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Comparative Effects of Congo Red and Liver Extract on Reticulocytes in Pigeons.

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The remissions produced in pernicious anemia by Congo red[1-4] are of interest, not only therapeutically, but also etiologically as regards the pathogenesis of the disease. Mermod and Dock[2] have used the antianemic action of this dye in support of the view that pernicious anemia is caused by a hemolytic toxin, and suggested that the remissions may be due to adsorption of the toxin on the colloidal surfaces of the dye. They reported that, in normal guinea pigs, intraperitoneal injection of Congo red caused an increase in reticulocytes just as did liver extract. This has been confirmed by Lassen, Jacobsen and Nielsen.[5] However, Wakerlin, Bruner and Kinsman[6] reported recently that Congo red did not increase reticulocytes in pigeons, but liver extract did. Since these authors injected the Congo red intramuscularly, and it is not absorbed readily from the tissues, due to its colloidal properties, and moreover is used clinically by intravenous injection, their report could not be considered final unless it agreed with more crucial tests. The object of this report is to present the results of such tests, that is, repeated intravenous injections of the dye, which guaranteed systemic action, compared with injections of liver extract under similar conditions. In addition, the range of reticulocytic variation was determined in normal pigeons which have been used considerably in bioassays of antipernicious anemia agents, so as to rule out possible errors in determining the reticulocytogenic actions of Congo red.

Healthy grain-fed pigeons were used throughout, each bird being confined in a separate cage. Daily reticulocyte counts were made according to Mermod's[7] method. Blood was obtained by punc-

1 Massa, M., and Zolezzi, G., *Klin. Wchnschr.*, 1935, **14**, 235.

2 Mermod, C., and Dock, W., *Science*, 1935, **82**, 155.

3 Lendvai, J., *Klin. Wchnschr.*, 1936, **15**, 1034.

4 Gualdi, A., *Riv. osp.*, 1936, **26**, 323.

5 Lassen, H., Jacobsen, E., and Nielsen, A., *Acta path. et microbiol. Scandinav.*, 1936, **13**, 543.

6 Wakerlin, G. E., Bruner, H. D., and Kinsman, J. M., *J. Pharm. Exp. Therap.*, 1936, **58**, 1.

7 Mermod, C., *J. Clin. Invest.*, 1936, **15**, 559.

ture of a small leg vein, using not over 0.05 cu. mm. Only those cells were counted as reticulocytes in which the reticulum completely surrounded the nucleus, as was done by Wakerlin, *et al.*[6] Congo red was used as a 1% solution in 5% dextrose solution, boiled for 5 minutes and then filtered through quantitative filter paper. This procedure avoids precipitation (aggregation) of the colloidal dye, which occurs in physiological and other salt solutions. Injections were made into a wing or leg vein. Liver Extract Concentrated—Lilly (N.N.R.) was injected intramuscularly. The effects of the 2 agents were compared by alternate injections in the same birds, and continued injections of Congo red alone in other birds for the continued periods of alternate injections. The injections were always preceded by 3-day periods of control reticulocyte counts.

Distribution of Reticulocytes. One hundred counts of reticulocytes were made in 30 normal pigeons and a distribution curve charted. The mean for the entire series was 7.8%, standard deviation, ±2.39, standard deviation of the mean, ±.239, and the probable error of the mean, ±0.1613. Thus, there was a considerable variation, but for the most part, the reticulocytes were grouped around 8.0%.

Congo Red and Liver Extract. The results on 3 groups of 9 pigeons are presented as curves of averages for the groups in Chart 1. The first group of 3 pigeons received 1.0 cc. of liver extract per kg., intramuscularly, daily for 5 days. There was a prompt rise in reticulocytes, followed by a fall to normal. At this point, 4 daily intravenous injections of Congo red (50 mg. per kg.) were given, total 200 mg. per kg. Daily reticulocyte counts were made until it was certain that no rise occurred. A second group of 3 birds received, after a control period, 4 daily intravenous injections of Congo red (50 mg. per kg.), total 200 mg. per kg., and, when it was apparent that no rise occurred, 5 daily injections of liver extract (1 cc. per kg.) were given intramuscularly. A third group of 3 birds received 4 daily injections of Congo red alone (50 mg. per kg.) intravenously, total 200 mg. per kg., and the counts continued for the same period as that of the other 2 groups.

