Testing the effects of natural products on hair growth in stumptailed macaque

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Abstract

The effect of natural products(drug 1, drug 2) on hair growth of frontal scalp of ten stumptailed macaques was investigated in period of 12 months. Drug 1 conteins extracts of Angelica gigantis Radix etc. and drug 2 conteins extracts of Corni Fructus, etc. Photographs of close view of the frontal scalp were taken once every month and folliclogram analysis has been done at pretretment and on 5th, 10th and 12th month after treatment. Drug 1 showed only slight effect or no effect on hair growth and drug 2 exhibited a significant degree of hair regrowth, but the control group exhibited a definite degree of regrowth and increasing density of vellus hair. Also, the physiological parameters, such as body weight, blood pressure, heart rate, serum levels of androgens, hematological measures during the treatment of drug 1, drug 2, and vehicle, were within normal limits. It indicates the drug 2 is the possible appearence of new summit of hair growth.

1. Introduction

The frontal alopecia developing in the scalp of postadolecent stumptailed macaques is in many aspects similar to the common baldness (androgenetic alopecia) of human and is considered to be a pertinent animal model for the study of human baldness.

The frontal alopecia that develops in postadolescent stumptailed macaques is a unique hereditary phenomenon in this species. Signs of the secendary sexual development in these animals appear between 3 and 4 years of age and of both sexes and serum concentrations of testosterone in both male and female animals also begin to elevate around the age of 4 years. This chronological age of the stumptailed corresponds with late pubescence in humans. In most of the animals the first sign of frontal alopecia(thinning for hair) appears shortly after age 4 years.

Histologically, the phenomenon of baldness manifests it self as a diminution of the size of hair follicles per se – that is, tranformations of the follicles from terminal to vellus type, and there are no signs of pathological changes in the hair follicles, epidermis, or dermal structure[6,7].

These biological features, as well as the testosterone dependency of the balding process in the stumptailed correspond well with nature of human male pattern baldness(androgenetic alopecia). In this aspect, it has been fair to test the effect of natural products on hair growth in this macaque model.

The principle aim of this study is to examine the effect of topical application of drug 1 and drug 2 – natural products develped and provided by Pacific R & D Center, Pacific Corporation, Korea, on hair regrowth in the forntal bald scalp of the Stumptailed Macaque.

Drug 1, drug 2 and vehicle solution were provided by Pacific R&D Center in different times, that is: on 27, Jan., 1994; 11 July, 1994; 29 Sept., 1994; 1

Dec., 1994 and 15 Dec., 1994. The test solutions of 0.2ml were approximately 50 cm^2 central triangular area of the bald frontal scalp once every day, 7 day per week.

2. Materials and methods

Ten adult stumptailed macaques, 5 males and 5 females, ranging in age from 8 to 15 years were used in this study(table I). Three of them, 2 females and 1 male - are for vehicle; four of them, 2 males and 2 females for drug 1; and another three, 2 males and 1 female for drug 2.

Drug 1 conteins 5% of the extracts mixture of Angelica gigantis Radix, Persicae Semon, Alismatis Rhizoma, Moutan Radicis Cortex, Glycyrrhiza Radix and Caryophylli Flos which were sellected *in vitro* and *in vivo* studies, in vehicle. Drug 2 conteins 5% of the extracted mixture of Corni Fructus, Eclipta Herba, Mori Fructus, Sanguisobae Radix, Achyranthes Radix, Salviae Radix, Cuscutae Semon and Pharbitdiis Semon which were sellected *in vitro* and *in vivo* studies, in vehicle. Vehlcle conteins 1% of polyoxiethylene hydrogenated caster oil and 0.5% of glyceroylmonooleate in 30% of ethanol.

All animals were kept in door single cages in a room maintained over 10 ℃ and with natural windows light. The animals were fed on standard diet of the *Macaca mulatta* with water ad-libitum and supplemented with maize and fruit(apple) or vegetables (cucumber, cabbage and carrot).

2.1. Topical Application

Drug 1, drug 2 and vehicle solution were provided by Pacific R&D Center in different times, that is: on 27, Jan., 1994; 11 July, 1994; 29 Sept., 1994; 1

Dec., 1994 and 15 Dec., 1994. The test solutions of 0.2ml were approximately 50 cm^2 central triangular area of the bald frontal scalp once every day, 7 day per week.

2.2. Photographic recording

Photographs of the frontal scalp in two magnification, large(0.81X) and small(0.40X), were taken once every month.

2.3. Histology and morphometric study of hair folliculogram

A skin biopsy (4mm punch) was taken on 24th January, 11th July, 16th December 1994 and 9th February 1995, from the frontal scalp under ketamine HCl anesthesia (10mg/kg). The biopsy was taken alternately from the right and left side of the upper-to middle region of frontal scalp near the mid-sagittal line. The skin specimens were fixed with Bouin solution for 2 hours at room temperature, and the edge of the tissue was trimmed making a flat sur face parallel to the slant of the hair folicles under a stereomicroscope. Following dehydration with graded alcohol, the tissures were embedded in paraffin wax. Serial sections (10μm thick) were cut vertical to the skin surface; the yield was approximately 200 sectins for each biopsy sample. All sections were stained with hematoxylin and eosin.

Histological observations of the epidermis, hair follicles, dermis, cutaneous appendages and morphometric analysis of the folliculogram were performed on all section[1].

