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MECHANISM OF THE ACTION OF β-GLUCURONIDASE INHIBITOR UPON APOCRINE SWEAT AND SEBACEOUS GLANDS AND ITS DERMATOLOGICAL APPLICATION

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Abstract. The effect of glucaro(1→4)(6→3)dilactone and (1→4)monolactone, β-glucuronidase inhibitors, upon the apocrine sweat and sebaceous glands and their clinical application to several dermatoses were investigated. (1) The growth-promoting effect of testosterone upon the sebaceous gland of the rat was inhibited by the administration of glucaro(1→4)monolactone. (2) The oral intake of glucaro(1→4)(6→3)dilactone or its topical application (0.1% dehydrated lanolin ointment) reduced the β-glucuronidase activity in the axillary sweat and osmidrotic skin. (3) The oral administration and the topical application of dilactone to patients with osmidrosis resulted in complete deodorization in 28 out of 69 cases and alleviation of odor in 33 cases. In addition, no significant difference was observed between the clinical effect and existence of superficial bacterial infection of axillary skin. Oral administration and local application of the dilactone to acne vulgaris and steroid acne showed a favorable clinical effect in 26 out of 30 cases. In verrucae planae juveniles, the oral administration of this dilactone showed an excellent effect in 25 out of 29 cases. (4) A possible mechanism of action and the metabolic regulation of β-glucuronidase inhibitor upon the gland tissues, and especially the relationship between the inhibitor and sexual hormone are proposed.

β-Glucuronidase (β-G) which catalyses the hydrolysis of β-D-glucuronide, is well known for its important role in the catabolism of mucopolysaccharides and transportation of aglucuron such as bilirubin or sexual hormones (10), although the physiological significance of its existence in almost all the tissues is not well clarified.

On the other hand, D-glucaro(1→4)lactone shows complete inhibition of β-G at a concentration of 10^{-7} M (20, 21) and this inhibitory effect is 5 times stronger than in the case of glucaro(1→4)(6→3)dilactone in vitro, whereas in vivo the effect is the reverse since the dilactone is more easily absorbed from the intestinal canal, and its degradation products, (1→4)- and (6→3)lactones, induce a prolongation of its inhibitory effect (13, 14, 29).

There are, however, many reports describing a high activity of β-G in inflammatory tissues and neoplasms.

Glucaro(1→41)lactone has also been used for the treatment of carcinoma of the urinary bladder because high β-G activity in urine or cancer tissues increased concomitantly with the amount of carcinogenic aglucurones liberated from glucuronide in the bladder (2, 3).

In the dermatological domain; (1) high β-G activity was reported not only in the human epidermis but also in the apocrine sweat and sebaceous glands (4, 18, 24, 26). Its physiological significance, however, has not yet been documented. Furthermore, (2) the pilosebaceous system was shown to be regulated by androgens, and experimental investigations showed that the administration of various androgenic hormones, especially testosterone, caused hyperplasia or hypertrophy of the sebaceous gland in rats, rabbits, etc. (8).

Considering both (1) and (2), we investigated the effects of the β-G inhibitor upon the sebaceous and sweat glands, and demonstrated its clinical application to some dermatoses such as osmidrosis, acne, verrucae, etc.

MATERIALS AND METHODS

Sodium glucaro(1→4)lactone and glucaro(1→4)(6→3)-dilactone diacetate were obtained from Chugai Pharmaceutical Company by courtesy of Dr Hirasaka (16).

For oral administration, we used 50 mg/kg of the dilactone diacetate daily, and for the external administra-

Table I. *Effects of glucarolactone upon the sebaceous gland of female rat treated with testosterone*

	Control	Testosterone	Testosterone + glucarolactone
Volume of the sebaceous gland ($1000\ \mu^3$)	87 ± 36^a	332 ± 92^a	185 ± 61^a
S.E.	11	29	19
β-G activity (U)	132–156	198–224	82–118

[a] S.D. $= \sqrt{\frac{\Sigma X^2 - (\Sigma X)^2/(N)}{N-1}}$ $N = 10$.

tion, dehydrated lanolin ointment containing 0.1% dilactone diacetate was prepared. In the animal experiment, injection of 0.5 mg/g of Na-glucaro(1→4)lactone was employed.

