Mean green pod weight (kg) per 10 plants as affected by different treatment combinations

Age of plants at spraying (weeks)	GA concentration (ppm)					Mean	SEm	F ratio	C.D. at 5%
	0	75	150	225	300				
4	1.395	1.477	1.635	1.730	1.874	1.622	± 40.7	Significant	0.118
8	1.396	1.265	1.270	1.201	1.143	1.255			
Mean	1.395	1.372	1.452	1.465	1.508				
SEm		± 57.6							
F ratio		Not significant							

(value of regression coefficient = − 0.7601); whereas the reverse was true when the crop was sprayed at the pre-flowering stage (value of regression coefficient = + 1.6143). A unit increase in GA concentration spray at the pre-flowering stage resulted in about 2.7 kg of extra total mean yield per 10 plants as compared to those sprayed at the flowering stage. Further work is, however, needed to determine the cause of this increase in yield[4].

Zusammenfassung. Die Applikation von Gibberellinsäure bei *Pisum sativum* bewirkt im vegetativen Ent-wicklungsstadium eine Ertragssteigerung, im Blühsta-dium eine Ertragsverminderung.

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Hormone Dependency of Sex-Linked Feathering of Female Hybrid Chick Embryos (Cross New Hampshire ♂ × Light Sussex ♀)

The F_1-generation of this cross shows a sex-linked difference in down colour: on hatching the males are white and the females reddish-brown. The pigmentation of the females becomes visible on the 11th day of incubation when a yellow stripe appears on the back and neck. By the 14th day of incubation, the brown colour has spread over wings, thighs and occiput (Figure 1).

In a previous communication (GROENENDIJK-HUIJ-BERS[1]) evidence was presented in favour of the concept that the red pigment of the female down cannot be formed when no ovarian hormone is circulating: after hemi- and subtotal castration on the 4th day of incubation, the down colour of the 14-day-old female embryos ranged from a pale brown to almost white. The shortage of ovarian hormone was also manifested by the partial or full preservation of the right Müllerian duct (Md). A detailed report on this matter is in preparation. The results obtained in castration experiments agree with the observation of HAMILTON[2] that melanophores of skin ectoderm from 6- to 7-day-old embryos of the Rhode Island Red and New Hampshire breeds, cultivated in vitro, did not produce red pigment unless gonadal hormones (either oestrogens or androgens) were added to the culture medium. The latter studies suggest substituting the ovarian hormone of the subtotally castrated female embryos with
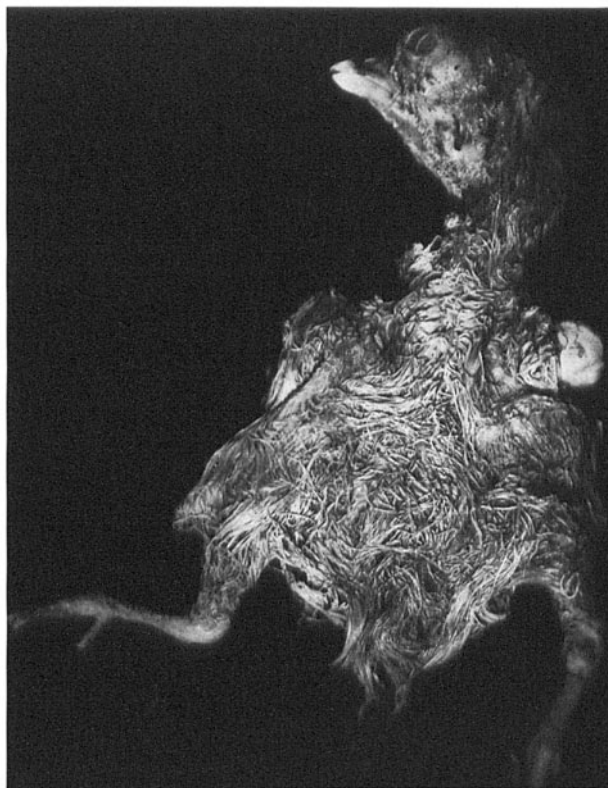

Fig. 1. 14-day-old female control embryo; crown-rump length 5.7 cm. Feathers are brown, particularly on back, thighs, wings, neck and occiput.

[1] MARGOT M. GROENENDIJK-HUIJBERS, Experientia 22, 302 (1966).
[2] H. L. HAMILTON, Anat. Rec. 78, 525 (1940).

oestrogenic or androgenic compounds. The preliminary results of this investigation built upon this idea are communicated here.