2.4. Physiological and labortory examinations

2.4.1. Body weight

The body weight was measured monthly.

2.4.2. Blood pressure and heart rate

Systolic, diastolic, mean arterial pressures, and heart rate were monitored, together with on the time of taking photographs, by a cloth cuff connected to a blood pressure meter and by an electrocardiograph on 6 September, 6 October and 3 November.

2.4.3. Laboratory tests

Peripheral blood was collected on 1 February 1994, 3 May 1994, 6 September 1994, 2 December 1994 and 6 February 1995. Hematology and blood chemistry were determined.

The serum was used to assay for testosterone and dihydrotestosterone.

3. Results

3.1. Gross observation

Photgraphs of the entire frontal scalp (S) (0.40X) and a close view of the frontal scalp (L) (0.81X) were taken once every month.

At pretreatment all ten animals showed a moderate degree of baldness or early bald being furnished with short, fine and less pigmented hairs.

3.1.1. Control Group

All 3 animals treated with vehicle showed thickening of less pigmented vellus hair in the frontal scalp, and showed gradual advancement of baldness(Fig. 1.).

3.1.2. Drug 1 Group

All 4 animals treated with drug 1 showed lengthenning and thickening of hairs, and brownish in color, particulary in the upper region after 2 months of treatment. Hair regrowth appeared mostly in the mid-central portion of the bald frontal scalp after 3 months of treatment. Continued treatment induced lengthening and thickening of the regrown hair and showed the summit of the hair regrowth progress at treatment of 5 months(Fig. 2.).

3.1.3. Drug 2 Group

All 3 animals treated with drug 2 showed thickening of hair from short vellus to medium-sized hair or maintenance of hairiness in short time in the frontal scalp. hair regrowth was appeared obviously mostly in the mid-central portion of the bald frontal scalp after 2 months of treatment and showed the summit of the hair regrowth progress at treatment of approximately 3 months.

The effect was more obvious in the #16 animal than in #19 animals. However, after approximately 8 months of treatment, hair growth was not maintained. Hair dnsity decreased, new grew hair became less pigment and thinning, that is more obvios near portion of root of hair. It seems some hair root became thickened, and brownish in color at 12 months of treatment(Fig. 3.).

3.2. Histological Observation and Sequential Analysis of Folliculograms

At pretretment and after 5, 10, 12 months of treatment histological changes were observed in serial sections of drug 1, drug 2 and vehicle cases. The changes observed in the epidermis, dermis, cutaneous appendages, and hair follicles were all recorded in each section and traced as three - dimensional

images.

The folliculogram represents a histogram of the proportional distribution (percentage in abscissa) of hair follicles belonging to different cyclic phases(T = telogen, A3 = early to mid anagen, A5 = late anagen, and C = catagen), and the sizes of these follicles (lengths, mm, in ordinate).

The hair follicles in telogen and late anagen phasee were further divided into vellus and terminal types: telogen follicles of a size less than 1 mm, and anagen follicles less than 1.5mm, were classified as vellus type, and follicles larger than the above size as terminal type.

3.2.1. Control group

Fig. 4 is the sequential representation of folliculograms in the vehicle case. The #9, #14 and #22 animals showed regressive changes on teratment of 5 months, 10 months and 12 months.

3.2.2. Drug 1 group

Fig. 5 is the sequential representation of folliculograms in the drug 1 case. The #10, #15, #23 and #24 animals showed signs of progression from telogen (T) to mid-anagen (A3) and from mid-anagen to late anagen (A5) after treatment of 5 months. These changes in the folliculogram persisted at treatment of 10 and 12 months with exception of #15.

3.2.3. Drug 2 group

Fig. 6 is the sequential representation of folliculograms in the drug 2 case. The #13 and #16 animals showed progression from telogen (T) to mid-anagen (A3) and from mid-anagen to late anagen (A5) after treatment of 5 months, 10 months and 12 months. But #19 animal showed little regression of follicle growth.

3.3. Physiological examinations

3.3.1. Body weight

During the entire experimental period, the body weight, ranging from 4.5-6kg in females and 6.0-11.0kg in males showed a gradual increased from 1.4-2.9kg in females and 2.6-5kg in males in vehicle, drug 1 and drug 2 treated animals(Table II).

3.3.2. Blood pressure and heart rate

The mean vales of values of systolic and diastolic pressures during the treatment of drug 1, drug 2 and vehicle were 128/90.4(120-130 /90-92)mmHg. That indicated no significant effect of treatment on blood pressure.

Heart rate, ranging from 153 to 214 per minute (183.5 mean), showed no specific changes during the treatment periods in either vehicle or drug 2 cases.

3.3.3. Blood sampling data

Testosterone values measured at pretretment as well as during the treatment showed it is in the normal range with wide individual variations, from 2.47-16.2nmol/L in adult males and from 0.91-3.31nmol/L in females(Table III).

The results of conventional serum tests were within normal limits (Table IV).

4. Discussion

The effect of natural products(drug 1, drug 2) on hair growth of frontal scalp of ten stumptailed macaques was investigated in period of 12 months. Photographs of close view of the frontal scalp were taken once every month and folliclogram analysis of biopsied skin has been done at pretretment and on 5th, 10th and 12th month after treatment.

In the case of drug 1 treatment, animals no. 10 and no. 15 exhibited moderate degree of hair regrowth from 5th month to 8th month after treatment. Both gross observation and follulogram analysis showed that vellus follicles in both telogen and anagen phases of the bald scalp enlarged into medium and terminal size. However, after 8th month of treatment drug 1 showed only slight effect or no effecton hair growth.