Animal experiment

Eight-week-old female rats were divided into 3 groups. The first group was treated with single intramuscular injection of 500 U of testosterone propionate depot and 0.5 mg/g of Na-glucaro(1→4)lactone every day. The second group was treated with testosterone proprionate depot only and the third was a control group. After 30 days, a histological examination of cutaneous tissue taken from the back of each rat was made. The size of the sebaceous gland was measured with a planiometer in the photographs of these serially sectioned specimens, and its volume was calculated in accordance with Ebling's method (8).

Human experiments on sweating

Volunteers were treated for 6 hours with the dilactone lanolin ointment on various skin regions, or for 4 days with oral administration of the dilactone diacetate. Then filter-papers were applied to the measurement regions (axilla, chest, etc.). They were covered by a paraffin film and the edges were tightly sealed with adhesive tape. The volume of sweat was calculated by weighing the filter-paper before and after the experiment. The experiment was carried out at a room temperature of 32°C and 80% humidity. Immediately after weighing the filter-paper, sweat was extracted into the acetate buffer (0.1 M, pH 4.5) and β-G activity of the sweat was measured by the method of Fishman et al. (9). (U denotes liberated phenolphthalein μg/h/ml of sweat.) β-G activity in cutaneous tissue was measured according to the method of Matsuura (24). Histochemical demonstration of β-G was made in accordance with Hayashi et al. (15).

Drug administration to patients

A dose of 0.1% dilactone-lanolin ointment or 0.1% glucaro(1→4)monolactone-lanolin (in cases of verrucae) was applied once or twice a day to the skin lesion and the placebo (lanolin only) was also applied, for a blind or a double blind test. In the oral administration, 50 mg per kg body weight of glucaro(1→4)(6→3)dilactone diacetate was administered daily. No other local or systemic treatment was given during the investigation.

RESULTS

Mechanism of action of β-G inhibitor upon the apocrine sweat and sebaceous glands

Effects of glucaro(1→4)monolactone upon sebaceous gland of rats treated with testosterone. Hypertrophy of the increased gland cells can clearly be observed in the group treated with testosterone, compared with the non-treated group. This change was less marked in the group treated with both testosterone and glucarolactone as compared with the group given testosterone only. The volume of ten sebaceous glands randomly chosen in each group and the β-G activity of the skin is shown in Table I.

The volume of the sebaceous gland, $t\ [(MN_1 - MN_2)/(SE_1^2 + SE_2^2)] = 7.8$ was shown between the control group and the testosterone group: $t = 4.1$ between the testosterone group and the testosterone + glucarolactone group, and $t = 4.2$ between the control group and the testosterone + glucarolactone group. It was confirmed that glucarolactone inhibits the growth-promoting effects of testosterone on the sebaceous gland. The cutaneous β-G activity in the dorsal region of the testosterone + glucarolactone group demonstrated lower activity than in the testosterone group and the control group, but β-G activity was revealed histochemically in the sebaceous glands of all three groups, and we could not demonstrate any difference in its concentration in the specimens.

After the 20th day of the experiment the testosterone group showed a decrease in body-weight in comparison with the control group, and the glucarolactone group showed yellowish and lustreless changes of the hair and dried skin as if they were on a low-lipid diet.

Effects of glucaro(1→4)(6→3)dilactone upon intensity of sweating and β-G activity of sweat. The results obtained from the measurement on the axilla, the chest, and the back of healthy adult men, are shown in Table II.

