of the *A. oryzae* transglucosidase (*7*) and the enzyme involved in these reactions. In the former, glucose units of maltose are transferred to the cosubstrates glucose, maltose, isomaltose, and panose, whereas in the latter galactose units of lactose are transferred to the cosubstrates glucose, galactose, lactose, and Compound I. Radioactive cosubstrates have been used to substantiate the proposed transgalactosidic mechanism of action of the new enzyme. Thus C^{14}-glucose included in the digest appeared in Compounds I and IV, and C^{14}-galactose appeared in Compound II. These studies are being continued in our laboratories.

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Manuscript received August 20, 1952.

Chromatographic Separation of Estrone, Estradiol, and Estriol

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The estrogens appear to be present in human and cattle blood in extremely small concentrations, of the order of 3–8 μg/l whole blood (*1, 2*). The determination of the three natural estrogens in blood requires quantitative methods for their separation and estimation. The object of this report is to present a method suitable for routine use for the quantitative separation of estrone, estradiol, and estriol at this microlevel. The following work has been carried out with pure crystalline estrogens, as a preliminary to the application of the method to the estrogens in blood.

Partition chromatography has been used for the separation of closely related compounds that show a difference in partition coefficients (*3*). Recently a method for the separation of estrone and estradiol by partition chromatography was reported (*4*). A NaOH solution adsorbed on Celite forms the stationary phase, and benzene the moving phase. A similar system, also using NaOH-Celite and benzene, was used earlier by another group (*5*) for the separation of certain estrogen isomers. The method proposed by the British workers proved to be more effective in our hands in accomplishing the estrone-estradiol separation.

It seemed valuable from the standpoint of the number of manipulations, time, and magnitude of losses, to effect a separation of all three estrogens on one column in one operation. Because of the more hydrophilic character of estriol, as compared with estrone and estradiol, it is not eluted from the NaOH-Celite phase by benzene. It was recognized that a more favorable partition system was necessary for the separation of estriol. This can be achieved by two methods: a change in the moving phase, and/or a change in the stationary phase. A separation of all three estrogens has recently been reported by the British group (*6*), utilizing a change in the moving phase to accomplish the separation of estriol. The eluent used was chloroform-butanol (3 : 1). This procedure, or the use of acetone, ethylene dichloride, or methanol as eluents, did not prove as satisfactory as the method reported below, because of the elution of a large amount of butanol-soluble material in the estriol fraction. Accordingly, the second approach, a change in the nature of the stationary phase, has been used in the technique developed in our laboratory. It was discovered that estriol could be eluted by benzene from a less alkaline stationary phase consisting of Celite to which water rather than NaOH had been added. It occurred to us that after use of the NaOH-Celite column to separate estrone and estradiol, it could be changed to the less alkaline water-Celite or $NaHCO_3$-Celite column by reaction with gaseous HCl or CO_2. Estriol is then readily eluted by benzene from these systems. These modifications were carried out successfully, and, because of the greater safety in handling CO_2, it has been used routinely to modify the partition characteristics of the column.

The chromatographic conditions of Swyer and Braunsberg (*4*) were used, and the columns were prepared according to the careful description of Haenni, Carol, and Banes (*7*) for preparations of this type. A West-type straight condenser (ID, 10.8 mm), with stopcock at the reciving end, was employed as a chromatographic tube. This allowed the temperature of the column to be controlled at 24° ± 2° C by circulation of water of the appropriate temperature through the condenser. The column is filled with benzene (CP benzene-thiophene–free is shaken 6 times with sulfuric acid and redistilled), and fine glass wool (Fiberglas, Corning) is packed into the constriction above the stopcock. Three g Celite 535 (Johns-Manville) and 25 ml benzene in a small beaker are thoroughly mixed with 2.4 ml 2.3 *N* NaOH for several minutes. The stopcock is opened enough to permit slow drainge, and the Celite mixture is transferred to the tube in small portions, with a spatula. A flocculent suspension is formed by slowly working the packing rod (a glass rod flattened at one end to a circular head with a clearance of 1.0 mm in the adsorption tube) up and down as a piston through the Celite mixture. The Celite is then gently compressed with the rod to form a uniform pack with a sharply defined level surface. The mixture fills the tube to a height of 125 mm ± 5 mm.

Suitable aliquots (containing 2–10 γ) of solutions

of crystalline estrone,[1] estradiol,[1] and estriol[2] in alcohol-toluene (1 : 19) are transferred to a small round-bottom flask, and the solvent is evaporated to dryness *in vacuo* at room temperature. The residue is dissolved in a small volume (1–2 ml) of benzene and discharged onto the column just as the benzene drains below the top surface of the Celite. The transfer is completed by repeating with an additional portion of benzene. The tube is then filled with benzene and the stopcock adjusted so that a flow rate of 2.5–3.0 ml/min is attained.