In the case of drug 2, all three animals exhibited a significant degree of hair regrowth as early as 2nd and 3rd month of treatment: increase in hair length and hair density, which can be seen in the gross observation, but not in folliculogram, because first postreatment folliculogram has been done at 5th month after treatmen. Animals no. 13 and no. 16 exhibited progressing increase of late anagen follicles seen in the folliculogram analysis at 10th and 12th month of treatment. That indicated the possible appearence of new summit of hair growth.

In control group all three animals exhibited a definite degree of regrowth and increasing density of vellus hair. During the period of experiment a tendency of a progressive baldness and no signs of medium and terminal hair has been observed. Folliculogram revealed a transformation from anagen 5 and catagen into telogen.

Its seems that the effect of drugs on hair regrowth exhibited a fluctuation. In case of drug 2, the summit of hair regrowth appeared at 2nd or 3rd month of treatment and possibly the second summit falled on 12th month and thereafter. In case of drug 1 summit appeared at 5th month of treatment.

However, In case of vehicle, animals exhibited progressive baldness without any fluctuation.

The physiological parameters, such as body weight, blood pressure, heart rate, serum levels of androgens, hematological measures during the treatment of drug 1, drug 2, and vehicle, were within normal limits.

References

1. Uno, H, Kemnitz, JW, Cappas, A, Acachi, K, Saknma, A and Kamoda H: The effects of topical diazoxide on hair follicular growth and physiology of the stumptailed macaque. Journal of Dermatological Science, 1: 183-194 (1990).

2. Uno, H, Cappas A: Action of topical minoxidil in the bald stumptailed macaque. Journal of the American Academy of Dermaltology, 16(3):657 -668 (1987).

3. Parker, LN, Lifrak, ET, Odell, WD : Lack of a gonadal or adrenal anagen mechanism for the hypertrichosis produced by diazoxide, phenytoin and minoxidil. Biochemical Pharmacology, 31:1948-1950 (1982).

4. Uno, H, Allegra, F, Adachi, K, Montagna, W: Studies of common baldness of the stumptailed macaque (Macaca Speciosa). I. Distribution of the hair follicles. Journal of Investigative Dermatology, 49: 288-296 (1967).

5. Uno, H: The stump-tailed macaque as a model for baldness. International journal of Cosmetic science, 8:63-71 (1986).

6. Uno, H : Biology of hair growth. In Seminars in Reproductive Endocrinology : Andrology in Women, Vol 4. Edited by L. Speroff, Thieme-Stratton, New York, 131-141 (1986).

7. Rittmaster, RS, Uno, H, Povar, ML, Mellin, TN and Loriaux, DL: The

effect of 4-MA, a 5-alpha-reductase inhibitor and anti-androgen, on the development of baldness in the stumptailed macaque. J. Endocrinol. Metab. 65 : 188-193 (1987).
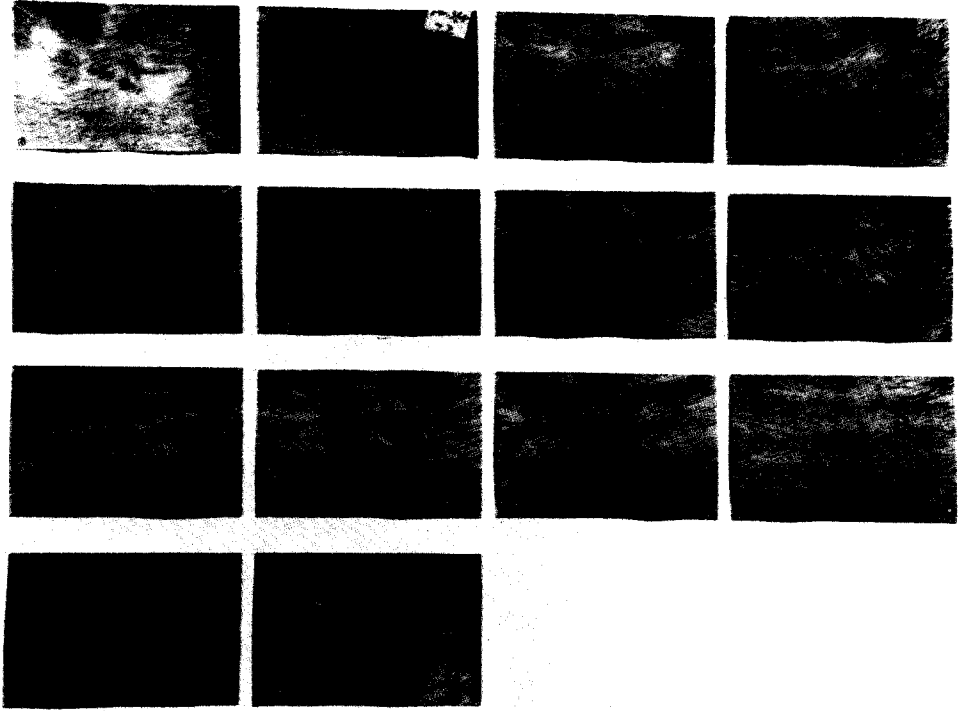
Evaluated ~~at:~~

