An intermediate transfer in this way between chromatographic runs with different solvents has certain advantages over two-dimensional paper chromatography. In the usual technique, at the end of the first development the spots have increased in size and they become still more diffuse during the second run. By the transfer technique the original spot size is recovered. Also, separate treatment of the individual spots makes it feasible to use different solvents for different components in the second run.

Reference

1. WYATT, G. R. *Biochem. J.*, **48**, 581 (1951).

Manuscript received December 11, 1952.

Comparative Effects of Total Body and Tail Heating on the Peripheral Leukocyte Count of the Rat[1]

B. H. Ershoff and J. G. Gaines

Emory W. Thurston Laboratories, Los Angeles

A reduction in the peripheral lymphocyte count has been widely employed as an index of adrenocortical activation in the rat. It is becoming increasingly apparent, however, that a number of factors other than the amount of circulating corticoids may alter the peripheral lymphocyte count and hence may affect the validity of this measurement as an index of adrenocortical activity. Marked variations have been reported as to what constitutes a "normal" leukocyte count in the peripheral blood of the rat (*1–6*). Such factors as whether the blood specimen was taken from an anesthetized or unanesthetized rat, the type of anesthetic employed, varying techniques in restraining the animal and in obtaining the blood specimen, all may affect the peripheral leukocyte count. Data are presented on the comparative effects of total body versus localized tail heating (when employed as experimental procedures in obtaining the blood specimen) on the peripheral leukocyte count of the rat.

The animals employed in the experiment were female rats of the Long-Evans strain. Animals were placed at weaning on the following diets, and blood counts were taken when rats had attained the weights indicated in Table 1. Two experimental rations were employed: diet A and diet B. Diet A was a natural food ration;[2] diet B was a purified ration of the following composition: sucrose, 61%; casein,[3] 24%; salt mixture,[4] 5%; cottonseed oil (Wesson), 8%; and wheat-germ oil (Vio Bin), 2%. To each kg of the above diet were added the following synthetic vitamins: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; nicotinic acid, 60 mg; calcium pantothenate, 60 mg; biotin, 5 mg; 2-methylnaphthoquinone, 5 mg; folic acid, 10 mg; ascorbic acid, 100 mg; *p*-aminobenzoic acid, 400 mg; inositol, 800 mg; vitamin B_{12}, 100 μg; and choline chloride, 2 g. To each kg of diet were also added 6000 USP units of vitamin A[5] and 600 USP units of vitamin D[6]. The vitamins were added in place of an equal amount of sucrose.

When taking the blood specimens, animals were divided into 2 groups. Half the animals had blood specimens taken following localized tail heating; the remainder after total body heating. The procedures differed as follows: in the localized tail-heating group, unanesthetized rats were wrapped in a towel with only the tail protruding. Free-flowing blood was collected from the tail after warming it in hot water and snipping a bit from the end with scissors or scalpel. Total body heating was administered by placing rats in small metal cages with screen sides and bottom, before an electric heater for a period of 20–30 min. The procedures employed in taking the blood specimens in

TABLE 1

COMPARATIVE EFFECTS OF TOTAL BODY HEATING AND TAIL HEATING ON THE PERIPHERAL BLOOD COUNT OF THE RAT

Group	Number of animals	Body wt. (g)	RBC		Total WBC		Lymphocytes	Granulocytes	
			Av. (in millions)	Range	Av.	Range		%	Total
Diet A									
Tail heating	12	113	7.5	(7.0–8.1)	9,740	(7,750–13,600)	7,987	18	1,753
Total body heating	12	107	6.9	(6.1–7.9)	5,462	(2,700– 8,250)	3,933	28	1,529
Diet B									
Tail heating	20	166	7.4	(6.9–8.1)	10,266	(8,150–14,400)	8,315	19	1,951
Total body heating	20	164	7.3	(7.0–8.1)	6,143	(3,150– 8,200)	4,546	26	1,597
Diet B									
Tail heating	8	114	6.6	(6.0–7.1)	10,112	(8,900–11,300)	8,191	19	1,921
Total body heating	8	114	5.9	(5.3–6.7)	4,625	(4,200– 6,000)	3,654	21	971

Data for RBC, total WBC, total granulocytes, and lymphocytes are expressed as cells/mm³.

[1] This study was supported in part by the Office of Naval Research.

[2] Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo., supplemented twice weekly with lettuce.

[3] Vitamin-Free Test Casein, General Biochemicals, Chagrin Falls, Ohio.

[4] Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, Ohio.

[5] Myva-Dry Powder, Distillation Products, Rochester, N. Y.

[6] Hy-Dee Powder, Standard Brands, New York, N. Y.

this group were the same as in the first series, except that localized tail heating was omitted. Erythrocyte and leukocyte counts were made with the usual pipets and diluting fluids; differential counts were made on smears stained with Wright's stain, 100 cells on each of 2 slides being employed for each analysis. Results are summarized in Table 1.

