THE ACTION OF HANSON'S THYMUS EXTRACT ON THE MOUSE

By M. LAFON

(From the Department of Genetics, University College, London)

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In a recent paper Hanson (1934) described important results as to the effect of extracts of the thymus gland on growth and morphological differentiation. In his experiments, albino rats from an inbred strain were daily injected during a long period with a thymic extract, the preparation of which is fully described by him in a previous paper (1930). Four successive generations were bred, the young being treated from the 16th day, and the treatment continuously applied even during pregnancy. Comparison with the controls shows a striking acceleration of growth, quite conspicuous from the growth curves, an earlier appearance of such morphological changes as the growth of the hair and the teeth, the opening of the eyes and the ears, and an earlier sexual maturation, as manifested by the external descent of the testes and the opening of the vagina. The injections have a cumulative effect, the results being most striking in the F_2 animals, with a slight increase of effect through the following generations. The average time for the opening of the vagina may be reduced, from the 60th to 70th day in the controls, to the 18th to 19th day in F_4, the appearance of the hair from the 14th to 17th day to the 3rd day.

Both parents were treated in all these experiments and non-treated offspring were not available for comparison. These results could be interpreted, either as due to some modification of the gonads induced by the hormone and affecting the development of the offspring, or as a simple physiological action on the treated individuals themselves. In this latter case the hormone might or might not be transmitted through the placenta.

It was the aim of the present research to resolve this question, while the use of a different species served to show how far Hanson's results could be generalised.

MATERIAL AND TECHNIQUE

The animals used belong to an inbred strain of mice from the Little's black agouti stock, kept in the Department of Zoology of University College, London. Experimental and control animals were close relatives, sometimes litter-mates, more often cousins. They were both supplied with the same diet and the same conditions of environment throughout the experiment. Inbreeding was continued through the F_1 and F_2 generations.

The extract was prepared, following quite accurately Hanson's (1930) prescription. Thymus from young calves (2–6 weeks) was freed from any extracapsular connective tissue, finely ground, then stirred up in a beaker with a solution of 0·5 per cent. HCl (600 gm. of thymus being added to 1000 c.c. of the solution). The milky emulsion was slowly and gradually warmed for 1½ hours to 94° C., while constantly stirred. The fibrous tissue was removed from time to time, pressed between Pyrex plates, and the liquid secured was finally added to the extract. Then the temperature was slowly brought down to 80° C. (in ½ hour). After filtration, the extract was made up to 990 c.c. by pouring distilled water at 96° C. on the filter. The extract was then placed in a sterilised beaker and stirred by a sterilised stirrer. It was gradually brought to 95° C. and then the flame was immediately removed. At the end were added, drop by drop, 10 c.c. of a solution of 3 per cent. menthol in 97 per cent. absolute alcohol. The extract, a greenish yellow liquid of *p*H 5·4, was kept in sterilised tubes.

The animals had a daily intraperitoneal injection: 0·03 c.c. from the 16th to the 25th day, and 0·06 c.c. after the 25th day.

These injections were continued without any apparent trouble, during 4 months for the parents, 3 months for the F_1 and 2 months for the F_2. Any attempt to increase the daily dose failed, larger doses stopping growth and causing serious disturbances in the behaviour of the animals.

The young were daily examined and weighed, until the end of the growth period.

RESULTS AND DISCUSSION

Negative results

No acceleration was found in growth, the growth curves of the controls, the F_1 and the F_2 being sensibly the same.

The appearance of the hair, the eruption of the teeth, and the opening of the eyes and the ears, did not occur earlier in the treated animals (see Table I).

Table I

	Teeth	Hair	Eyes	Ears
Controls	5·8 ± 0·3	6·8 ± 0·2	13·6 ± 0·4	14·2 ± 0·4
F_1	5·9 ± 0·3	6·7 ± 0·3	13·4 ± 0·5	14·0 ± 0·5
F_2	5·8 ± 0·2	6·8 ± 0·2	13·5 ± 0·3	14·0 ± 0·3

Positive results

A definite acceleration of sexual maturation was found in F_1 (see Table II): the opening of the vagina took place from the 53rd to the 63rd day in the controls, from the 32nd to the 40th day in the F_1 animals, the descent of the testes from the 47th to the 57th day in the controls, from the 30th to the 39th day in the F_1 animals.

