

pyruvic acids, and in smaller amounts from fumaric and malic acids. From these results it is concluded that the conversion of succinic acid to propionic acid proposed by Wood, Stone and Werkman¹ probably takes place by direct decarboxylation and not by way of fumaric, malic, and oxalacetic acids as intermediates.

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The Bill of the Sparrow as an Indicator for the Male Sex Hormone. I. Sensitivity.*

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Research on the male sex hormone has long been hampered by the lack of indicators which at the same time are sufficiently sensitive, inexpensive and simple from a technical viewpoint. Keck¹ reported the fact that pigmentation of the male sparrow bill depends on the presence of the male sex hormone. Using a preparation which had been extracted in our laboratory from male urine and standardized by the rat seminal vesicle test, he concluded that in the bill of male castrates as little as 1/15 of a daily rat unit gives a distinct reaction by the eighth day.

Since the sparrow can be kept with little expense even in small laboratories and the color change is so distinct and directly observable, it seemed worthwhile to investigate the availability of this reaction as a hormone indicator. We here report on a quantitative study, comparing the sparrow unit with the capon and the rat units.

Castration causing permanent depigmentation is easily performed in the sparrow. Even in the unoperated male, the bill is light brown outside of the breeding season, as is the bill of the female, with few exceptions, during the whole year. It would considerably facilitate the use of the test if such unoperated specimens should react the same way as the male castrate. This possibility has been thoroughly investigated as well as the question whether the duration of castration has some influence on the color response.

¹ Wood, H. G., Stone, R. W., and Werkman, C. H., *J. Bact.*, 1935, **20**, 84.

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¹ Keck, W. N., *J. Exp. Zool.*, 1934, **67**, 315.

The writer is greatly indebted to Professor F. C. Koch of the University of Chicago, who readily consented to collaborate with us by the supply of 3 samples of standardized male hormone preparations obtained from human urine, namely:

Sample 1—UVC4: 45 BU/cc. (approximately 0.1 cc. = 0.75 r.u.)
 " 2—UVC5: 15 BU/cc. (" 0.1 " = 0.25 r.u.)
 " 3—UVC6: 6 BU/cc. (" 0.1 " = 0.1 r.u.)

According to Koch, the strength of these preparations was standardized directly in "bird units (BU)" (we would prefer the terms *comb* or *capon* unit) while the rat units (r.u.) given represent only a calculated estimation. Table I contains the results obtained by the injection of this material into castrate and non-castrate males and females. Since the experiments were carried out in the month of December and with birds kept indoors for several months, all bills were of the very light brown color indicating absence of male hormone at the time when the injections were started.

TABLE I.

Bird No.	Sex	Time Castrated, mo.	Daily Injection, .1 cc. of prep.	Reaction after		
				5 days	10 days	20 days
450	♂	3.5	UVC4	III	IV	*
434	"	3.5	"	II	IV	•
500	♀	3.5	"	III	IV	IV
440	"	3.5	"	II	III	*
4	♂	—	UVC5	II	III	IV
16	"	—	"	II	III	IV
17	♀	—	"	I	III	III
18	"	—	"	I	II	II
425	♂	18	UVC6	II	III	IV
463	"	3.5	"	II	III	IV
497	"	3.5	"	III	IV	IV
386	♀	3.5	"	0	II	III
429	"	3.5	"	0	III	*

0, no reaction; I, darkened brown; II, light blue; III, dark blue; IV, black; *, killed.

The data presented in the table show that the pigment reaction appears in both sexes, castrates as well as non-castrates. Two of the females (500, 386) were less than one year old but responded as well as the adult ones. However, the present experiment confirms our general experience that the bill of the female sparrow is slower in response and does not give as complete a reaction as the male bill. Some regions, especially the under surface of the lower bill, always stay brown, due to the lack of melanophores. It is truly remarkable that the female bill responds at all in view of the

fact that in the normal life cycle, pigmentation of such extent never occurs. The histological study of the reaction which will be presented in a following paper provides a full explanation of this situation.

Normal males and females of the quiescent sex period (birds No. 4, 16, 17, 18) respond nearly as well as castrates. The sex glands are not activated by the hormone injections and it is not probable that they contribute hormonally in any way to the reaction. Considering, however, that increasing illumination might easily stimulate the testes and thus bring about a pigmentation of the bill interfering with the testing procedure, it seems advisable to make use only of castrate males in the quantitative assay of hormone preparations.

With the exception of one, the castrates listed in the table were injected $3\frac{1}{2}$ months after the operation. Male 425, which had been castrated for 18 months reacted nearly as readily and fully as completely. Keck in his above quoted work had used 14 castrates 6 months after the operation. In these as in other series of experiments, we have never observed a distinct change in sensitivity with the age of castration.

As a rule 10 daily injections give a full color reaction. Discontinuation of treatments at this time is still followed by further darkening of the bill color, due to the movement of the pigment layer toward the surface of the bill. About 20 days after the first injection the pigment reaches the surface. In the third week after the last injection it begins to wear off, the unpigmented layers appearing first as a white ring around the base of the bill. Five weeks after the last administration of the hormone the bill has practically lost all its pigment again.

It is of importance that the reaction in its first stage is not merely quantitative but consists in a characteristic change from light brown to blue. The blue tint is a physical effect, due to the deposit of melanin granules below the cornified strata of the epidermis.

The outcome of the experimental series with Professor Koch's standardized preparations proves the correctness of our previous evaluation of the sparrow unit (SU) as the equivalent of one-tenth (or even less) of the rat unit and approximately one-half of the Chicago capon unit (BU).