β-G activity in sweat taken from the axilla was generally lower than that of the serum (8–12 U), and its activity in sweat of the chest or the back

Table II. *Effects of glucarodilactone upon the intensity of sweating and the β-G activity of human sweat*
U = μg of liberated phenolphthalein/hr/ml of sweat. Parentheses denote ml of sweat/50 cm²/h

Pat.	Sex	Age (y.)	Habitually handed side	Axilla Right U	Axilla Left U	Chest U	Back U	Upper arm U
No treatment								
S. S.	♂	46	right	2.6 (1.60)	1.9 (0.90)	0.4 (1.48)		0.9 (1.46)
T. O.	♂	38	right	2.3 (1.20)	1.4 (0.62)	0.2 (1.02)	Trace (0.14)	
N. M.	♂	36	left	5.2 (0.24)	3.1 (0.63)		Trace (0.33)	
N. K.	♀[a]	26	right	6.4 (0.34)	7.9 (0.18)			
Oral administration								
S. S.	♂			1.5 (1.70)	0.9 (1.44)	0.2 (1.50)		
T. O.	♂			0.1 (1.32)	0.3 (1.21)	0.1 (1.64)		
0.1% ointment								
S. S.	♂			0.0 (0.76)				
T. O.	♂			0.0 (0.90)				

[a] Patient with osmidrosis.

was considerably lower than that of the axilla. One case of osmidrosis showed the highest β-G activity, i.e., the enzyme is excreted more easily from the apocrine gland.

After application of dilactone lanolin ointment to the axilla for 6 hours, β-G activity disappeared totally in sweat but the 4-day oral administration of glucarodilactone revealed about 40% reduction of the β-G activity in sweat of the axilla and the chest, although complete inhibition could not be obtained.

It was noticed that the inhibitor showed no effect on the amount of sweating by either oral or external administration. We also observed that the sweating of the axilla was more intense on the habitually handed side (mainly right) than on the contralateral side and we concluded that it was unreasonable to treat the other side as the control in such experiments.

Behaviour of β-G activity in axillary cutaneous tissue caused by application of glucarodilactone. Twenty-four hours before operation on a patient with osmidrosis, dilactone lanolin ointment was applied on one side of the axilla, and the other contralateral was treated with lanolin only. Permeability of the inhibitor and its inhibitory effect on β-G were determined using the skin lesion obtained after the operation. Table III shows these results. As stated above, β-G activity of the gland on one axilla is not the same as on the other side. In this table, all of the habitually handed sides were treated with inhibitor and β-G activity of the total skin layer was decreased to about one-third of that of the other side.

Examination of the β-G activity in the epidermis and dermis separately showed that the treated side activity in epidermis was less than 20% of that in the control, whereas the dermis of the treated side still retained 60 to 70% of the activity of the control side. The inhibitory effect on β-G activity is attributable chiefly to that in the epidermis and the permeation of the inhibitor into the deep layer of the dermis is difficult even when applied for 24 hours.

The histochemical investigations revealed that β-G activity remained in the lumina and the gland cells of the apocrine glands but it was markedly reduced in the epidermis and the upper portions of the excretery ducts.

Table III. *β-G activity of the axillary skin after the application of glucarodilactone ointment*

U = μg of liberated phenolphthalein/h/g of wet tissue

Pat.	Sex	Age (y.)		Left (control, treated lanolin only) U	Right (treated with inhibitor) U
T. T.	♀	16	Total skin layer	3 510	1 380
			Epidermis	1 314	130
			Dermis	3 330	1 074
Y. O.	♀	16	Total skin layer	3 720	1 775
			Epidermis	792	279
			Dermis	3 615	2 922
M. N.	♀	19	Total skin layer	8 160	3 270
			Epidermis	1 776	750
			Dermis	8 640	6 660
Y. S.	♂	21	Total skin layer	6 980	2 294
			Epidermis	1 310	121
			Dermis	6 774	3 962
N. H.	♂	21	Total skin layer	3 872	1 235
			Epidermis	640	156
			Dermis	3 460	1 920

Clinical application

From the above experiments, evidence suggested that the blockage of β-G might change the metabolism of the secretory gland to free it from the testosterone effect. On the other hand, β-G might be liberated as one of the lysosomal enzymes from such tissue as inflammatory tissue or a neoplasm. We therefore examined the clinical effects of glucaro(1→4)(6→3)dilactone diacetate either applied externally or administered orally upon some skin diseases, such as osmidrosis axillae representing the abnormal secretion of the apocrine gland, acne vulgaris representing hyperplasia and hypersecretion of the sebaceous gland, verrucae as viral neoplasia, and a few inflammatory dermatoses.