Materials and methods. As in the previous experiments[1], the gonadal primordia of 4-day-old F_1-hybrid embryos of this cross were destroyed by local electrodiathermic coagulation. In addition, on the 5th day a single dose of either testosterone propionate in corn oil (Orchisterone, Frosst) or oestradiol benzoate in arachis oil (Dimenformon, Organon) was administered by instilling the chorion through the shell window. In other cases an 18-day-old donor testis was implanted according to Dossel's method[3] along the allantoic stalk. At autopsy on the 14th day, the embryos were prefixed and exsanguinated by intracardial perfusion with 5% mercuric chloride, and, after processing of the sex organs, stored in 70% alcohol. The latter fluid washes out the blood contained in skin and feather papillae, so that the pigmented feathers sharply contrast with the white skin. The embryo's sex organs as well as back and occiput were photographed. The gonadal remnants were fixed in Maximow's fluid, containing 2% osmic acid, for 48 h at 25 °C. The sex of the embryos was verified by histological examination of the complete series.

The mortality rate of the combined procedures is extremely high. The embryos of the particular cross used are feebler than those of, for example, the White Leghorn.

During the natural hatching season, the mortality rate varied between 80 and 90%; out of season it was nearly 100%. Most embryos died on the 8th or 9th day of incubation, due to failing allantoic development, but even embryos that died on the 13th day had to be discarded as unreliable. This explains the tardy progress of the investigations.

Results. The results are summarized in the Table. Out of the 8 hormone-treated female castrates kept alive until autopsy on the 14th day of incubation, 6 were definitely brown (Figure 5); that is, darker than could be expected from the size of the ovarian remnants (Figures 3, 4 and 6). In 2 of these cases, the regression of the right Md was absent and the ducts were fully preserved (Figure 6); in the other cases, the cranial end of the right Md was situated at or above the lower third of the mesonephros, which indicates a low level production of the ovarian hormone. The brown colour was seen only after administration of high doses of gonadal hormones (150 γ oestradiol or 1250 γ testosterone). Lower doses (100 γ oestradiol or 500 γ testosterone) were ineffective: the colour remained pale brown as in the untreated castrates. This suggests a local influence of the hormones on the feather-pigmenting melanophores. Also a testicular implant,
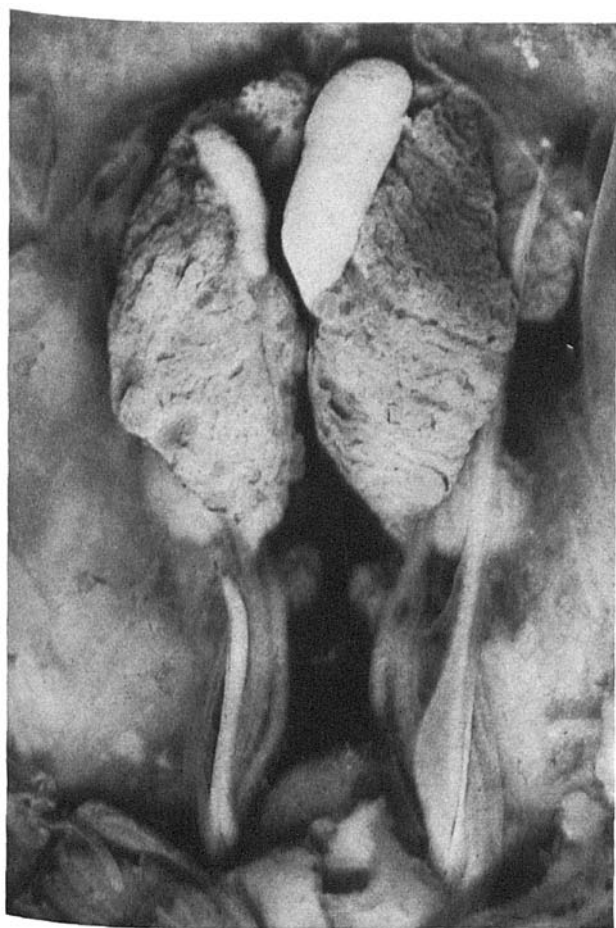
[3] W. E. Dossel, Science *120*, 262 (1954).

Fig. 2. Internal sex organs of 14-day-old female control embryo. Right ovary is rudimentary. Right Müllerian duct (Md) has regressed till below lower pole of mesonephros. Left Md complete with shell gland dilation.

Fig. 3. No. 41167. 14-day-old subtotally castrated female; crown-rump length 4.9 cm. Feathers are light brown on lower back and neck, but white on thighs, wings and occiput.

which had developed well on the allantoic membrane, promoted the formation of brown pigment. Apparently the testicular hormone substituted for the ovarian hormone. The male embryos remained white under all experimental conditions.

