.

Upon operation, the animals lost weight. Five days after surgery the weight at operation had been reached, but 8 and 12 days after grafting another weight reduction was observed. This secondary weight reduction might be caused by the intervention performed around 7 days and 10 to 11 days after surgery, when dressings were changed or removed in ether anesthesia. Later on, the animals continuously gained in weight.

Discussion. Forty-eight hours after intraperitoneal injection in rats of radioactively labeled sulfate, according to Boström and Gardell(4) practically all demonstrable ^{35}S in skin is found in sulfomucopolysaccharides. A negligible proportion is found in inorganic sulfate(4) or cystine(5). Consequently chemical isolation of the mucopolysaccharides before radioactive measurement is unnecessary, if one is not concerned about specific activities. The turnover rate of the total sulfomucopolysaccharide molecule is identical with that of the sulfate group taken alone (6). The radioactive measurements in this study, therefore, represent synthesis of sulfomucopolysaccharides.

The uptake of ^{35}S-sulfate in uninjured skin from grafted animals was significantly lower than that in skin of non-operated animals, except for grafted animals 28 days after surgery. The interpretation of these unexpected findings remains more or less speculative. In rats, injuries of various kinds, for instance excision of a patch of skin from the back or exposure to cold, brings about an increase in weight of the adrenals(7,8). The content of corticoids in blood is known to be increased in rats stressed from exposure to cold(8,9). Synthesis as well as turnover of hyaluronate and chondroitin sulfates in skin of intact rats are inhibited after 4 days' treatment with cortisone and hydrocortisone(10,11). For at least 10 days after grafting the animals in this study must be considered influenced by stress from surgical procedures, anesthesia and wearing and changing of dressings. The decreased uptake of ^{35}S-sulfate in uninjured skin of grafted animals may then in part be explained by an inhibition in the postoperative period of the synthesis of sulfomucopolysaccharides by corticoids produced in excess as a possible result of a stress reaction to injury. Twenty-eight days after surgery the stress reaction appears to have subsided. The lowered uptake in uninjured skin of animals 54 and 103 days after operation may be a reflection of a slower sulfate-sulfur turnover in acid mucopolysaccharides with age, as reported by Dziewiatkowski(12).

Despite the alterations in metabolic activ-

ity of sulfomucopolysaccharides in intact skin of operated animals, it appears justified to compare the uptake of ^{35}S-sulfate into autografts to that into symmetrical skin of the same animals. The influence of stress upon mucopolysaccharide metabolism affects both graft tissue and uninjured skin equally, and the influence of age must also be considered a general phenomenon.

The uptake of ^{35}S-sulfate into grafts was compared with the data on mucopolysaccharide biochemistry and on vascularization previously reported(1,13). The low synthesis of sulfomucopolysaccharides early after surgery is in keeping with the reduction in content of sulfomucopolysaccharides in 5-day-old grafts and the compromised vascularization during this period. Concerning changes in the rate of breakdown of acid mucopolysaccharides in grafts early after surgery, the studies presented here do not allow conclusions to be drawn.

About one week after surgery, simultaneously with the restoration of blood supply (13), an increase in ^{35}S-sulfate uptake into the grafts occurs. This increase is well pronounced during the following 6 weeks. One hundred and three days after surgery, however, the synthesis of sulfomucopolysaccharides in grafts is of the same order as that in symmetrical skin samples. The alterations in synthesis are reflected in similar alterations in content of acid mucopolysaccharides(1).

The alterations in ^{35}S-sulfate uptake into grafts resemble similar investigations on repair tissue in healing linear wounds(3) and granulation tissue(14). The present study emphasizes the role of acid mucopolysaccharides in the dynamics of biology in skin autografts of rats. The response to the grafting procedure consisting in synthesis of sulfomucopolysaccharides must be considered part of a connective-tissue response to injury. This response is characterized primarily by edema, and next by mucinous and fibrous organization of water(15).

Summary. Synthesis of sulfomucopolysaccharides, represented by uptake of ^{35}S-sulfate into full-thickness skin autografts and skin of rats 48 hours after intraperitoneal injection of radioactively labeled sulfate was studied. Three and 5 days after surgery, the uptake into grafts was reduced as compared to that into symmetrical skin. This period was followed by an increased uptake into the grafts, demonstrable as long as 54 days after surgery. One hundred three days after operation, the uptake into the grafts was of the same order as that into symmetrical skin. The alterations in uptake of ^{35}S-sulfate into the grafts are considered part of a connective-tissue response to injury. The uptake of ^{35}S-sulfate into uninjured skin of operated rats was reduced as compared to that into skin of non-operated animals, except for skin of grafted animals 28 days after surgery. These findings are discussed in the light of a possible influence of stress and age upon the metabolic activity of sulfomucopolysaccharides.

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