A significant difference was observed in the total WBC and lymphocyte count of the peripheral blood of rats, when blood specimens were taken following total body heating, as distinct from localized tail heating. In the latter group, the data for total WBC and lymphocytes in peripheral blood were comparable to those reported by other investigators for the rat (*2*); animals subjected to total body heating, however, had a significant reduction in the total WBC and lymphocyte count. The reduction was apparently not due to hemodilution, since little, if any, decrease occurred in the erythrocyte count. A reduction was also noted in the number of granulocytes per cubic mm of peripheral blood following total body heating. This decrease, however, was less marked than the reduction in lymphocyte count. Similar findings were obtained on both experimental rations. Determinations were also made of the leukocyte count of peripheral blood after localized tail heating and 20 min later on the same animals after total body heating. A reduction in the peripheral leukocyte count after total body heating was observed in these animals of the same order of magnitude as that reported above.

It has been established that the white cell count of peripheral blood is significantly greater than that of heart blood (*5–8*). This condition is caused, according to Quimby and Goff (*6*), by the "damming up" of white cells in the peripheral areas of the vascular system as a result of the normal resistance of the arterioles and capillaries to the flow of the blood. When rats were anesthetized with ether, the difference in white cell count between heart and peripheral blood was abolished, presumably because "ether and other substances which relax the contractile elements of the blood vessels and increase their lumen diameter reduce the resistance offered by the peripheral vascular bed to the flow of blood cells and result in a more even distribution of the leukocytes between the large and small vessels" (*6*). In the present experiment, total body heating similarly resulted in a significant reduction in the leukocyte count of peripheral blood to values comparable to that of heart blood (*5–6*). It would seem likely that an increase in circulation rate and small vessel dilatation (*9–11*) resulting from total body heating were responsible, at least in part, for the observed effects.

Total body heating has the following advantages over tail heating as an experimental procedure for obtaining blood specimens in the rat: (1) less handling of the animal is required in this procedure, since localized warming of the tail is omitted, and as a result, blood specimens may be obtained more quickly (once the animal is wrapped in the towel) with a minimum of restraint to the animal; (2) alterations in the blood count due to the stressor effects of warming the tail in hot water and the longer period of restraint in animals subjected to localized tail heating are minimized when total body heating is employed (this advantage would be particularly marked when multiple counts are made over an extended period of time); (3) the flow of blood on severing the tip of the tail, after total body heating, is more profuse than that generally obtained after localized tail heating. Total body heating would appear to have particular value, therefore, in obtaining blood specimens from nutritionally deficient or "toxic" animals from whom adequate amounts of free-flowing tail blood are difficult to obtain following localized tail heating.

References

1. DONALDSON, H. H. *The Rat.* Memoirs of the Wistar Institute, Philadelphia (1924).
2. SCARBOROUGH, R. A. *Yale J. Biol. & Med.*, **3**, 267 (1931).
3. JORDAN, H. E. *Handbook of Hematology.* Vol. II, 703. H. Downey, Editor. New York: Hoeber (1938).
4. CRESKOFF, A. J., FITZHUGH, T., and FARRIS, E. J. In: Griffith and Farris: *The Rat in Laboratory Investigation,* 351. Philadelphia (1942).
5. QUIMBY, F. H., SAXON, P. A., and GOFF, L. G. *Science,* **107**, 447 (1948).
6. QUIMBY, F. H., and GOFF, L. G. *Am. J. Physiol.*, **170**, 196 (1952).
7. KLIENEBERGER, C., and CARL, W. *Die Blut-Morphologie der Laboratoriums-Tiere.* Leipzig: Barth (1912).
8. ROOFE, P. G., LATIMER, H. B., MADISON, M., MAFFET, M., and WILKINSON, P. *Science,* **111**, 337 (1950).
9. GOODMAN, L., and GILMAN, A. *The Pharmacological Basis of Therapeutics,* 67. New York: Macmillan (1941).
10. MANN, F. C., ESSEX, H. E., HERRICK, J. F., and BALDES, E. J. *West. J. Surg.*, **43**, 177 (1935).
11. FOREGGER, R. *Anesthesiology,* **4**, 392 (1943).

Manuscript received November 13, 1952.

Estrogen Excretion in Women with Mammary Cancer before and after Adrenalectomy[1]

Thomas L-Y. Dao

Ben May Laboratory for Cancer Research, University of Chicago, Chicago

This paper presents data on the excretion of estrogen in the urine of women with cancer of the breast, before and after both adrenal glands were excised. Estrogens are known to be importantly involved in the genesis of mammary neoplasms in mice (*1*). It is known (*2*) that ovariectomy sometimes causes a remission of this disease in women. It has been further established that bilateral adrenalectomy (*3*), with maintenance on cortisone acetate, causes a significant regression of mammary cancer in ovariectomized women in certain cases.

It has been shown (*4*) that estrogen is produced in the adrenals of certain strains of mice in which the gonads were excised in early life. Also, estrone has

[1] This investigation was supported by grants from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council and from the Jane Coffin Childs Memorial Fund for Medical Research.