Table IV. *Effects of glucarodilactone upon patients with osmidrosis*

	No. of cases	♂/♀	Markedly effective	Effective	Ineffective
0.1% ointment	48	5/43	26	16	6
Oral administration	21	4/17	2	17	2

Table V. *Effects of glucarodilactone ointment upon the axillary odor and superficial bacteria separated from axillary skin*

	No. of cases	Markedly effective	Effective	Ineffective
Superficial[a] bacteria (+)	11	8	2	1
Superficial bacteria (−)	7	6	1	0

[a] Staphylococcus albus 9, Corynebacterium 2.

Osmidrosis axillae. The clinical experiences with the inhibitor against 69 cases of osmidrosis are shown in Table IV.

"Markedly effective" means that more than 2 examiners could not notice any odor, and "effective" means improvement judged form the complaints of the patients and comments of their families. Concerning the external application, 42 out of 48 cases (87%) showed improvement, mostly 1 day after the treatment, but if the therapy was interrupted the odor reappeared, mostly within 1 to 3 days. The placebo had no effect and when it was substituted for the inhibitor, reappearance of the odor was recognized. In cases of oral administration, 19 out of 21 cases (90%) showed favorable results. The effects were noticed after the second day of administration, and remained for 2 days to 3 weeks after interruption of the treatment, depending on each individual case.

In 11 out of 18 patients with osmidrosis, the bacterial infections were revealed by culture from the axillary skin surface, as shown in Table V.

Table VI. *Effects of glucarodilactone upon acne*

Mode of administration	No. of cases	♂/♀	Markedly effective	Effective	Ineffective
Acne vulgaris					
Externally and orally	24	1/23	6	15	3
Externally only	4	0/4	2	1	1
Steroid-acne					
Only externally	2	0/2	2	0	0

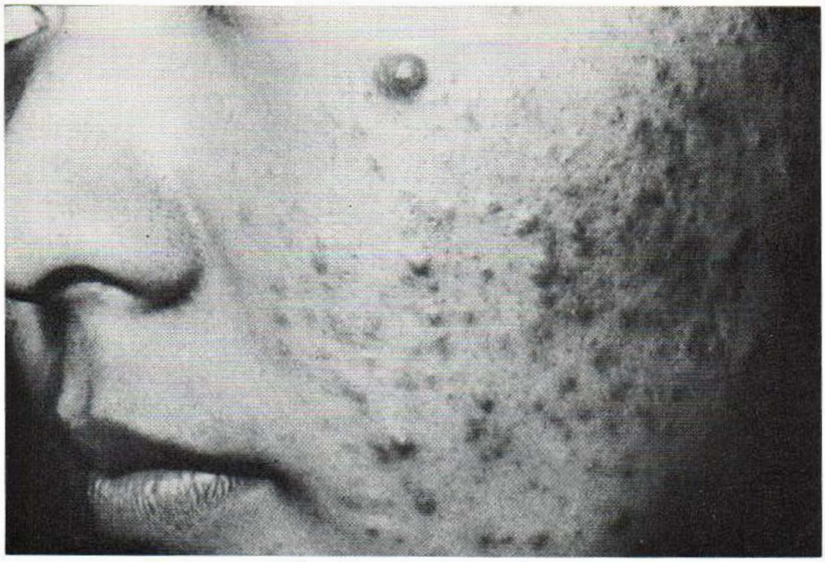

Fig. 1. Acne vulgaris, pretreatment.

However, no significant difference on the effect of the β-G inhibitor was observed between the bacteria-positive and the negative group.

Acne vulgaris and steroid acne. Twenty-eight cases of acne vulgaris, especially the premenstruation flare-up type, and 2 cases of steroid acne were treated with external application alone or with combined oral administration (results shown in Table VI). Most of the cases of acne vulgaris (24 out of 28) showed marked improvement and decrease of papules within 4 weeks. Six cases showed no recurrence of the eruption for more than 8 weeks and the treatment could be considered as markedly effective (Figs. 1 and 2).

In the 2 cases of steroid acne, external application only elicited the remission of erythema and papules on the second day, and the eruption disappeared after 1 week.