Half of the F_1 animals (Section A) were treated in the same manner as were the parents, both father and mother being injected; but no further increase was ob-

Table II. *Dates of maturity of controls and* F_1

Controls				F_1			
Males		Females		Males		Females	
No.	Day	No.	Day	No.	Day	No.	Day
4	49	3	57	12	32	11	33
6	51	2	60	13	31	21	33
7	47	10	54	26	30	24	37
11	54	14	61	22	33	25	35
12	57	13	62	23	34	32	39
21	47	22	54	31	39	33	40
32	50	23	53	42	36	41	36
33	55	34	63	43	35	51	34
41	52	42	58	52	34	53	35
51	53	52	57	61	38	62	39
53	48			72	31	71	32
54	53			73	31	74	33
				81	32	82	35
						83	34

served in F_2, the results being sensibly the same as in F_1 (Table III). The other half (Section B), including several litter-mates of the preceding, were divided into two groups.

Table III. *Dates of maturity of* F_2

Males F_2		Females F_2	
No.	Day	No.	Day
1	35	3	34
2	33	4	36
11	30	5	32
13	31	12	33
22	36	21	38
31	38	23	40
32	39	24	38
41	35	42	36
43	34	43	36
45	33	46	35
51	35	52	33
61	32	53	34
62	31		
63	31		

In the first group (B 1), fathers only were injected. F_2 animals behaved like the controls.

The earlier sexual maturation could not be ascribed to a genetical modification, since the father had no part in the transmission of the effects of the thymic hormone.

In the second group (B 2), mothers only were injected. A proportion of the young were injected from the 16th day; these behaved like the F_2 of Section A.

Another fraction of the young, litter-mates of the preceding group were left untreated, then separated from the mother on the 28th day. They behaved like the controls. One litter of five injected individuals derived from uninjected ancestors showed accelerated maturity, like the animals of Tables II, III and V, but this

Table IV. *Group B* 1

Males F_2		Females F_2	
No.	Day	No.	Day
111	50	101	54
112	52	113	55
114	51	121	59
122	55	123	60
124	49	125	55
131	54	132	61
133	53	134	58
135	51	141	54
142	48	143	55

Table V. *B* 2. *Mothers and young injected*

Males F_2		Females F_2	
No.	Day	No.	Day
202	31	201	34
203	32	204	33
211	30	212	32
213	35	221	36
226	35	227	37
231	38	232	39
233	34	234	36
242	33	241	35
243	34	244	34
245	37		

Table VI. *B* 3. *Mothers injected, young untreated*

Males F_2		Females F_2	
No.	Day	No.	Day
205	48	206	55
207	49	222	58
214	53	223	61
215	51	224	60
225	50	235	57
246	52	247	58
251	55	252	54
253	52	254	59
		255	58

experiment has not yet been performed on a large scale. The mean ages at maturity with their standard errors are given in Table VII. It will be seen that the ages for each sex fall into two strikingly constant groups, those maturing early having themselves been injected, while the injection of parents only had no effect whatever. The injections have no cumulative effect, and they do not cause any acceleration of the early growth and the concomitant morphological changes.

This wide difference from Hanson's results may be ascribed either to the use of a different species or to the fact that Hanson's hormone is not a definite compound,

Table VII. *Means and their standard errors*

	Males	Females
Controls	51·3 ± 0·89	57·9 ± 1·11
F_1 } (both parents treated)	33·5 ± 0·75	35·4 ± 0·65
F_2 }	33·6 ± 0·71	35·4 ± 0·55
A (fathers only injected)	51·4 ± 0·72	55·7 ± 0·89
B 1 (mothers and young injected)	33·8 ± 0·75	35·1 ± 0·67
B 2 (mothers only injected)	51·3 ± 0·74	57·8 ± 0·70

and, although his directions were very accurately followed, the physiological action of different preparations may vary. This work therefore calls for fresh experiments to resolve the problem posed by Hanson.

SUMMARY

1. The injection of an acid extract of the thymus gland to mice accelerated the descent of the testes by 18 days and the opening of the vagina by 22 days.

2. No effect on growth was observed, nor was the effect on maturity transmitted to the uninjected progeny of injected individuals.

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REFERENCES

HANSON (1930). *J. Amer. med. Ass.* **94**, 653.
—— (1934). *Science*, **80**, 274.