0 time:	early bald ,
	vellus hair
	two bald spots obvious
1M:	vellus grew
7M:	vellus hair
	density increased
8M:	vellus thinning
11M:	density decreased
	vellus thinning
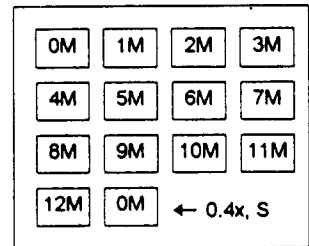
Evaluation:

no effect

progressive thinning

vellus hair

less pigment

0M	1M	2M	3M
4M	5M	6M	7M
8M	9M	10M	11M
12M	0M	← 0.4x, S	

Arrangement of photos

Fig 1 Frontal scalp of vehicle-treated macaque no. 22
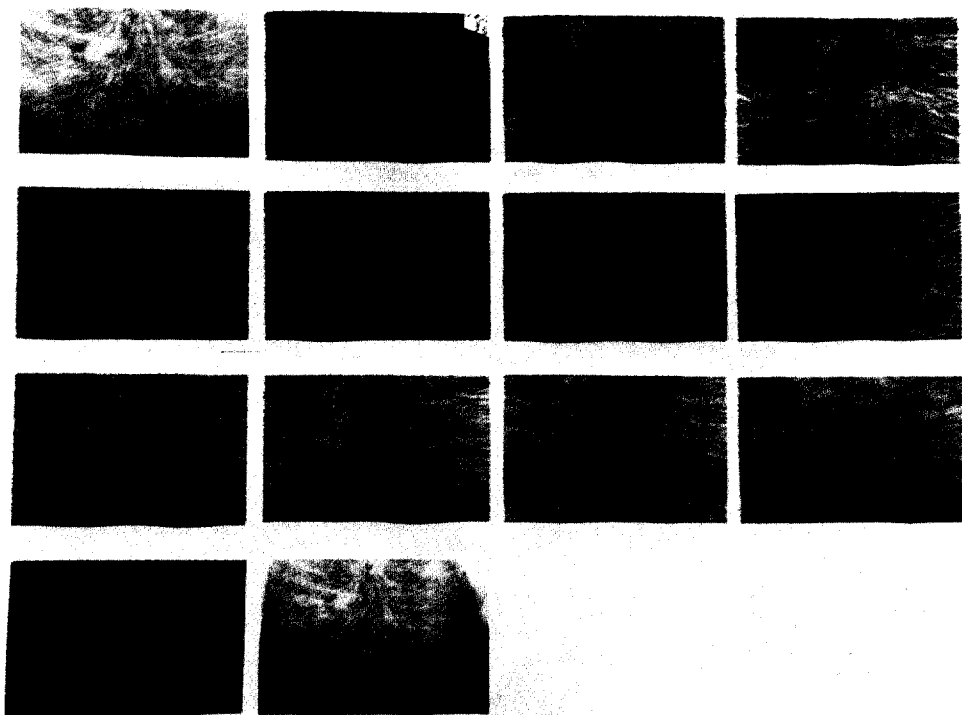
Evaluated at:

0 time:	early bald	
	three bald spots obvious	
	thin hair	
1M:	vellus grew	
2M:	intermediate hair grew	
	bald spots not obvious	
3M:	density increased	
4M:	hair longer	
5M:	the summit of regrowth	
10M:	density ↓	

0M	1M	2M	3M
4M	5M	6M	7M
8M	9M	10M	11M
12M	0M	← 0.4x, S	
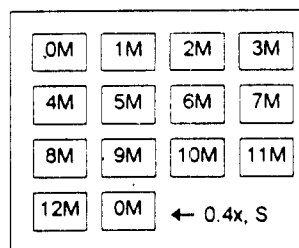
Arrangement of photos

Evaluation:

moderate effect

summit of effect in 5M

some intermediate hair maintaining

Fig. 2 Frontal scalp of drug 1-treated macaque no. 15
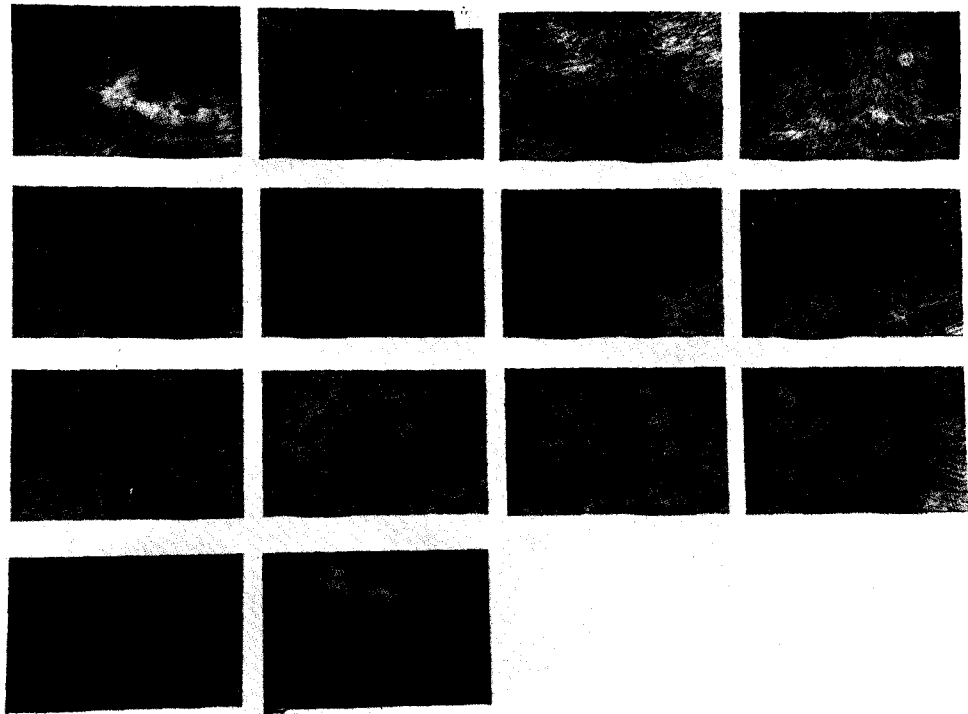
Evaluated at:

