that of the antibiotic is relatively low, or in some instances where the exposure of the organism to the antibiotic may resemble that of a surface growth.

The sudden acquisition of extreme resistance by isolated colonies on surface plates suggests that in addition to the gradual increase in resistance shown by organisms exposed to streptomycin, some bacterial cells may suddenly become highly resistant while exposed to comparatively low concentrations. This phenomenon, occurring as it does in material derived from a single picked off colony might offer a partial explanation of the manner in which resistance is acquired *in vivo*, making it unnecessary to postulate the presence of initially resistant strains prior to treatment in those cases where rapid development of extreme resistance is encountered during streptomycin therapy. Failure to observe any sudden increase in resistance in the broth transfer series, however, might have been related to the smaller size of the inoculum used, as judged in terms of numbers of bacteria. The average inoculum in the broth series contained 100,000 to 150,000 organisms while a loopful of surface culture used as the inoculum in the agar plate series contained several billion.

Bacteria developing resistance after prolonged exposure to sublethal concentrations of streptomycin showed impaired growth characteristics. On the other hand, resistant strains from patients under therapy and strains which developed resistance rapidly *in vitro* could not otherwise be distinguished from the strain originally isolated from patients prior to streptomycin therapy.

Conclusions. It was possible to increase the resistance of Gram-negative bacilli to over 50,000 units per ml, in all cases studied, by passage through increasing concentrations of streptomycin in broth and on agar plates.

In most instances resistance increased gradually, the change being more rapid on surface plates than in broth.

In the course of transfers on streptomycin agar, 4 instances of sudden increase in resistance of isolated colonies from relatively low values to over 50,000 units per ml were noted.

The organisms made resistant by exposure to streptomycin in broth showed marked pleomorphism and in some instances suffered changes in their biochemical reactions. When resistance developed as a result of exposure to the agent on surface plates, however, there was less pleomorphism, and no changes in biochemical reactions were noted. Resistant organisms obtained from the patient during or after treatment showed no morphological or cultural differences from the original sensitive strains isolated from the same patient.

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Effects of Sodium Fluoride on Hair Growth in the Rat.

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Shour and Smith[1] have shown that intraperitoneal injections of 0.3 cc of 2.5% sodium fluoride at 48-hour intervals induced hypocalcified stripes in the enamel and dentin of rats. When the injections were given at intervals of 24 hours or the dosage was increased, cumulative effects were produced which severely disturbed the formation of enamel and dentin. The effects produced by adding NaF to the diet were essentially similar to the effects produced by injections. Since the cytoplasm of the ameloblasts showed an abnormal character and disturbance of its globules, Shour and Smith suggested that fluorine exerted a direct local action on the enamel-forming cells.

[1] Shour, I., and Smith, M. C., *University of Arizona Agr. Exp. Sta. Tech. Bull.*, 1934, **52**, 69.

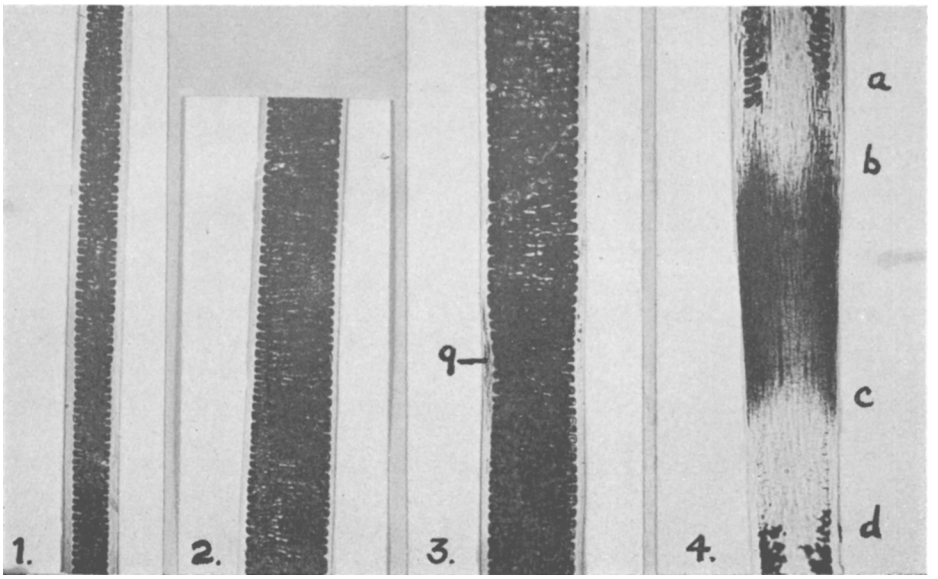

FIG. 1. Coarse hair from a rat whose diet contained 0.1% NaF.

FIG. 2. Coarse hair from a control litter mate. Photographs 1 and 2 were taken the same distance from the tips of the hairs.

FIG. 3. This photograph shows an interval of a hair which was affected by the intraperitoneal injection of 0.15 cc of 2.5% NaF. The medulla consists of fewer cells and granules (g) have formed in the cortex.

FIG. 4. The affected area resulting from the intraperitoneal injection of 0.2 cc of 2.5% NaF extends from a to d. The medulla is transparent due to little air between the cells and the granules in the cortex (b to c) mask the central medullary cells.

All photographs have the same magnification of 110 ×.

