9. The type of percolation is not important.

10. Defatting the drug before percolation is not important.

11. The activity of ergot is extracted more completely by acid alcohol than by neutral alcohol.

12. Higher percentage alcohol is more efficient for percolation of ergot than low percentage alcohol.

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THE TESTING OF ERGOT.

BY HOWARD H. CROSBIE.

In the course of investigating the breakdown rate of liquid preparations of ergot, we have in this laboratory been using all three usual methods of testing, *i. e.*, the Broome-Clarke rabbit uterus method, the Allport-Cocking color reaction, and the Cock's Comb method, with a distressing want of correlation, driving one to the verge of despair. We have experimented with a photographic modification of the Cock's Comb reaction that we think it worth drawing to the attention of other workers.

The method is to photograph the bird, before injection, by means of an appropriate light filter and red sensitive plates so that blue registers as black and red registers as white. The bird is then injected and after $1^1/_4$ hours is again photographed on the same plate, consequently the two photographs get the same development. The resultant prints although not necessarily good pictures of birds do pick up differences that are not visible to the unaided eye.

Before making an assay, one prepares two pairs of reference prints, one pair with a dose of some standard (in this case Ergotoxine ethanesulphonate solution $^1/_2$ mg. per cc.) of such size as to produce a minimum effect as in Fig. 1. Another reference photograph is made of the same bird with a larger dose and more pronounced effect as in Fig. 3. In assaying a sample marked "A" a first trial was made on the assumption that it was probably over-strength and a lesser dose of "A" was given than had been given to the same bird in Fig. 1 with the result shown

in Fig. 2. The dose in Fig. 2 being seven-eights that of No. 1 one can say that had the effect been the same, Sample A would be 114% of the standard, but the effect is more, therefore the strength of "A" is more than 114% of the standard.

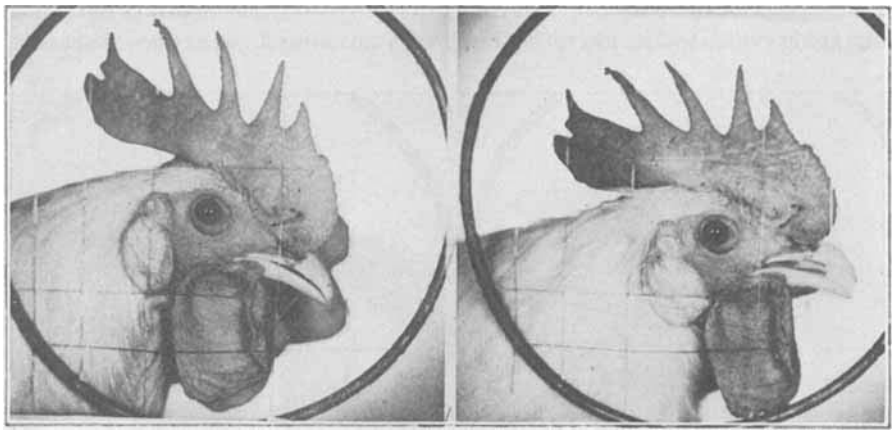

Fig. 1.—*Reference print. Minimum effect* (not visible to the eye). *Left,* Before injection. Animal No. 57. Sample No. E. E. S. Ampul. *Right,* After injection of 1.75 cc.—Sept. 26, 1934.

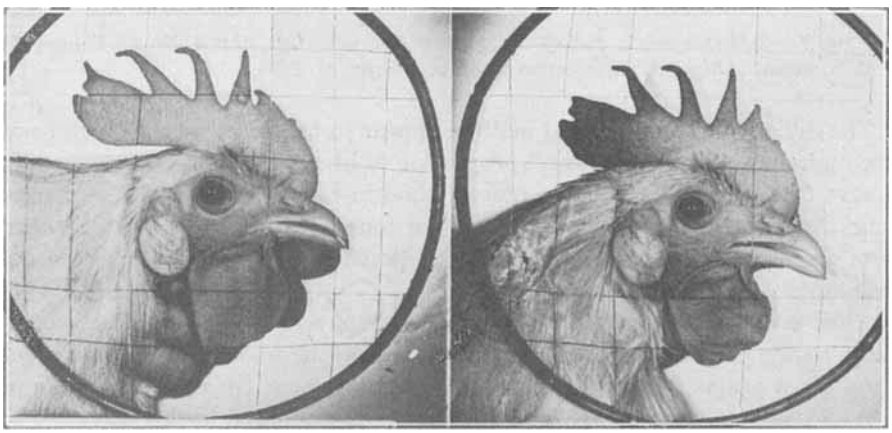

Fig. 2.—*The unknown. Left,* Before injection, Animal No. 57. Sample No. A—*Right,* After injection of 1.75 cc.—Sept. 26, 1934.