Verrucae planae juveniles and verruca vulgaris. As shown in Table VII, oral treatment was given to 29 patients with verrucae planae juveniles and complete disappearance of the eruption was observed in 24 cases within 4 days to 4 weeks (Figs. 3 and 4). However, relapses were observed in 2

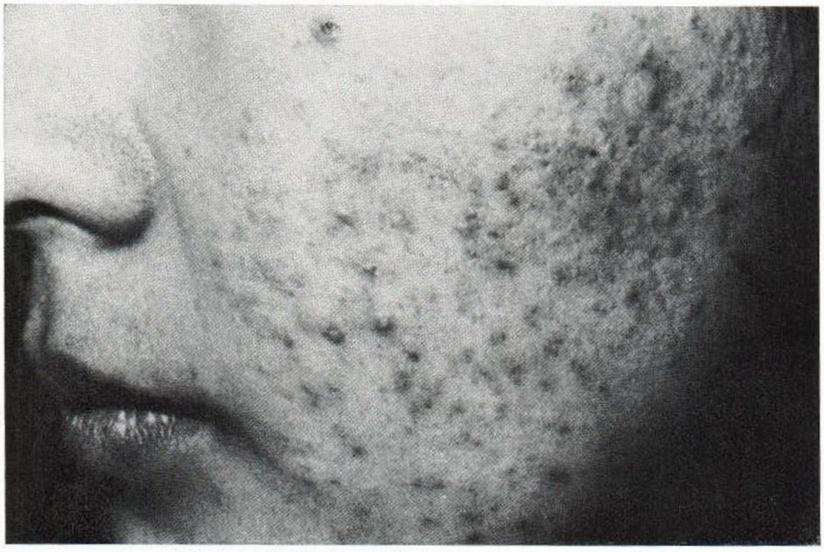

Fig. 2. After 4 weeks per oral treatment of Fig. 1.

Table VII. *Effects of glucarodilactone upon warts*

Mode of administration	No. of cases	Markedly effective	Effective	Ineffective
Verrucae planae juveniles				
Orally	29	24	1	4
Verruca vulgaris				
Orally	12	0	4	8

of these 24 cases after 1 or 2 months of discontinuance of the therapy. Those who received several other treatments showed strong resistance to this therapy, and furthermore, the older the eruption was, the more delayed was the manifestation of the effect. External application of the glucarodilactone or monolactone-ointment revealed no favorable effect at all.

Of 12 patients with verruca vulgaris treated orally, 4 showed a complete flattening or disappearance of warts after 4 to 8 weeks (Table VII). The remaining 8 cases showed no noticeable change after 8 weeks and the treatment was thus considered ineffective. Neither glucarodilactone- nor monolactone-ointment showed any effect. As to the injection, we have not tried it yet due to the severe pain it causes.

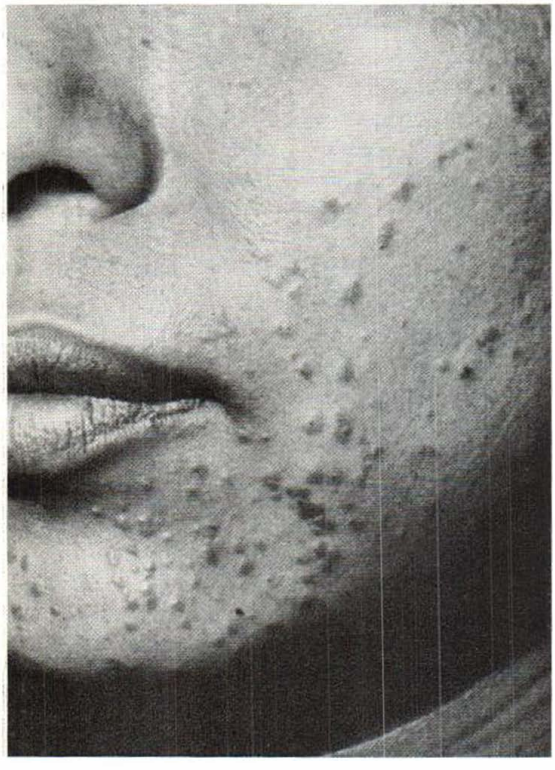

Fig. 3. Verrucae planae juveniles, pretreatment.