0 time:	early bald
	intermediate hair
	thin hair
	a bald spot (large and wide area) obvious
2M:	vellus and hair grew
3M:	summit of effect
5M:	thinning
9M:	density decreasing
10M:	density decreasing
12M:	more regrew hair maintaining

0M	1M	2M	3M
4M	5M	6M	7M
8M	9M	10M	11M
12M	0M	← 0.4x, S	
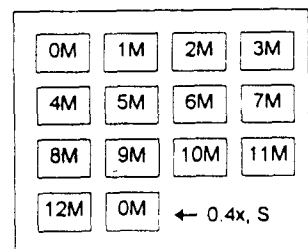
Evaluation:

slight effect

summit of effect in 3M

more thin and intermediate hair maintaining

Arrangement of photos

Fig. 3 Frontal scalp of drug 2-treated macaque no. 13
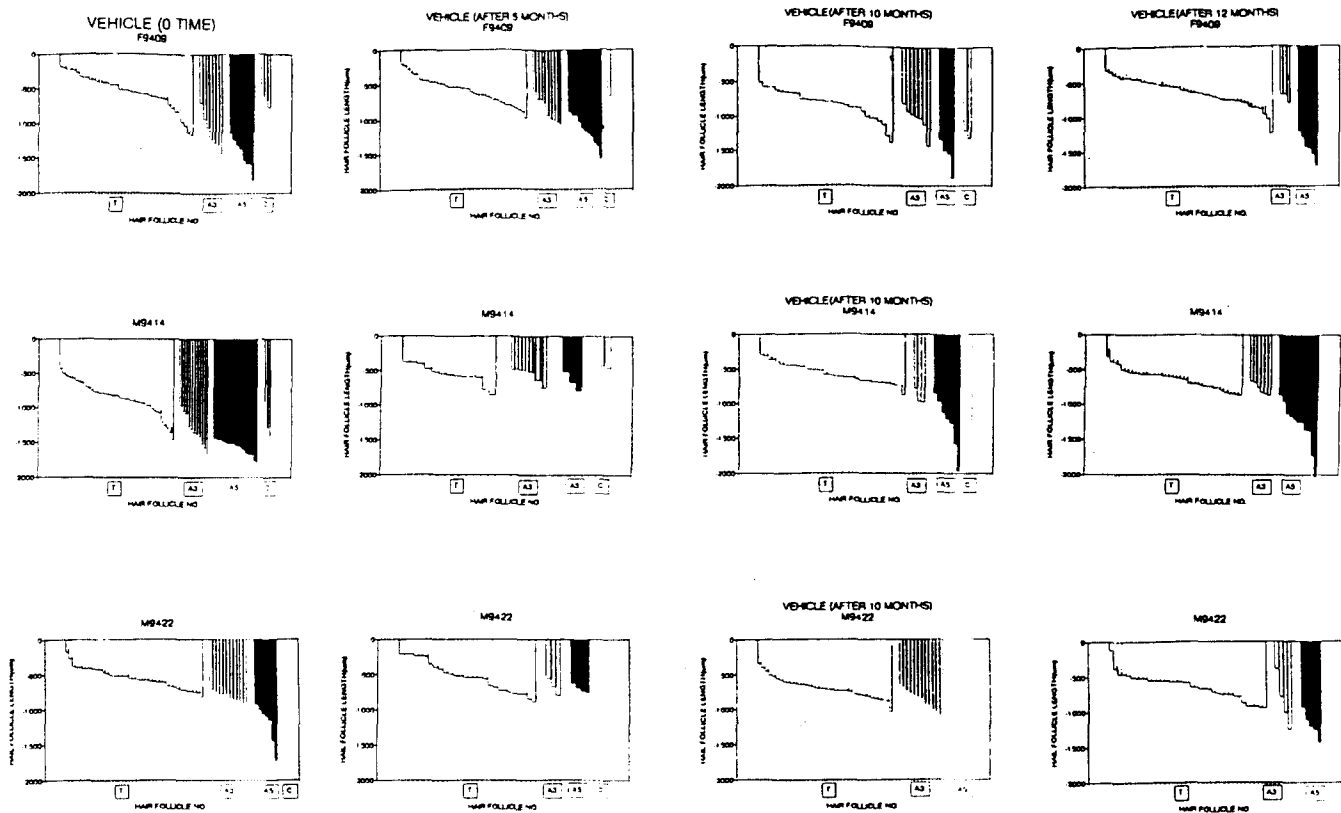
Fig. 4. Sequential representation of folliculograms in the vehicle case. The #9, #14 and #22 animals showed regressive changes on teratment of 5 months, 10 months and 12 months.

