The present experience provides data suggesting that SV_{40} when given by the respiratory route induces subclinical infection in man, manifested by presence of virus in the throats of a few of the subjects 7 to 11 days after exposure, and by development of specific neutralizing antibody in about two-thirds of the volunteers in the weeks following inoculation. The generally low levels of serum antibody detected in the volunteers after exposure to SV_{40} and failure to recover virus from most of the volunteers in the present work suggest that SV_{40} induces only a low grade infection in adults when introduced by the respiratory route.

Summary. SV_{40} virus present as a contaminant in a pool of respiratory syncitial virus prepared in cultures of rhesus monkey kidney cells was inoculated by the respiratory route into 35 adult volunteers: 8 of these received SV_{40} virus preparation in which the RS virus component was neutralized by addition of RS antiserum. None of the test subjects developed signs or symptoms attributable to inhalation of SV_{40}. SV_{40} virus was recovered from throat swab specimens of 3 of the volunteers 7 or 11 days after exposure. SV_{40} neutralizing antibody in titers ranging from 1:5 to 1:80 appeared in the blood of 22 of the 35 test subjects.

$TCID_{50}$ of SV_{40} per ml. None of the 12 pairs of serum, however, contained SV_{40} neutralizing antibody when examined at a 1:5 dilution against 100 $TCID_{50}$ of our strain of SV_{40} virus.

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Effect of Acid Mucopolysaccharides on Hair Growth in the Rabbit.* (26844)

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Histologic studies on the cyclic hair growth in animals have shown a close correlation between activity of the hair follicles and metachromatically staining material in and around the hair bulb(1). The same areas are heavily labeled on injection of $S^{35}O_4$ in the rat(2), though not in the mouse(3). The nature of the presumed sulfated mucopolysaccharide (MPS) has never been determined. The starting point of the present studies was the observation that most, if not all, children with Hurler's syndrome had extremely thick hair and appeared to have a faster rate of hair growth than children of comparable age. Since in Hurler's syndrome there is a characteristic storage and urinary excretion of 2 sulfated polysaccharides, chondroitin sulfate B and heparitin sulfate(4), the possible effect of intradermal injection of these and other polysaccharides on hair was studied in pigmented adult rabbits.

Experimental. The rabbits were kept in individual cages and fed a standard laboratory diet. The hair of the back was clipped. The rabbits were used only if the underlying skin was uniformly unpigmented, to insure as much as possible that all areas of the skin were approximately at the same stage of the hair cycle(5). Several areas of about 2 × 2 cm were marked off in each rabbit. 0.5 ml of different solutions of polysaccharide in

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TABLE I. Total of 34 Injection Sites on 8 Different Rabbits, Each Site Injected Daily with 0.5 ml of Substance Indicated at Concentration of 1.0 mg/ml.

	Substance								
	Hep.S.	ChS-B	H.A.	ChS-C	ChS-A	NaCl	Air	Calcium alginate	Ovalbumin
No. of inj. sites	7	8	6	2	2	4	1	2	2
Avg rating	2.9	1.6	1.0	1.0	.5	0	0	0	0

Rating: 0 = no growth; 1 = min growth; 2 = mod growth; 3 = max growth.

TABLE II. Each Rabbit Received One ml of the Indicated Suspension or Solution Intracutaneously. Hair growth evaluated 3 to 4 weeks after injection. Substances 1 to 6 are suspensions made up by mixing 0.5 ml of polysaccharide solution at 20 mg/ml with 0.5 ml of protamine sulfate solution at 20 mg/ml.

	Substance inj.									
Rabbit	1. Hep.S.–prot. sulf.	2. ChS-B–prot. sulf.	3. 1:1 mixture of 1 & 2	4. H.A.–prot. sulf.	5. ChS-C–prot. sulf.	6. Ca alginate–prot. sulf.	7. ChS-B, 20 mg/ml	8. Hep.S., 20 mg/ml	9. Protamine sulfate, 20 mg/ml	10. 1:1 mixture of 7 & 8
1	2	0		1	0	0				
2	2	0		1	1	1				
3	2		1				0	0	0	0
4	0		2				0	0	0	0
5	2			1	0					
6	2			1	1	0				

