

contents of one chamber). There are now two distinct spectra, one of methemoglobin above and one of oxyhemoglobin below.

When a drop of 3% hydrogen peroxide solution is instilled into the methemoglobin solution in the upper chamber, the upper spectrum immediately reverts to that of oxyhemoglobin, identical in intensity and in coincidence of bands with the spectrum of unaltered oxyhemoglobin below.

The passage of illuminating gas through both chambers causes a change of both spectra to that of carbon monoxide hemoglobin and again these bands are identical in position and intensity.

The experiment proves that the compound formed, in this instance, by the reaction of hydrogen peroxide on methemoglobin is actually oxyhemoglobin and not a compound with superficial similarity of absorption bands.

The opinion is advanced that it was a mixture of oxyhemoglobin with the methemoglobin-peroxide compound that led Haurowitz³ to the statement that the reaction product of methemoglobin and peroxide resembled in its spectroscopic appearance NO-hemoglobin.

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The Comb of the Baby Chick as a Test for the Male Sex Hormones.

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This report describes a method of utilizing the 6-day chick in the bio-assay of the male sex hormone, of androsterone particularly. Much of this study was occupied in eliminating pitfalls which obscured assay. Ruzicka¹ first suggested the chick as a test object, utilizing external application to the crest region as described by Fussgänger.² The latter claimed that external application of an extract containing only 1/50 of a capon unit gave a reaction as great as 50 times this amount, given intramuscularly to the capon.

The white leghorn responds best. Chicks younger than 6 days show a high mortality. Ten days' treatment has been found the best.

³ Haurowitz, F., *Z. Physiol. Chem.*, 1931, **198**, 9.

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¹ Ruzicka, M. L., *Bull. Soc. Chimique de France*, 1935, **5**, t. 2, 1497.

² Fussgänger, R., *Mediz. u. chem. Abteil. aus den med. chem. Forschungsstätten der I. G. Farbenindustrie Act. Ges.*, 1934, **2**, 1934.

One-half-inch mesh screen must be used as flooring to automatically eliminate droppings. Controls must be segregated to avoid both contact and the possibility of swallowing droppings. Failure to avoid this mistake is evident in our earlier controls which showed comb reactions. (See Graph S 1, S 2, S 4.) Only "chick starting mash" and plenty of water are required. Crowding must be avoided. Sick and underweight chicks rarely respond well or uniformly. Ten chicks were found to be an adequate number for each group. Sex of chick plays a minimum rôle, although males tend to give a slightly higher response in comb weight.

Suggested technic. From the 6th day on, groups of 10 chicks were treated once daily with given amounts of crystalline androsterone dissolved in 0.1 cc. of sesame oil (the same sample of sesame oil has been used throughout all experiments). The oil was applied by means of a blunt hypodermic needle attached to a tuberculin syringe, 1/20 cc. along the base of each side of the comb. The amount of oil used is so small that it does not spread or soil the chick. The daily treatments were continued for 10 days. The chicks were then killed with chloroform on the following day, the 17th day after birth.

Immediately after death the chick is weighed. The comb is now removed by making bilateral cuts close to and along the base of the comb down to the skull with a sharp knife. The comb is then freed by undercutting directly on the skull. It is weighed to mg. *at once* to avoid loss of weight by drying. Body weight, comb weight, and sex are recorded. The average comb weight of each group of 10 chicks is recorded graphically.

Sesame oil and Progynon B gave no reaction. Testosterone propionate and dihydro-androsterone gave some reactions but no attempt at careful titration was essayed in this study. Male urine extracted by the method of Gallagher, Koch and Dorfman³ gave definite response.

The graphs show high comb weight of the controls in Series 1, 2, and 4, in which no attempt to segregate the controls was made. The opposite applies to Series 3, 5 and 6 in which the controls were carefully isolated.

We are not yet prepared to offer a characteristic curve of response. According to Butenandt⁴ 150-200 γ of crystalline androsterone equals one capon unit. The following examples demonstrate

³ Gallagher, T. F., Koch, F. C., Dorfman, R. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 440.

⁴ Quoted from F. C. Koch, *J. Urol.*, 1936, **35**, 382: "Not until 1933 did he (Butenandt and his associates) obtain enough material to establish its formula correctly as $C_{19}H_{30}O_2$ and its potency as 150-200 γ per capon unit."

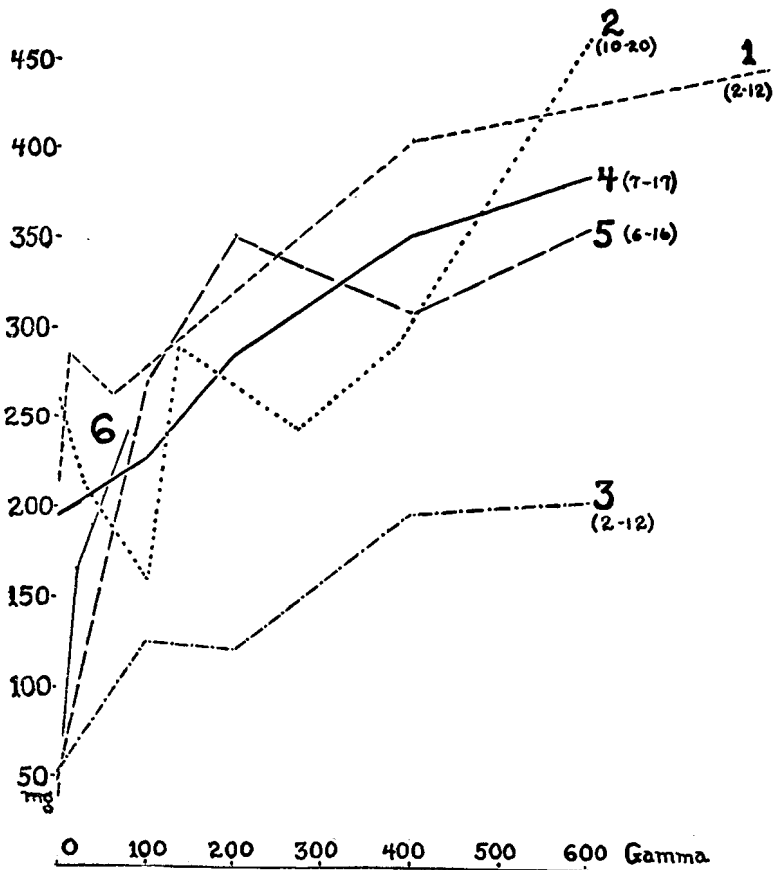


FIG. 1.

the results obtained with this technic: 100 γ androsterone in 10 chicks gave an average comb weight (c.wt.) of 277.1 mg. (heaviest 377, lightest 188); 200 γ androsterone in 10 chicks, average c.wt. 329.7 (heaviest 520, lightest 140). Twenty-seven untreated isolated controls, average c.wt. 49.1 mg. (heaviest 83, lightest 23).

The delicacy of the reaction is evidenced particularly when small dosage is employed (see flattening of ascent above 200 γ of androsterone). Twenty-two untreated controls, average c.wt. 39.3 mg. (heaviest 110 mg., lightest 12 mg.). Ten chicks, total dose 20 γ androsterone (0.1 c.u.) a.c.wt. 164.4 (heaviest 230, lightest 97). Ten chicks, total dose 40 γ androsterone (0.2 c.u.) a.c.wt. 179.5 (heaviest 260, lightest 110). Ten chicks, total dose 80 γ androsterone (0.4 c.u.) a.c.wt. 237.7 (heaviest 380, lightest 170).

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