Discussion and conclusions. In the Brown Leghorn breed, administration of large doses of oestradiol to the embryo did not feminize the down pigmentation of the cockerels (TRINKAUS[4]). This observation moved the latter author to conclude that the feather-pigmenting melanophores of the Brown Leghorn embryo are insensitive to and independent of sex hormones. The conclusion is probably correct, if it is not generalized. TRINKAUS, however, discussing HAMILTON's communication quoted above[2], argued that HAMILTON's observations must be 'non-specific in nature', as the red-feathered breeds show no apparent pigmentary response to sex hormones in the

[4] J. P. TRINKAUS, J. exp. Zool. *109*, 135 (1948).

Fig. 4. Internal sex organs of case No. 41167. Small remnant of right ovary, no trace of left ovary. Rmd's cranial extremity at lower $1/8$ of mesonephros; large shell gland dilation.
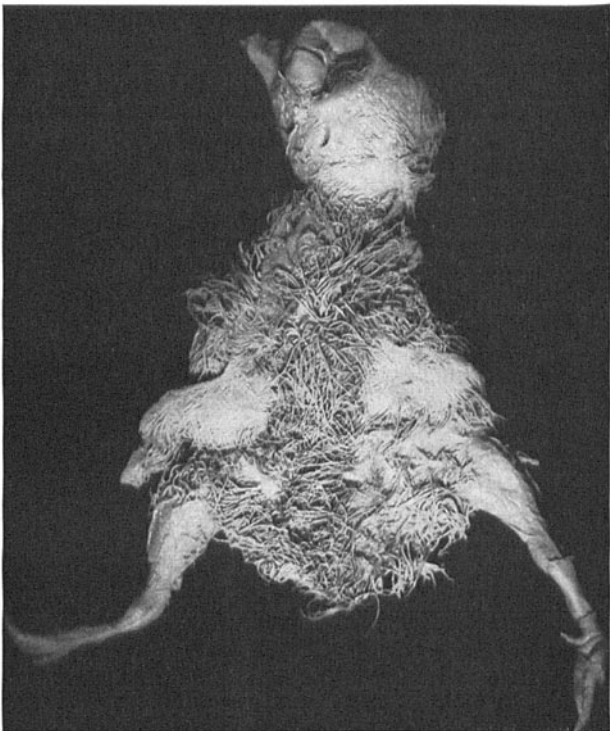

Fig. 5. No. 4885. 14-day-old subtotally castrated female, which received on the 5th day of incubation 1250 γ orchisterone (Frosst); crown-rump length 5.0 cm. Feathers are brown on back, thighs, neck and occiput.

Influence of experimental conditions on down colour of F_1-hybrid chick embryos (New Hampshire ♂ × Light Sussex ♀)

Colour of down	Sex	Controls		Castrates			Oestrogen treatment				Testosterone treatment				
		Normal	With corn oil[a]	Ovarian remnants			Controls		Castrates with small gonadal remnants		Controls		Castrates with small gonadal remnants		
				Medium	Small	Minute	50 γ	100 γ	100 γ	150 γ	500 γ	1250 γ	500 γ	1250 γ	Testicular implant
Brown	♀	12	3	6			3	3	1	2	1	2	1	2	1
Pale brown	♀				11				1				1		
Almost white	♀				1	5									
White	♂	12					3	1	1		1	8	2	2	

[a] These controls received on the 5th day of incubation 0.04 cm³ corn oil as sham treatment.

adult, whereas in the Brown Leghorn adult the differentiation of the feather-pigmenting melanophores is markedly affected by oestrogens. Although this argument was never substantiated by experiments, it was generally accepted, and the studies of HAMILTON were discarded[5].

The experiments reported here offer the in vivo duplication of HAMILTON's in vitro experiments. Substitution of the ovarian hormone by oestrogens, androgens or embryonic testicular hormone restores the production of red pigment by the feather-pigmenting melanophores. The degree of restoration depends upon the dose of gonadal hormones administered. This suggests a local influence of the hormones on the melanophores. The latter do not distinguish between oestrogens and androgens. This explains why in the experiments of WILLIER and RAWLES[6] skin ectoderm of 72-hour-old chick embryos of various crosses with a sex-linked down, transplanted into hosts of the same age, invariably developed the feather pigmentation of the genetic sex, independently of the sex of the host. In the case of the Rhode Island or New Hampshire breed, the implanted skin ectoderm always encountered a hormonal environment in the host, either male or female.

The melanophores of the genetic males of the cross New Hampshire ♂ × Light Sussex ♀, apparently lack the potency of developing red pigment due to their genetic constitution[7,8].