Fig. 5. Sequential representation of folliculograms in the drug 1
case. The #10, #15, #23 and #24 animals showed signs of progression from
telogen (T) to mid-anagen (A3) and from mid-anagen to late anagen (A5)
after treatment of 5 months. These changes in the folliculogram
persisted at treatment of 10 and 12 months with exception of #15.

Fig. 6. Sequential representation of folliculograms in the drug 2 case. The #13 and #16 animals showed progression from telogen (T) to mid-anagen (A3) and from mid-anage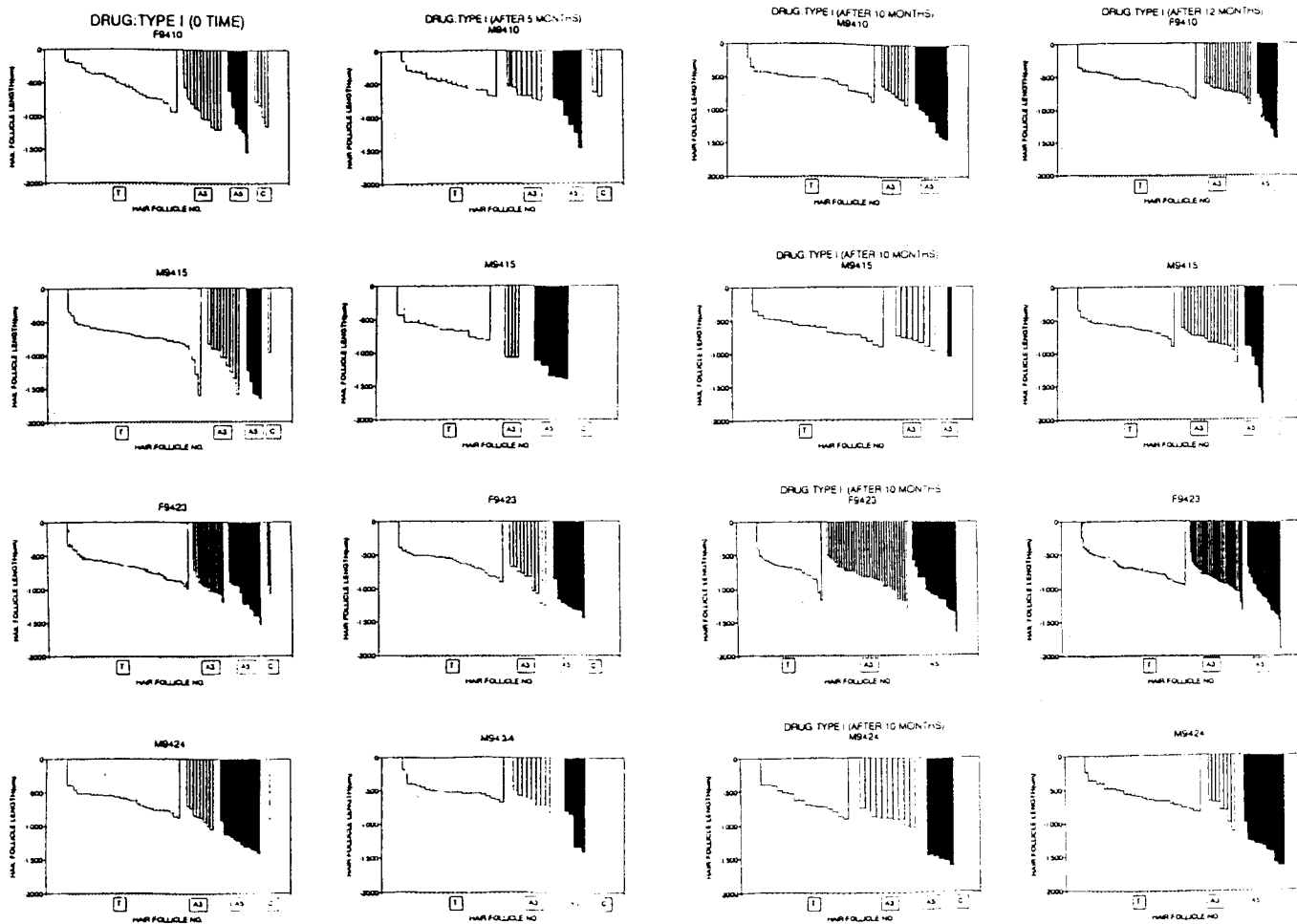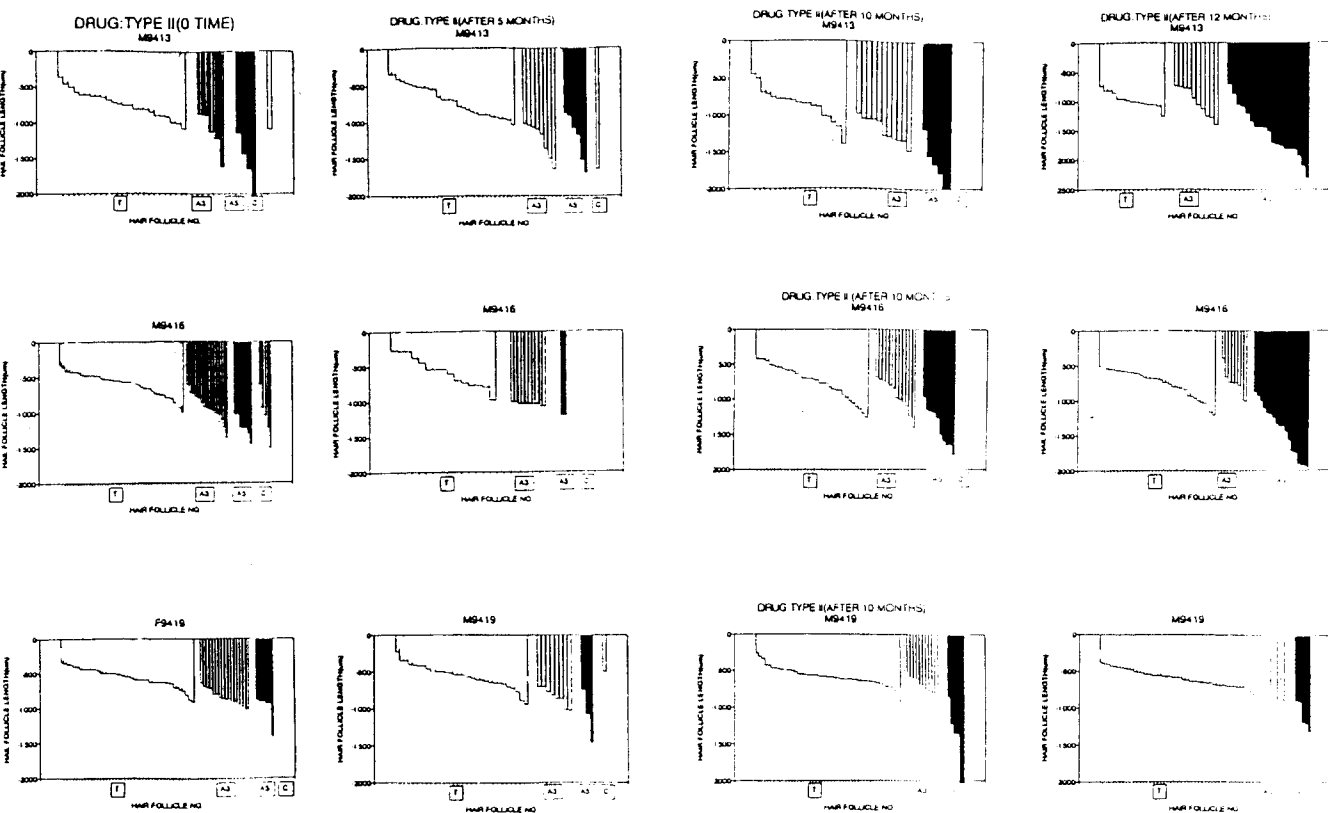n to late anagen (A5) after treatment of 5 months, 10 months and 12 months. But #19 animal showed little regression of follicle growth.