The curves in Fig. 1 leave no doubt that Congo red was not reticulocytogenic in pigeons, when acting systemically, while liver extract produced the usual prompt rises, which reached a peak in about 6 days after the first injection. Thus, these crucial tests confirmed the claim of Wakerlin, *et al.*

It is worthy of note that the increase in reticulocytes in each bird

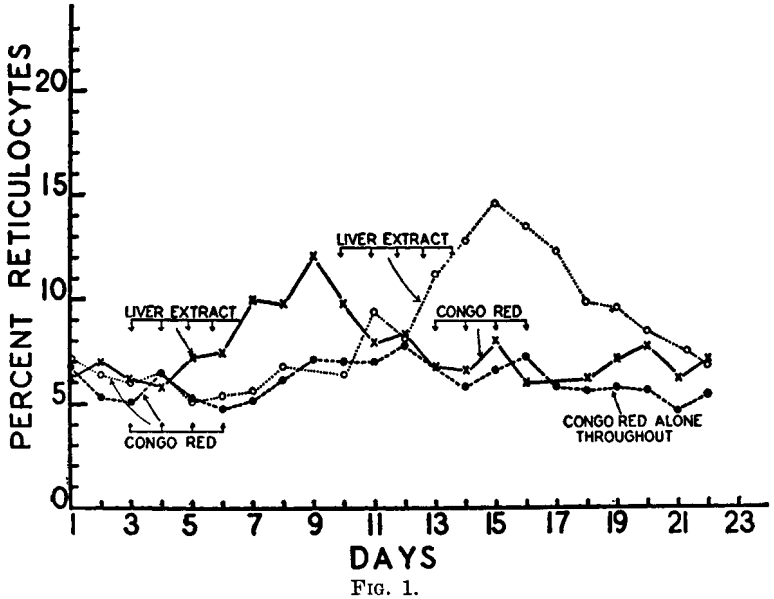

FIG. 1.

Comparative effects of Congo Red intravenously and liver extract concentrated intramuscularly on reticulocytes in normal pigeons.

Doses are given in the text; heavy bars indicate periods of medication in days, and arrows, individual doses of agents injected; each curve represents averages of results in 3 pigeons.

was not higher than that occurring occasionally in normal birds, and although the odds were very high that the response obtained was not due to chance variation, it would appear that the liver may produce only a response of such magnitude as might occur normally. In other words, liver extract may only be a "trigger", which sets off an otherwise normal variation. A number of agents are known to be reticulocytogenic for the pigeon, but ineffective therapeutically in pernicious anemia. Among these are extracts of beef steak[8] and normal urine,[10] and several amino acids.[9] In view of these facts, it is possible that the reticulocytogenic response to an impure material like liver extract may be due to some non-specific substance. Accordingly, the use of normal animals for bioassay of antipernicious anemia agents is unreliable and unsatisfactory.

On the other hand, the negative response of normal pigeons to Congo red does not mean that this dye may not be effective in pernicious anemia. For, if this disease is caused by a hemolytic toxin,

8 Vaughan, J. M., Muller, G. L., and Zetzel, L., *Brit. J. Exp. Med.*, 1936, **11**, 456.

9 Muller, G. L., *New Eng. J. Med.*, 1935, **213**, 1221.

10 Wakerlin, G. E., and Bruner, H. D., *Arch. Int. Med.*, 1936, **57**, 1032.

and Congo red can detoxify the toxin, then evidence of reticulocytogenic action, or of stimulation of bone marrow, could not be expected in a normal animal, where the toxin is not acting. It is possible that the toxin of pernicious anemia is not as readily detoxified by Congo red as are certain poisons and bacterial toxins.[11] The interesting fact remains that Congo red can act beneficially in a pathological state of the blood (pernicious anemia), and obviously non-specifically. Barker's recent negative report[12] is not final without more crucial and exhaustive tests.

Conclusions. 1. The variation of reticulocytes in normal pigeons has been determined. 2. Concentrated liver extract, injected intramuscularly, produced the usual rise in reticulocytes in normal pigeons, while Congo red, injected intravenously in the same and other pigeons for days, did not. This does not mean that Congo red can not act beneficially in pernicious anemia. The difficulties of bioassaying antipernicious anemia agents in normal animals are real and not always appreciated.

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Sensitization of Guinea Pigs to Cyclic Compounds and Effect on the Hematopoietic System.

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In recent years much experimental investigation of agranulocytosis has given rise to the view that "chemicals containing the benzene ring, may so depress the bone marrow that leucopenia results, and bacterial infection of the mouth may develop in consequence."[1] It has been the contention of Madison and Squier[2] that agranulocytosis is the result of drug *hypersensitivity* (to amidopyrine).

Landsteiner and Jacobs,[3] reported sensitization of guinea pigs to simple benzene ring compounds injected into, or spread on the skin of those animals for a number of consecutive days. Among those

11 Hanzlik and Butt, *J. Pharm. Exp. Therap.*, 1928, **33**, 260.

12 Barker, W. H., *Am. J. Med. Sci.*, 1937, **194**, 293.

1 Boyd, W., *The Pathology of Internal Diseases*, 2nd Ed., Lea & Febiger, Phila., 1935, p. 621.

2 Squier, T. L., and Madison, F. W., *Wis. Med. J.*, 1935, **34**, 175.

3 Landsteiner, K., and Jacobs, J., *J. Exp. Med.*, 1935, **61**, 643.