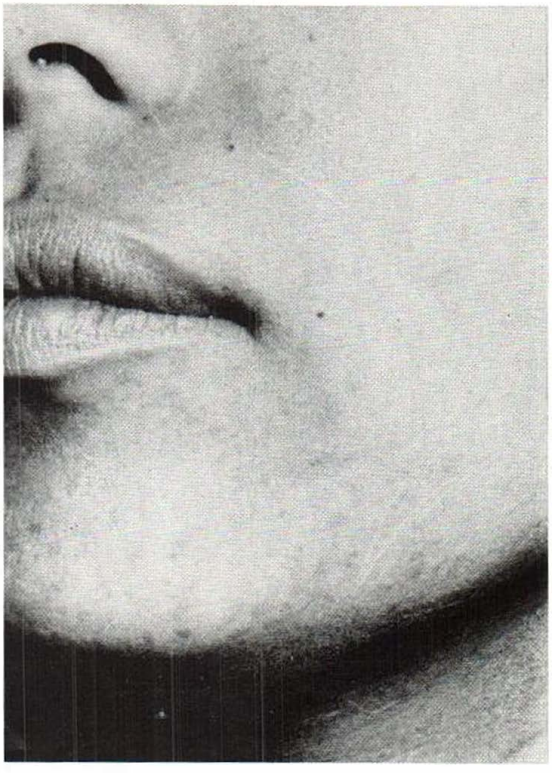

Fig. 4. After 2 weeks per oral treatment of Fig. 3.

Other diseases. In addition to the above-mentioned, several dermatoses shown in Table VIII were treated with external application and/or oral intake of the β-G inhibitor. Among them, pityriasis capitis and seborrhoea oleosa were markedly improved by the oral administration but no effect was shown in the case of rosacea.

The external application of dilactone-lanolin was very effective in one case of incurable Fox–Fordyce's disease in the improvement of severe itching, and effective in one case of Darier's disease.

Side effects. Oral administration of glucaro(1→4)-(6→3)dilactone diacetate which lasted for a few weeks, the longest duration being 12 weeks, caused no such adverse effects as gastrointestinal disturbance. A 0.1% lanolin ointment of this

compound was not irritative and long-term application resulted in no such side-effects as itching or erythema.

DISCUSSION

As stated earlier, the biological significance of β-G has not yet been fully elucidated, but it is considered to play an important part not only in the metabolism of mucopolysaccharides but also in the transport of aglucurons in body fluid. If a biologically active compound is produced by a certain organ and acts upon its remote target organ, it must be transported to the target as an inactive complex by a carrier, and be liberated from the carrier and reactivated at the target organ.

Such a phenomenon is not only characteristic in glucuronic acid conjugation, but sulphate conjugation also plays an important role in the transport of estrogen between fetus and mother, by means of the potent sulphatase existing in placenta which regulates these processes (19, 25). Various kinds of circulating hormone glucuronides (17) are known to exist in blood and testosterone glucuronide is recognized in human sera (5), liver (11), and urine (1, 12).

On the other hand, it is now well known that the sebaceous and the apocrine glands are markedly affected by the gonads, and Kuroda (18) and Matsuura (24) in our laboratory have already reported the strong β-G activity in these gland tissues. Since we were able to obtain the strong β-G inhibitor, we carried out a few fundamental experiments mainly on the role of the above-mentioned hormone glucuronides and β-G in the pilosebaceous system and tried to apply it to some cutaneous diseases.

At first, glucaro(1→4)monolactone showed a definite inhibitory effect on the hypertrophic proliferation of the sebaceous gland of female rat produced by testosterone. And glucaro(1→4)-(6→3)dilactone diacetate, which is used clinically for acne vulgaris and steroid acne, caused the reduction of seborrhoea and improvement or disappearance of acne.

Matsuura (24) reported the measurement of increased β-G activity in the apocrine glands of patients with osmidrosis, and we have also confirmed its intense activity. We also revealed that β-G activity of axillary sweat taken from the normal adult and the patient with osmidrosis is much more intense than that of the sweat from the chest or back, and the local application of the β-G inhibitor actually reduced the β-G activity.