Rating: 0 = no growth; 1 = intermediate growth; 2 = maximum growth.

a concentration of 1 mg/ml were injected intracutaneously into the marked areas. A total of 34 different injection sites was used in 8 rabbits. In the first experiments, injections were made daily for periods of 3 to 7 weeks or until there was obvious regrowth of hair in at least one injection site. The MPSs used‡ were purified in this laboratory by standard procedures(6). The results of this experiment are shown in Table I. Rating of the effect was based on number, length, thickness and intensity of pigmentation of the hair in injected sites. 0 indicates no visible effect as compared to a non-injected site. Maximum effect was scored as 3. All the injected MPSs exhibited some stimulation of hair growth. However, Hep. S. was more potent than any other polysaccharide, followed by ChS-B. Physiological saline, air, Ca alginate (1 mg/ml) or ovalbumin (1 mg/ml) had no effect. In 6 out of 7 sites, the effect of Hep. S. was maximal. The effect is illustrated in a composite photograph of 4 areas in one rabbit 4 weeks after start of injections. This shows the types and intensities of the reaction (Fig. 1). Scores of the sites were recorded as A = 1, B = 2, C = 3, D = 0.

To provide a depot of the MPSs, suspensions of mucopolysaccharide and protamine sulfate were made up as described in Table II, and 1 ml was given intracutaneously only once. In this experiment Hep. S. again was most effective. Injection of twice the concentration of polysaccharides without protamine sulfate or protamine sulfate alone had no effect.

The effect of Hep. S. in one rabbit is shown in Fig. 2. Attempts to reproduce these effects in albino rabbits gave inconclusive results. Plucking instead of clipping hair prior to injection caused intense inflammatory reactions followed by rapid regrowth of the hair within one week.

Biopsy specimens were obtained from the injection areas of the skin of 6 of the 14 rabbits, at least 3 days following last injection. In the case of the MPS-protamine complex,

‡ The following abbreviations are used: Chondroitin Sulfate = ChS; Hyaluronic Acid = H. A.; Heparitin Sulfate = Hep. S.