Zusammenfassung. Die rote Daunenpigmentierung der weiblichen F_1 Hybriden der Hühnerrassen Cross New Hampshire ♂ × Light Sussex ♀ entsteht nicht ohne Ovarialhormone, wie Kastrationsexperimente beweisen. Sie kommt aber zustande, wenn man ganz oder subtotal kastrierten Embryonen Östrogene oder Androgene zuführt oder embryonale Keimdrüsen implantiert.

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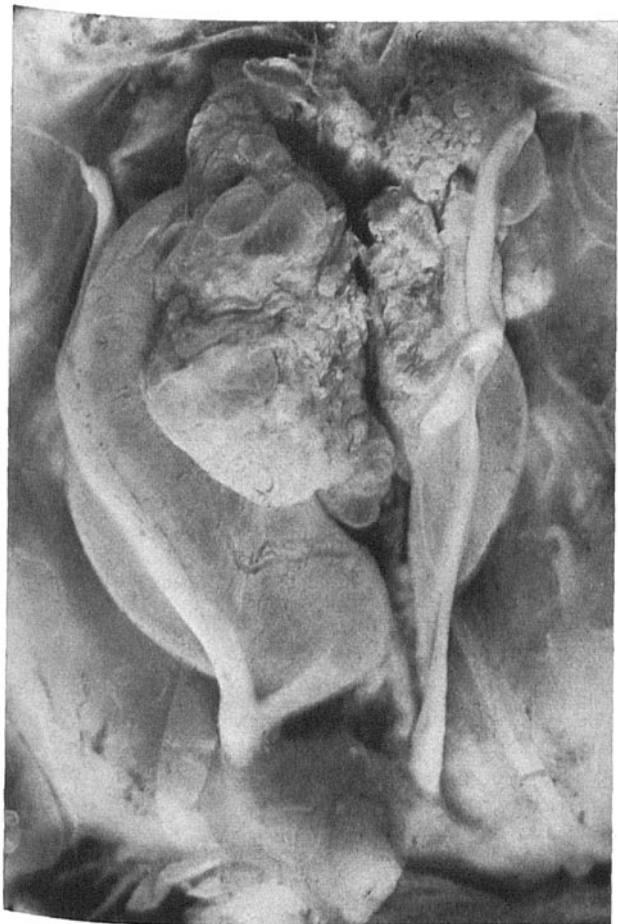

Fig. 6. Internal sex organs of case No. 4885. Minute remnant of right ovary. Right Md is fully preserved, the left Md is interrupted in its mid-portion due to mechanical damage. The Wolffian ducts are tremendously distended as a result of the action of the testosterone propionate; both mesonephroi show multiple cysts.

[5] B. H. WILLIER, Archs Anat. microsc. Morph. exp. *39*, 269 (1950).
[6] B. H. WILLIER and MARY E. RAWLES, Physiol. Zool. *13*, 177 (1940).
[7] It is a pleasure to thank Prof. Dr. W. K. HIRSCHFELD, Chairman of the Department of Poultry Breeding and Husbandry of Utrecht University, for his kind cooperation in supplying eggs from the cross New Hampshire ♂ × Light Sussex ♀, used in these experiments.
[8] The generous supply of Orchisterone by Frosst Company and of Dimenformon by Organon Ltd. is gratefully acknowledged.

Ciliated Biliary Epithelial Cells in the Livers of Non-Human Primates

Cilia[1] in epithelial cells of intrahepatic bile ducts (ductular cells) have been observed in normal livers of just one species (rat)[2]. They have been found much more frequently in a variety of conditions of pathological nature affecting livers of not only rat[3,4] but also man[4,5]. Cilia in normal liver of man or any other primate have not been described as a regular feature of the biliary tree. This paper reports the constant occurrence of ciliated cells in livers of squirrel monkeys (*Saimiri sciureus*,

Voigt 1831) shortly after admission to the colony and before any treatment was instituted.

We have studied liver biopsies from 14 young male *S. sciureus* monkeys, which were obtained with the

[1] E. D. DE ROBERTIS, W. W. NOWINSKI, and F. A. SAEZ, *Cell Biology* (W. B. Saunders Co., Philadelphia 1965), p. 385.
[2] J. W. GRISHAM, Proc. Soc. exp. Biol. Med. *114*, 318 (1963).
[3] J. W. GRISHAM and E. A. PORTA, Expl Cell Res. *31*, 190 (1963).
[4] J. W. GRISHAM and E. A. PORTA, Expl Molec. Path. *3*, 242 (1964).
[5] I. STERNLIEB, J. Microsc. *4*, 71 (1965).