As a consequence of our findings, we applied 0.1% lanolin ointment of this inhibitor to patients with osmidrosis, and obtained a favorable clinical effect in almost every case.

Among many theories about the nature of the odor in osmidrosis axillae, bacterial infection is widely believed to be an important factor which enhances the odor, but as seen from our cases, half of the osmidrosis was revealed to be bacteria-negative and the effect of the β-G inhibitor was the same as bacteria-positive. Indeed, we cannot directly correlate the odor with the β-G activity,

Table VIII. *Effects of glucarodilactone upon other various skin disease*

	Mode of administration	No. of cases	Effective	Slightly effective	Ineffective
Pityriasis capitis or Seborrhoea oleosa	per os	4	4	0	0
Acne rosacea	per os	3	0	0	3
Fox–Fordyce disease	ointment	1	1	0	0
Darier's disease	ointment	1	1	0	0
Hyperidrosis palmaris	per os	6	0	0	6
Syringoma	per os	3	0	0	3
Pustulosis palmaris et plantaris	per os and ointment	3	2	0	1
Psoriasis vulgaris	per os and ointment	6	1	0	5
Ichthyosis vulgaris	ointment	5	0	1	4
Keratosis palmaris et plantaris hereditaria	ointment	4	0	1	3
Lichen pilaris	per os and ointment	5	1	0	4
Fixed drug eruption (Antipyrin)	per os and ointment	3	0	0	3

but it seems probable that the inhibition of β-G causes blocking of sexual hormone transport to the apocrine gland and thus decreases the metabolism in situ. In addition, topical application of the inhibitor in Fox–Fordyce's disease, although only one case, was proved effective by the marked disappearance of severe itching. This result is very significant when one considers that the disease is related to the sexual hormone and the accumulation of mucin-like substance (27) in the apocrine gland is accompanied by high β-G activity.

The next problem is cellular proliferation and destruction, and the behaviour of β-G activity. Carr (6, 7) observed a marked elevation of β-G activity in Ehrlich's tumour in mice and in other experimental tumours and reported that the addition of glucaro(1→4)lactone blocked the proliferation of these tumour cells. It is also well known that urinary β-G activity is high in cancer of the urinary tract, and the injection of β-G inhibitor may be directed at the focus of bladder cancer (30). We have never used the inhibitor against malignant skin tumours, but oral administration was carried out with favorable effects on verrucae planae juveniles and verruca vulgaris. In cases of lesions such as warts, which have no inclination to moistening, 0.1% glucaromonolactone-lanolin was substituted for dilactone ointment, because in such cases dilactone could hardly be expected to convert to monolactones without aqua.

Although we must consider that the treatment of verrucae planae is subject to such psychogenic influences as suggestion therapy, the rapid and dramatic effect in 25 out of 29 cases must be due to the medicinal properties of the inhibitor and the mechanism of this action is very interesting. Up to now, no one has reported that β-G inhibitor suppresses viral proliferation. Nusbacher et al. (28) reported recently that the release of lysosomal enzymes resulted in the segmentation of chromosomes and the acceleration of viral growth, but it seems rather unreasonable to speculate that the inhibition of β-G, one of the lysosomal enzymes, causes the suppression of the viral proliferation. The mechanism of its action remains to be clarified.

Finally, we must not neglect glucuronolactone dehydrogenase, the main enzyme of glucaric acid synthesis.

$$\text{D-glucuronolactone} \xrightarrow[\text{NAD}^+]{\text{glucuronolactone dehydrogenase}} \text{D-glucaric acid}$$

According to Marsh (23), this enzyme activity in human liver is much lower in the fetus and infant than in the adult; therefore the high β-G activity in the infant liver cannot be controlled and the resulting hydrolysis of bilirubin-glucuronide causes neonatal jaundice. On the other hand, urinary glucuronolactone dehydrogenase activity is greatly reduced in animals and patients with cancer (21, 22), and this may conversely be the cause of the elevation of β-G activity. We are studying the pathway from glucuronolactone to glucaric acid and glucuronolactone dehydrogenase in normal and pathological skin tissues, and we will report the results elsewhere.

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