Table I Status of experimental animals before treatment

No. of animals	Sex	Age(years)	Body Weight(kg)	Use for
9	F	9	5.0	Vehicle
10	F	10	5.0	Drug 1
13	M	12	11.0	Drug 2
14	F	11	4.5	Vehicle
15	M	9	6.5	Drug 1
16	M	11	8.0	Drug 2
19	F	11	5.5	Drug 2
22	M	13	8.0	Vehicle
23	F	15	6.0	Drug 1
24	M	9	6.0	Drug 1

Table II Changes in body weight of experimental animals

	animal No.	sex	body weight monthly (kg)												
			0 Time	1M	2M	3M	4M	5M	6M	7M	8M	9M	10M	11M	12M
vehicle	9#	F	5.0	6.5	6.0	7.0	7.0	6.5	7.0	7.0	7.5	7.5	7.5	7.6	7.5
	14#	F	4.5	6.5	5.0	6.5	7.0	6.3	6.0	6.0	6.2	6.5	6.3	6.3	7.0
	22#	M	8.0	9.5	8.0	9.0	9.0	10.5	12.0	12.0	11.5	12.5	12.8	11.6	11.0
average body weight			5.83	7.5	6.33	7.5	7.67	7.77	8.33	8.33	8.4	8.83	8.87	8.5	8.5
Drug 1	10#	F	5.0	6.5	6.0	6.5	6.0	6.0	6.0	6.0	5.2	5.5	5.5	5.8	6.1
	15#	M	6.5	7.5	7.0	8.0	9.0	9.5	11.0	11.0	11.5	12.0	12.0	12.0	11.5
	23#	F	6.0	7.5	6.0	7.0	7.2	7.2	8.0	7.2	8.8	9.5	9.2	8.9	8.9
	24#	M	6.0	6.5	6.0	7.0	8.0	7.8	8.5	9.0	9.5	10.0	10.2	10.0	10.0
average body weight			5.88	7.0	6.25	7.13	7.55	7.63	8.38	8.3	8.75	9.25	9.23	9.18	9.13
Drug 2	13#	M	11.0	12.0	12.0	12.0	13.0	12.0	12.1	12.8	13.5	14.5	14.5	13.5	13.6
	16#	M	8.0	9.5	9.0	10.0	10.2	11.0	11.7	12.0	12.0	12.5	13.0	13.5	11.7
	19#	F	5.5	5.5	6.0	6.0	6.1	6.2	6.5	6.5	6.0	6.5	6.5	6.3	6.9
average body weight			8.17	9.0	9.0	9.33	9.77	9.73	10.1	10.43	10.5	11.17	11.33	11.1	10.73