The present study has been made to see if another ectodermal derivative, the hair, was affected in any way by sodium fluoride. If NaF administration induced any effects on the hair, it also seemed worthwhile to learn if these effects differed from those resulting from inanition.

Hair was removed from the backs of 15 23-day-old rats. Five of these served as controls and received Purina Dog Chow while 10 were fed finely ground chow containing 0.1% NaF. Similar young rats receiving a 0.2% concentration in their diet failed to grow and survive well. The average gain in weight of those animals receiving 0.1% NaF in their diet was 10 g between the 23rd and 40th days of life, and the average gain of the 5-litter-mate controls over the same period was 45 g. Hair grew on the controls on the 35th or 36th day of life as described in a previous paper[2] while hair which grew slowly and evenly was retarded on the average of 10 days in the NaF-fed animals. A measurement of 50 of the coarser hairs of the treated animals showed that they had only 60% of the length and 60% of the diameter of similar hairs of the controls. Photographs of hairs from the NaF-fed rat (Fig. 1) and a control animal (Fig. 2) were taken the same distance from the tips of the hairs and are presented for comparison. The medulla of the hair of the NaF-fed animal consists of fewer cells while the cortex seems little affected. Histological sections fixed in Bouin's and stained with hematoxylin and eosin failed to show cellular disturbances in the hair bulb which would account for this small size. The teeth of the NaF-fed animals gradually became pigment-free and chalky white.

In order to compare the effects of inanition with the effects of NaF in the feed, 6 23-day-old rats were fed daily 5 g of finely ground chow as in previous experiments.[3]

[2] Butcher, E. O., *Anat. Rec.*, 1934, **61**, 5.

[3] Butcher, E. O., *J. Nutrition*, 1939, **17**, 151.

Such rats gained 8 g in 16 days as compared to the 10 g average gain made by the NaF-fed animals in a similar period. Hair growth was retarded about 10 days in the underfed animals and appeared in small areas over the dorsum in comparison to the even growth of 3 well-fed litter mates. A measurement of the diameters of 50 hairs showed that in many instances the hairs were as large as those of the controls and seldom were 10% smaller. All the hair follicles in the NaF-fed animals appeared to be affected while only localized areas of follicles in the underfed animals seemed to receive enough nutrition to produce hair. The teeth of the underfed animals erupted nearly as fast as those in the controls, keeping their typical translucent appearance. The eruption rate for the lower incisors in underfed and well-fed rats averaged about 0.6 mm daily. This rate varies from that reported by Addison and Appleton[4] (0.4 mm daily) and Smith[5] (0.45 mm daily).

Shour and Smith[1] injected 0.3 cc of a 2.5% solution intraperitoneally in 90- to 270-day-old rats. In the present experiments 35-day-old rats would usually tolerate 0.3 cc of a 1.2% solution intraperitoneally. It was found best to introduce 0.2 cc of this 1.2% solution and then to administer 2 more injections of 0.05 cc at half-hour intervals. Some animals did tolerate 0.2 cc of a 2.5% solution. Such injections were made in 38- to 42-day-old rats or when the second growth of hair was ⅛ to ¼ inches long externally (the first growth was removed at the age of 23 days with sodium sulphide). Forty-eight hours after the injection, hairs were plucked, dried, and mounted in clarite.

The administration of the NaF affected the hair while it was forming and when the affected area emerged from the follicle, the medulla was narrowed for a short interval (Fig. 3). The medulla of this interval consisted of fewer cells which is similar to the effects resulting from feeding the NaF. Injections every 48 hours caused successive narrowings in the hair. In many instances the effects were greater and the affected area was much longer as shown in Fig. 4 where it extends almost the entire length of the microphotograph. Fewer cells constituted the medulla and the cells often failed to shrink. Accordingly, there was less space for air between the cells. When hairs were mounted in clarite, the medulla in the normal region appeared black due to the large amount of air between the cells while the interval affected by the NaF appeared transparent.

In addition to the effects on the medulla there was in most cases formation of granules in the cortex (Fig. 3). In Fig. 4 the medulla is transparent from a to d due to the small intercellular spaces. The granules in the cortex are so numerous and black that they mask the central medullary cells from b to c.

The injections thus caused greater effects than the feeding. Nembutal anesthesia for many hours produced no effects on the hair similar to those caused by the injection of NaF.

Summary. Inclusion of 0.1% NaF in the diet of rats resulted in the retardation of the growth of hair and in smaller hairs. Underfeeding, so that the gain in weight was similar to that when NaF was included in the diet, did not produce a similar reduction in the size of the hair.

Intraperitoneal injections of NaF affected the growth of the hair and caused a narrowing of the hair for a short interval. The medulla of this interval consisted of fewer cells. The medullary cells also often failed to shrink, resulting in little intercellular space between them. In addition, the intraperitoneal injections caused the formation of granules in the cortex. Since NaF is commonly accepted as an inhibitor of acid phosphatase,[6] it may be that NaF affects hair growth through inhibition of this enzyme.

[4] Addison, W. H. F., and Appleton, J. L., *J. Morph.*, 1915, **26**, 43.

[5] Smith, M. C., *J. Dent. Res.*, 1934, **14**, 139.

[6] Moog, Florence, *Biol. Bull.*, 1944, **86**, 51.