Color standards of *Brom Cresol Green* were unaffected after several months' exposure in test tubes to usual laboratory conditions, while similar standards of methyl red had faded irregularly and become totally useless. *Brom Cresol Green* may be used directly in a bacteriological culture medium, for instance, while this would be out of the question for methyl red.

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Can fasting fowls synthesize glycocoll or ornithine?

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According to Suga[1] "starving hens (unlike well-fed birds) are unable to synthesize hippuric acid from benzoic acid and glycocoll; they conjugate injested benzoic acid with ornithine into ornithuric acid and excrete the latter compound." This is contrary to other authors, as Thomas[2] found that chickens kept on an inadequate diet could not even produce ornithine for conjugation with benzoic acid. Crowdle aand Sherwin[3] found that hens on a carbohydrate diet were able to synthesize ornithine under these conditions but found no glycocoll compounds; in other studies[4] where chickens were fed toxic organic compounds such as were detoxicated in the animal body by union with glycocoll, no combination with glycocoll was ever found. They concluded that glycocoll was never used by the chicken for detoxication purposes and these results were corroborated by the work of Yoshikawa[5] who fed chickens benzoic acid and glycocoll

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[1] Suga, T., *Kyoto Igaku Zasshi*, 1919, xv, 225; *Jap. Med. Lit.*, 1920, v, 46; *Chem. Abstracts*, 1921, xv. 881.

[2] Thomas, K., *Centralblatt f. Physiol.*, 1914, xxviii, 769.

[3] Crowdle, J. H., and Sherwin, C. P. Results unpublished.

[4] Sherwin, Carl P., and Crowdle, Jas. H., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 318.

[5] Yoshikawa, J., *Zeit. Physiol. Chem.*, 1910, lxviii, 79.

together and found that, in spite of this, benzoic acid was not conjugated with glycocoll but that it was burned in the body of the fowl and the benzoic acid conjugated with ornithine. Unfortunately this experimenter does not state whether his birds were "well fed" nor does he even mention the type of diet. It seemed worth while to follow up this clue regarding glycocoll synthesis in the organism of the fowl and at the same time we thought it might give some valuable hints as to the difference between the metabolism during fasting and while on a normal diet. We decided to place a few hens on a normal complete diet and then feed them benzoic acid in order to determine whether glycocoll might conjugate with the benzoic acid, thus causing the excretion of hippuric acid and then after investigating this point to take up the point of difference between Thomas and Suga regarding the power of the starving hen to furnish ornithine for the detoxication of benzoic acid.

In our work hens were provided with an artificial anus according to the method of Voeltz.[6] It was found unnecessary to provide the chicken with two different receptacles for urine and feces, as the chickens were seldom able to defecate and had to be given enemas both morning and evening. The urine, on the contrary, while usually a white pasty mass adhering to the feces, was excreted in considerable volume—ranging from 200 to 500 c.c. per day. The urine in this case consisted of a white muddy precipitate (uric acid) and a serous-like liquid. The hens were placed in a small metabolism cage provided with a coarse wire screen flooring and drain. The cage was washed at the end of each forty-eight hours with an 0.05 normal solution of sodium hydroxide to remove any traces of organic acids and to wash off, but not dissolve, the uric acid clinging to the parts of the cage. The diluted alkaline urine was then filtered by suction, exactly neutralized and evaporated to dryness at 40° with the aid of an eleectric fan.

It is claimed that the intestinal tract of the chicken may be completely evacuated in the course of six hours; we, however, found that from the nitrogen determinations made on the urine that only after two days of fasting had a stage of endogenous nitrogen metabolism been reached.

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[6] Voeltz, W., *Handbuch d. Biolog. Arbeitsmethoden*, 1922, Abt. IV, Teil 9, Heft II, 300.

At this stage we started feeding benzoic acid and gave each of the three hens one gram of benzoic acid per day for five days, during which time the hens received no food but plenty of water. The evaporated urines were united, acidified with phosphoric acid and extracted with alcohol in a rotatory extracting apparatus. This alcoholic extract was then evaporated to dryness by means of an electric fan and shaken several times with large quantities of ether. The ether extract was allowed to stand in the ice-box for nearly a month, then as no benzoyl-ornithine crystallized out, the ether was distilled off and the brown residue dissolved in hot water. On cooling, crystals appeared in the water solution which resembled those of benzoic acid. The crystals were removed by suction and recrystallized from hot water.

On drying the crystals melted at 120-121°, proving the substance to be unchanged benzoic acid. The amount of benzoic acid thus recovered amounted to 9.5 grams or 63.3 per cent. of the amount fed. The residue from the original alcoholic solution was again extracted but this time with a mixture of seven parts other and three parts alcohol at 30°. This extract was placed in a stoppered flask and kept at 0° and each day sufficient ether added to cut down the percentage of alcohol one per cent. When the percentage of alcohol had been reduced to three per cent., there appeared a granular white precipitate on the bottom of the flask. This substance was redissolved in alcohol and precipitated by the addition of ether, dried at 80° in vacuo and found to melt at 180-184°, but the total yield of the pure substance was slightly more than 0.1 grams. Nitrogen determination by Kjeldahl showed 8.06 per cent. instead of the calculated 8.23 per cent.

"Well fed" hens were also fed 15 grams of benzoic acid, that is, three different hens were given one gram of benzoic acid per day for each of five days. The urines were treated in exactly the same manner as described above.

As a result of this feeding we isolated 3.1 grams of benzoyl-ornithine from the urine of the hens and 8.3 grams of uncombined benzoic acid. Inasmuch as we found no trace of a glycocoll compound it seemed that perhaps hippuric acid had been excreted but had escaped detection. For this reason a hen was fed one gram hippuric acid per day for a period of three days. The urines were evaporated and extracted with the same alcohol ether mixture. From this extract or from the water solution of the residue of this extract, we recovered 2.3 grams of the ingested hippuric acid without difficulty.

It would seem then that starving hens do furnish a very small amount of ornithine when this is necessary for the detoxication of benzoic acid. Contrary to the claims of Suga however, we were unable to find even a trace of hippuric acid in the urine of well fed birds after the feeding of benzoic acid but instead only benzyl ornithine or free benzoic acid and like Yoshikawa we believe that birds are unable to furnish glycocoll for detoxication purposes and even unable to make use of it, if it is furnished them from exogenous sources.

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The experimental production of gall-stones in dogs, in the absence of infection, stasis, and gall bladder influence upon the bile.

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Numerous circumstances and influences which favor the development of gall-stones are now recognized, but uncertainty exists as to which of them are contributory in character and which critical, and as to whether indeed the decisive causes for cholelithiasis are to be found amongst them. In this connection, observations under controlled conditions in animals possess interest.

By a method elsewhere reported,[1] it is possible to join a rubber tube to the common duct of a dog and collect the bile under sterile conditions for months. The gall bladder should be removed at the time of intubation. Our animals thus treated remained in

[1] Rous, Peyton, and McMaster, P. D., *Jour. Exp. Med.*, 1923, xxxvii, 11.