Table III.Changes of testosterone

Unit: nmol/L

sampling at / No. of animal	0M	3M	7M	10M	12M
9 (F)	1.74	1.24	1.95	2.25	0.91
10 (F)	1.86	1.72	2.09	2.47	1.28
13 (M)	16.21	12.51	8.18	7.22	6.52
14 (F)	1.76	0.92	2.33	2.29	1.92
15 (M)	5.20	8.92	5.56	5.29	5.89
16 (M)	7.65	9.24	12.89	10.72	10.27
19 (F)	1.36	1.75	2.54	1.92	1.06
22 (M)	2.47	6.56	15.00	10.50	/
23 (F)	1.57	1.92	2.83	3.31	1.01
24 (M)	2.72	7.09	12.09	12.76	7.20

Table IV. The results of laboratory blood tests

No. of animal / item	9	10	13	14	15	16	19	22	23	24		Unit
Glucose	3.2	3.9	3.4	4.9	3.8	3.4	3.4	3.4	3.4	2.1		mmol/L
BUN	3.6	5.5	4.9	6.4	5.7	8.0	5.7	6.4	4.5	7.8		mmol/L
Creatinine	62	69	96	64	73	46	48	114	61	52		μmol/L
Cholesterol	3.6	3.3	2.5	3.2	3.7	2.6	3.0	2.4	2.7	2.5		mmol/L
SGOT	38	46	48	43	61	38	41	69	32	38		IU/L
SGPT	10	17	10	15	7	10	12	12	15	17		U
LDH	8	15	5	21	14	14	30	16	12	24		IU/L
AKP	29	25	26	57	64	42	53	48	40	58		IU/L
Total bilirubin	1.71	1.71	2.17	1.71	1.71	2.57	1.71	2.57	2.57	1.71		μmol/L
Ca	1.76	1.76	1.99	1.95	1.76	1.76	1.78	1.60	1.52	1.65		mmol/L
P	1.16	1.18	1.25	1.22	1.32	1.16	1.22	1.30	1.20	1.08		mmol/L
Na	141	133	136	141	133	99	130	138	136	138		mmol/L
K	4.6	4.2	3.5	4.2	4.6	2.9	3.3	4.3	4.6	4.5		mmol/L
Cl	102.7	97.6	97.6	97.6	92.5	92.5	95.0	92.5	92.5	105.3		mmol/L
Testosterone	1.74	1.86	16.21	1.76	5.20	7.65	1.36	2.47	1.57	2.72	M: 6.85±5.64 F: 1.63±0.22	nmol/L
Estradiol	All data <10 pmol/L										M: - F: -	pmol/L
RBC	4.25	4.78	4.09	3.24	4.09	4.70	4.34	4.92	3.44	3.79		million/mm
WBC	8.7	8.2	8.0	8.7	10.9	8.1	6.7	6.4	7.8	10.9		thousand/mm
Neutrocyte	74	70	78	72	76	71	78	74	70	78		%
Lymph-cell	22	26	21	26	22	26	18	23	26	20		%
Mononuclear	4	2	1	2	-	3	4	3	2	1		%
Acidocyte	-	2	-	-	2	-	-	-	2	1		%

No. of animal / item	9	10	13	14	15	16	19	22	23	24		Unit
Glucose	3.6	3.9	4.1	3.7	4.2	3.8	4.3		4.0	3.9		mmol/L
BUN	4.3	5.0	5.4	7.1	6.1	6.4	6.1		5.4	5.0		mmol/L
Creatinine	70	88	97	79	88	97	88		70	79		μmol/L
Cholesterol	4.0	4.1	3.6	3.8	3.4	3.5	4.5		4.1	3.4		mmol/L
SGOT	18.1	22.0	16.8	20.0	15.6	19.0	21.3		17.6	20.4		IU/L
SGPT	17	20	22	24	17	20	26		22	17		U
LDH	106	121	78	92	118	122	135		96	145		IU/L
AKP	67	78	67	80	77	82	76		67	85		IU/L
Total bilirubin	1.7	2.6	1.7	2.6	1.7	1.7	2.6		2.6	1.7		μmol/L
Ca	2.44	2.39	2.43	2.44	2.39	2.34	2.44		2.34	2.39		mmol/L
P	1.29	1.24	1.32	1.24	1.32	1.44	1.36		1.30	1.28		mmol/L
Na	142	144	145	146	145	140	142		138	141		mmol/L
K	4.7	5.1	5.4	4.8	5.0	4.6	4.4		4.6	4.9		mmol/L
Cl	107	111	110	112	106	107	107		105	109		mmol/L
Testosterone	0.91	1.28	6.52	1.91	5.89	10.27	1.06		1.01	7.20	M: 7.47 ± 1.94 F: 1.23 ± 0.40	nmol/L
Estradiol	323	155	40	329	125	256	326		200	436	M: 214.2 ± 172.5 F: 266.6 ± 82.9	pmol/L
RBC	4.26	4.07	4.09	4.46	3.57	4.11	4.13		4.44	3.81		million/mm
WBC	15.6	9.4	16.4	21.6	14.8	7.2	7.7		9.2	15.1		thousand/mm
Neutrocyte	57	56	71	76	70	58	52		68	72		%
Lymph-cell	38	36	26	20	26	38	44		28	22		%
Mononuclear	3	5	2	3	2	3	2		3	5		%
Acidocyte	2	3	1	1	2	1	2		1	1		%

Monkey No. 22 died.