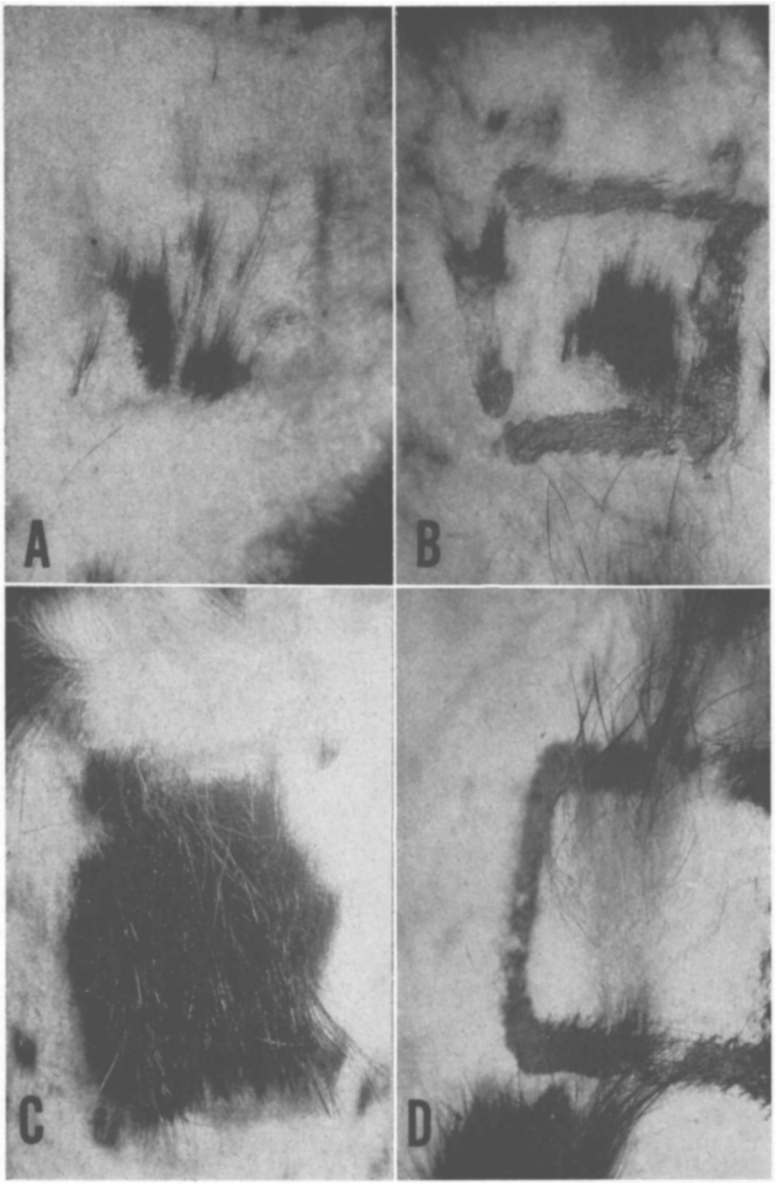

FIG. 1. Four inj. sites on back of shaved rabbit 4 wk after daily inj. of following substances in dose of 0.5 ml/day. A = ChS-A, B = H.A., C = Hep. S., D = Saline.

the time interval was 4 weeks after the single injection. Each area was divided into 2 portions, one-half being fixed in 10% formalin or Bouin's solution, the other in 96% alcohol. A typical example of the tissue reaction is pictured in Fig. 3. This picture was chosen since it shows an uninjected area adjacent to an area injected in this case with ChS-B daily for 4 weeks.

In control areas and in areas receiving H.A., no active hair growth or only a few actively growing hairs were found in the sections. In areas injected with Hep. S. and ChS-B, or in some with ChS-A, a most pronounced growth was seen histologically with the hairs thicker than normal. The papillae of some hairs were unusually large and contained numerous large histiocytes. In addition, some inflammation was seen in the corium and in the epidermis of all specimens.

The growth of single hairs in the control areas was apparently caused by plucking of these hairs during clipping.

Discussion. The pattern of hair growth undoubtedly is a complex phenomenon. The role of pigmentation and inflammation in the hair growth of the colored rabbit has been well described(5). According to the report of these authors, inflammation accompanied by crusting and scaling of the skin always is associated with effective chemical "irritation" and stimulation of the resting hair follicles. Hyperemia and erythema unassociated with crusting and scaling, as produced by mild irritants, did not initiate a hair growth cycle in the rabbit. In our experiments, no crusting or scaling of the skin during or after the injections was observed.

The correlation of active hair growth with presence in the dermal papilla of metachromatically staining material, presumably a sulfated MPS resistant to testicular hyaluronidase, has been well documented(7). It appears from the data reported here that sulfated MPSs and especially Hep. S., on intradermal injection, are able to initiate growth activity in quiescent hair follicles of the pigmented rabbit. We assume that metachromatic cells in and around the papilla normally produce similar substances and that inflammation acts similarly *via* cells producing metachromatic substances. Conversely prolonged treatment with heparin or especially heparinoids has been reported to lead to partial or total alopecia in a very high percentage of cases (in 99% of cases treated with a dextran sulfate(8)). These sulfated polysaccharides can be assumed to inhibit the reactions of the normal polysaccharide. The nature of the reaction or reactions involved in activation of the hair follicle is unknown. It certainly is not the furnishing of S-containing precursors of keratin suggested by Sylvén(9).

