followed, except that the amount of pancreatin was increased to 500 mg to provide a rapid rate of hydrolysis. From 20 g of interior bread crumb, 1220 ml of gas (± 2%) were obtained regardless of the age of the bread (up to 1 week at 30° C). Thus, the *in vitro* data are in agreement with *in vivo* data (*11, 12*) indicating that the extent of digestion of the starch in bread is not influenced by the age of the bread.

Further studies are underway concerned with the nature of the changes in the starch component accompanying the staling of bread, and with the nature of the mechanism involved in the favorable action of bacterial α-amylase in preventing or retarding these changes.

References

1. SCHULTZ, A. S., *et al. Cereal Chem.*, **29**, 200 (1952).
2. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. *Ibid.* **16**, 712 (1939).
3. JACKEL, S. S., *et al. Ibid.*, **29**, 190 (1952).
4. GEDDES, W. F., and BICE, C. W. *Q.M.C. 17–10.* War Department. Office of the Quartermaster General, Washington, D. C. (1946).
5. KERR, R. W. *Chemistry and Industry of Starch,* 2nd ed. New York: Academic Press (1950).
6. WHISTLER, R. L. *Purdue Univ. Agr. Expt. Sta. Rept.* File No. C-205 (1947).
7. ROSTORFER, H. H., KOCHAKIAN, C. D., and MURLIN, J. R. *J. Nutrition*, **26**, 123 (1943).
8. BARTELS, L. *Mehl u. Brot.*, **42**, 21, 41 (1943).
9. HABS, H., and PLAGEMANN, L. *Deut. Z. Verdauungs-u. Stoffwechselkrankh.*, **7**, 129 (1943).
10. HEUPKE, W. *Münch. med. Wochschr.*, **89**, 128 (1942).
11. HABS, H. *Deut. Z. Verdauungs-u. Stoffwechselkrankh.*, **7**, 201 (1943).
12. ABELIN, I., and BIDERBOST, A. *Biochem. Z.*, **247**, 429 (1932).

Manuscript received December 8, 1952.

A Simple Method for Making Transfers in Paper Chromatography

A. M. Moore and Joyce B. Boylen

Biology Division, Atomic Energy of Canada, Ltd., Chalk River, Ontario, Canada

A very efficient technique for elution of water-soluble compounds from paper chromatograms is that described by Wyatt (*1*). In this procedure the strip of paper to be extracted, cut to a point at one end, is developed as a descending chromatogram with water which is allowed to drip from the pointed end of the strip into a receiver. Compounds which travel with the front may be eluted in a few drops of water. We have been using a modification of this technique to transfer single components to another chromatographic strip for development with a different solvent. Transfer of the eluate in the ordinary way, after evaporation, is complicated by the requirement of keeping the size of the initial spot on the new chromatogram as small as possible. This necessitates performing the transfer in portions of a few microliters at a time and letting each portion dry before the next is applied. As numerous washings are required, this procedure may be very time-consuming.

It has been found possible to perform the entire

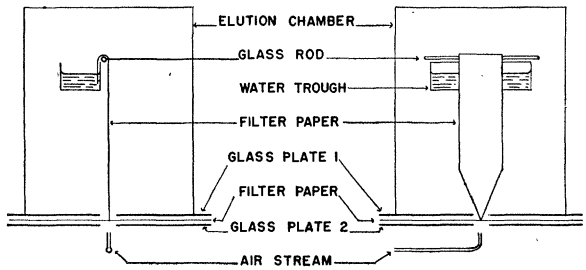

FIG. 1. Sketch of apparatus used for single stage transfer between chromatographic runs.

transfer as a continuous process by using the arrangement shown in Fig. 1. The strip of filter paper to which the transfer is to be made is sandwiched between 2 sheets of glass or plastic in which are matching round holes about 1¼ in. in diameter. The elution is performed in a small chamber resting upon these sheets so that the air in the elution chamber can be kept nearly saturated with water vapor—an essential condition if the elution is to proceed properly. The original strip is suspended from the developing trough so that its pointed tip just touches the new strip in the center of the round window. When the water front reaches this point and begins to spread on the new strip, a stream of air at room temperature, directed against the under side of the paper window, is sufficient to evaporate the eluate as rapidly as it flows. In this way, the spot size may be kept as small as is desired by regulating the air flow, and the time required for the transfer is just the time required for the elution.

Since the water flow is not observable under these conditions, the time necessary to complete the elution must be determined indirectly. The method we find most convenient is as follows. A 10-cm length of the strip (A) to be eluted is marked off in 0.5-cm divisions with lead pencil. The elution is then begun. When the water front reaches the tip of strip A and begins to spread on the new strip (B) a colored compound of known R_f value is applied at the graduation farthest from the tip of A. The subsequent travel of such a marker is linear with time during the evaporation. The R_f of the marker and the distance it has moved being known, together with the water-loading and the width of strip A, the volume of water which has been evaporated at any time may be calculated. If its R_f is sufficiently small, the indicator itself will not be eluted in the time it takes to evaporate the required 0.5 ml of water. The water-loading is determined by weighing strips of known area under similar conditions. We find about 12 mg of water/cm² in a wet strip of Whatman No. 1 paper during an elution.

As a marker an orange-colored indelible pencil (Mongol No. 962, Eberhard Faber Co.) is very convenient, since it may be inserted through a small hole in the lid of the elution chamber without altering the water saturation of the enclosed air, and the R_f of the orange dye has a suitable value—about 0.52 in our experiments.

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.