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Effect of Multiple Medication on Hemoglobin Content in Tranquilized Mice

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Effect of Multiple Medication on Hemoglobin Content in Tranquilized Mice¹

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J. AUGUSTINE, and J. HEYNE

Abstract. Varying doses of medication were given tranquilized white mice. The hemoglobin content was measured by means of a Spectronic 20 colorimeter, using the Cannan chemical technique, with percent transmittance from colorimeter reading plotted against concentration grams per hemoglobin per 100 ml. blood of the standard. Comparison of length of medication, dosage, sex of animals, age of animals, and length of time on tranquilizer before medication was started recorded. Included in medication were varying amounts of sex hormones, and effects noted.

MATERIALS AND METHODS

White mice of known ages were given varying amounts of the tranquilizer, reserpine, over a three-week period. Initially, half doses of the different medications were used to give the animal a chance to build up a tolerance to the drug and at the same time to check for toxic effects. After a three-week period, full dosage of medication was given. Finally a dosage of 0/25 mg. of reserpine daily in water was given to three groups of five mice each.

Since surprisingly little information is available on respiratory rate, oxygen consumption and basal metabolism in mice, we were not given the benefit of literature consultation. The little data available in *Spector's Handbook of Biological Data* was of practically no value.

We were interested in the effect of different drugs given with the tranquilizer. The same medications were used as in the studies on respiratory pattern reported in a previous paper (Shell et al., 1967). In addition, we were interested in what effect, if any, might be obtained by use of several hormones given in combination with the tranquilizer and the effect, if any, upon hemoglobin concentration.

Our interest in hemoglobin content was initiated by the high respiratory rate we obtained for Vitamin E in counts per minute.

The apparatus used for determinations was a Spectronic 20 Colorimeter. The principle involved was that when whole blood is diluted with potassium ferricyanide-cyanide solution which oxidized hemoglobin to methemoglobin, and which in turn converted to cyanmethemoglobin, the intensity of the cyanmethemoglobin can be measured photometrically. The procedure was standardized by use of standard solutions of cyanmethemoglobin, available commercially.

Standards were prepared according to the procedure of Cannan in *Clinical Chemistry* (1958), Connerty and Biggs (1962), and percent

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transmittance from the colorimeter reading plotted against the concentration grams per hemoglobin per 100 ml. blood of the standard.

RESULTS

Our results showed a higher percentage of hemoglobin in the blood of females with control Vitamin E, Nicotinamide, Sulfanilamide, Hykenone, Neonal, and Epinephrine, but the excess of the female over the male was very slight with the Sulfanilamide, Neonal, and Nicotinamide. With Vitamin C the male showed the greater percentage of hemoglobin per 100 ml. blood, and this was the only instance where the hemoglobin of the male exceeded that of the female.

Table 1

Hemoglobin Concentration in Tranquilized Mice with Various Medications

Medication	Dosage	Percentage of Males	Hemoglobin Females
Control	0.25 mg.	12.05 g.%	18.10 g.%
Vitamin C	2.40 mg.	17.50	16.15
Vitamin E	1.20 mg.	14.15 g.%	23.07 g.%
Nicotinamide	0.80 mg.	15.68 g.%	16.10
Sulfanilamide	0.80 mg.	13.00	13.60
Hykenone	0.50 mg.	13.70	16.20
Neonal	0.10 mg.	16.27	17.08
Epinephrine	0.15 mg.	11.00	13.80

In the case of Vitamin E, there was a tremendous increase in the amount of hemoglobin in the female. This was not unexpected because of the increase in respiratory rate we noticed in our physiograph determinations.

With the various sex hormones given in combination with reserpine the greater percentage of hemoglobin again was found in the female, except when progesterone was given. Altman and Russell (1961) has reported an erythropoietic effect of small doses of estrogenic substances. Although Jacobson and Doyle (1962) reported that leukemia could be induced with prolonged administration of estrogens, we did not notice anything of this nature in our determinations.

Table 2

Hemoglobin Concentration in Reserpine Treated Mice with Hormone Medication

Medication	Dosage	Hemoglobin in Gram Percent	
		Males	Females
Sandril	0.25 mg.	12.05	18.10
Testosterone	0.005 mg.	13.00	16.27
Theelin	0.70 I.U.	15.07	15.97
Progesterone	0.0015 mg.	13.73	11.86
Estrogenic Substance	1.5 I.U.	13.26	13.80

A study of the morphology of normal mouse blood using smears stained with Wright's stain was made. (The red blood cells appeared to have the normal biconcave shape, but showed a moderate amount

of polychromasia in all of the blood smears made.) Small clumps of platelets were seen in even distribution in all blood smears. Where polychromasia is abnormal in the human, it appears to be normal in the mouse.

In an effort to explain the high respiration rate and hemoglobin content in Vitamin E treatment, a regular red blood count was made. Normal red count with no medication was 1,500,000 per cubic centimeter, while in the case of the females treated with the Vitamin E, the value obtained was 2,300,000/cc. In the Vitamin E treated mice, blood cell morphology was the same as normal blood except there appeared to be a decrease in the amount of polychromasia.

Filmanowicz, working about the same time as Gurney (1966), reported erythropoiesis with Vitamin E as Gurney had noticed with estrogenic substances.

CONCLUSIONS

We concluded from our experiments that with the exception of Vitamin E, which appreciably raised the hemoglobin level of the female, and Vitamin C, which raised the level of the male, reversing the trend from female to male completely, no appreciable differences were noted with our medication. Lacking any literature to compare our results with previous work, we were not able to draw as many conclusions as might have been made otherwise.

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