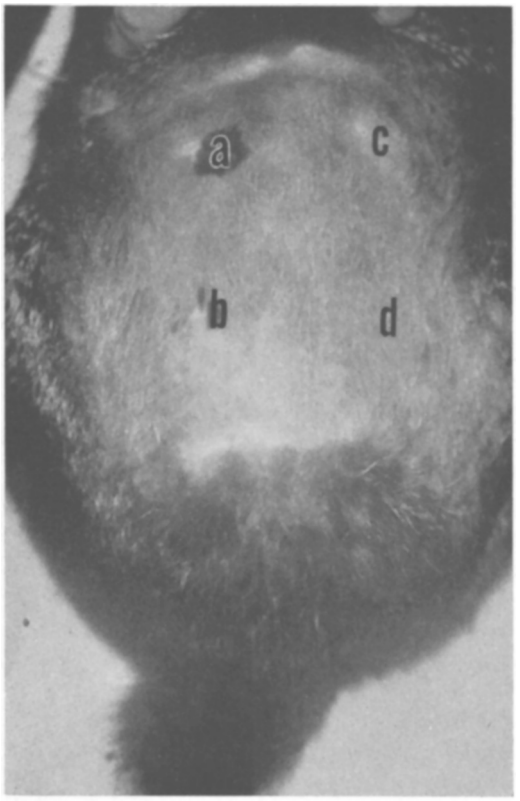

FIG. 2. Effect of 1 inj. of MPS-Protamine complex (10 mg of each) after 4 wk on the back of a shaved rabbit: a = Hep. S., b = ChS-A, c = saline, d = no inj.

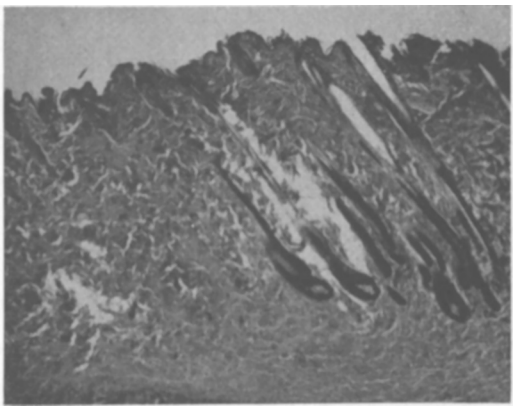

FIG. 3. Hematoxylin and Eosin section of rabbit skin showing uninj. area on left, and area inj. with ChS-B on right, containing many large, active hair follicles.

Summary. Repeated intradermal injection of MPS initiated a hair growth cycle in the pigmented rabbit. Hep. S. was the most active in the reaction, followed by ChS-B. A single injection of MPS applied as the insoluble protamine complexes also stimulated hair growth, again with Hep. S. the most active. These experiments suggest an essential role of a sulfated MPS in the normal hair growth cycle, as suggested previously from

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Disappearance of Human Diphtheria Antitoxin from Human Passive Transfer Skin Sites.* (26845)

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Kuhns(1) described a method for measuring the disappearance of human diphtheria antitoxin from passive transfer skin sites. The tests are carried out in Schick positive persons, and they depend upon intradermal antitoxin totally or partially to neutralize Schick toxin which is introduced later. Subsequent partial or total inhibition of toxic reactions indicate the degree of disappearance of antitoxin from skin sites. Further experiences with this method are described here. Precipitating, non-precipitating and skin sensitizing human antitoxic sera are compared as to their disappearance from passive transfer sites.

Materials and methods. Materials used in the Schick tests, evaluation of immediate reactions to Schick toxoid, and methods of hyperimmunization of Schick negative subjects have been described(2). Preparation and properties of the diphtheria toxin and toxoid used and the technics employed in carrying out the intracutaneous neutralization test in rabbits are described elsewhere(2,3,4). Human immune sera contained high titers of antitoxin as measured by rabbit skin tests. Precipitating and non-precipitating sera, and skin sensitizing and non-sensitizing sera have been used for purposes of comparison. Specimens from 6 individuals were utilized, designated as follows: Sp (non-precipitating – 15 Units/cc), She (precipitating – 80 Units/cc), Rie (precipitating – 50 Units/cc), Ni (non-precipitating – 100 Units/cc), Bo (precipitating skin sensitizing – 12 Units/cc), and Hu (non-precipitating skin sensitizing – 20 Units /cc). All serum samples to be tested were removed from the frozen state at 0°F and rapidly thawed to room temperature just prior to use. Dilutions of antitoxin were made in borate-NaCl buffer pH 7.4 containing 200 μg gelatin/cc to prevent surface denaturation. Methods of quantitatively measuring precipitation in a fluid and semi-solid medium have been described(5). Wheal reactivity was measured by the method of passive transfer (6). Toxoid and toxin preparations from the same source were employed for all testing purposes.

Neutralization of Schick Toxin by Intradermal Antitoxin: This test is based upon the ability of intradermal antitoxin to neutralize the toxic action of the equivalent amount of Schick toxin in Schick positive subjects. Recipients were used for no more

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