When we compare Figs. 2 and 3 we find the ratio of dose 175/250. Had the effect been the same, "A" would be 143% of the standard, but the effect is less, therefore the strength of "A" is less than 143% of the standard.

By taking another bird and administering a dose representing 128% (mean of above limiting values), then comparing the print with the print of this bird's reaction to the standard, one can say whether strength is above or below 128%. There seems to be no difficulty in getting results that can be relied on to an accuracy of within 10%.

A bird whose temper has been ruffled also ruffles its feathers and may give the impression that the exposures are not the same. Prints are best judged by holding at arm's length with partly closed eyes. It is also necessary to concentrate

one's attention on the combs alone and to neglect the human hand which shows black, not being in the spotlight.

Another question may arise, would another bird give the same result? We cannot trespass on the hospitality of the editor to reproduce the prints but we have photographic evidence that four other birds with this sample gave concordant results.

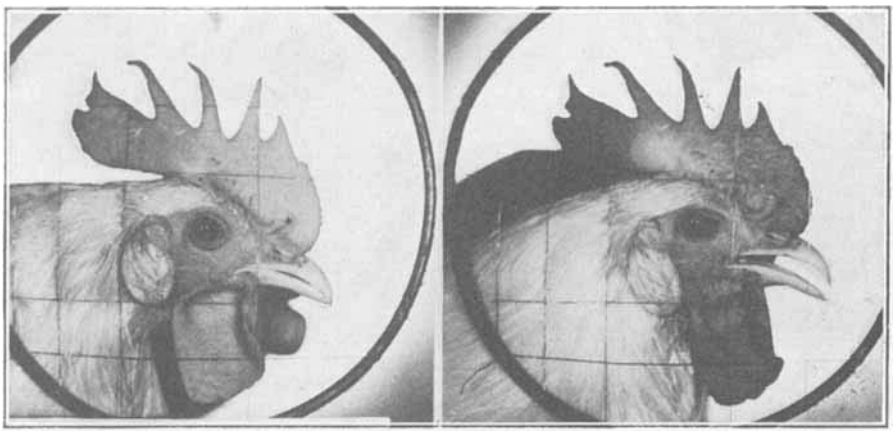

Fig. 3.—*Reference print. Full effect* (visible to the eye). *Left,* Animal No. 57, Sample No. E. E. S. ampul. *Right,* After injection of 2.5 cc.—Sept. 19, 1934.

The difficulties of the official method appear to be chiefly in the lack of power of the human eye to detect a small proportion of blue in the presence of strong red. We have been using this photographic method in routine testing of manufactured batches for a considerable time and we are convinced that much more accurate results are obtained thereby, with the additional advantage of having an easily translatable permanent record.

After taking more than 100 photographs we have come to the conclusion that reliable results can only be obtained by comparing the photograph of the effect of the unknown against the effect of a standard solution *on the same bird.* We have yet to find one pair that contradicts another when compared in this way.

The photographic technique is fairly simple, using an "A" filter and Wratten Panchromatic plates, an exposure of $^1/_{15}$ sec. at f/4.5 is sufficient when using two photoflood lamps.

The function of the ring screen showing in the prints is to avoid the necessity of arranging and refocusing each time, the ring and camera being fixed on a baseboard which also holds the two flood lights which have reflectors to concentrate the beams on the ring screen. This arrangement ensures similar lighting conditions for both exposures.

A repeating back is needed to obtain both images on the same plate and is of the usual type.

A white background is desirable but pointing to the sky is not practical as blue sky registers as black. We find a ground glass screen illuminated from behind gets over this difficulty.

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