Estrone exhibits the greatest rate of elution and appears in the eluate after a forerun of 30 ml of benzene (Fraction I). It is completely recovered in the following 80 ml of benzene (Fraction II). A clear zone of 10 ml of benzene (Fraction III) follows before the appearance of estradiol. An additional 90 ml of benzene (Fraction IV) is sufficient to elute the diol. This elution can be completed in a few minutes by applying N_2 under pressure to the column. Estriol cannot be eluted from the NaOH-Celite system by benzene, and the system is therefore modified to permit its recovery. Gaseous CO_2 is bubbled up through the chromatographic column at the rate of 30 cc/min for a period of 5 min; 100 ml of benzene (Fraction V) then elutes the estriol. The elution of estriol can also be facilitated by the use of N_2 or CO_2 under pressure to force the benzene through the column. The effluents are collected in graduated cylinders, transferred to round-bottom flasks, and evaporated to dryness under reduced pressure at bath temperatures not exceeding 50° C.

The residues are dissolved in a measured volume of ethyl ether, (purified daily by shaking with ferrous sulfate and redistilling), and an aliquot is transferred to a fluorometer tube for analysis. The ether can be evaporated to dryness in 15–20 sec on a warm (60° C) water bath, and the ether vapors exclude air from the tube during evaporation. This step avoids oxidative losses which occur when alcohol, the usual diluent in steroid procedures, is employed with or without the use of a stream of N_2 (*8*). Two tenths ml alcohol-toluene (1 : 19) is added to the residue in the test tube, and the estrogens are analyzed by the fluorimetric procedure of Engel *et al.* (*9*). The fluorescence is measured in a Colemen Model 12 photofluorometer with a lamp filter transmitting at 436 mμ (Corning #3389 and Corning #5113 glass filters) and photocell filter transmitting at 525 mμ (Baird 525 mμ interference filter + Corning #3385 glass filter). Standards were run in each fluorometric analysis. Estrone, estradiol, and estriol are the standards of comparison for the fluorescence exhibited by Fractions II, IV, and V, respectively.

The entire collection period requires either 1.0 or 2.0 hr, depending on whether pressure or gravity flow is used in the elution of estradiol and estriol; preparation of the column, 0.5 hr; fluorimetry, 0.5 hr; and evaporation of the benzene from the eluates, 0.5–0.75 hr. Thus, in either 3 or 4 hr, a quantitative separation and estimation of microquantities of estrone, estradiol, and estriol can be accomplished.

TABLE 1

PARTITION CHROMATOGRAPHY OF ESTROGENS ON NaOH-CELITE AND $NaHCO_3$-CELITE COLUMNS

Expt.	No. of trials	γ of estrogen chromatogrammed			Av. % recovery of estrogen in benzene fraction				
		Estrone	Estradiol	Estriol	I (30 ml)	II (80 ml)	III (10 ml)	IV (90 ml)	V* (100 ml)
A	7	10	0	0	0	99.7	—	—	—
B	6	0	10	0	0	0	0	99.4	—
C	3	0	0	10	0	0	0	0	99.9
D	1	10	10	0	0	90.5	0	102.0	—
E	1	5	5	0	0	113.0	0	103.0	—
F	5	2	2	0	0	104.0	0	95.5	—
G	2	5	5	5	0	97.1	0	95.5	99.8
H	6	2	2	2	0	93.4	0	91.6	102.1
			Mean			98.77		96.17	101.07
			Standard deviation			± 7.52		± 8.02	± 7.86
			Range			84.5–113.0		82.5–110.0	89.3–112.5

* Fraction V represents the eluent from the modified NaOH-Celite column.

Table 1 contains a summary of the results obtained when 2–10 γ amounts of estrone, estradiol, and estriol were chromatographed separately and together. It is apparent that the NaOH-Celite system is adequate for the separation of estrone from estradiol (Expts. A, B, D, E, F). The presence of a forerun and a distinct intermediate "clear" zone between the two estrogens should be a valuable aid in purification. Expt. C demonstrates that estriol is not eluted from the NaOH-Celite column by benzene. After modification to a $NaHCO_3$-Celite column with CO_2, the estriol is readily recovered in the benzene effluent. Expts. G and H illustrate the separation of 2 and 5 γ quantities of the three estrogens. The over-all accuracy appears satisfactory as judged by the means and standard deviations of the recovery of estrone, estradiol, and estriol.

The modified partition chromatography method described here quantitatively separates 2–10 γ amounts

[1] Estrone and β-estradiol were generously supplied by Edward Henderson, of the Schering Corporation.

[2] Estriol from Parke, Davis & Company.

of the three estrogens. The partition column is of small size and is simple, rapid, and uncomplicated in operation. The application of this method to the determination of the microquantities of estrogens present in blood is now in progress.

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Manuscript received August 18, 1952.

Induced Emigrations Among Small Mammals

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This study of the effect of intensive trapping over a large area upon the members of the surrounding population consists of two experiments. One of them was conducted on Mount Desert Island, Me., during the summer of 1950; the second, in the Huntington Wildlife Forest near Newcombe, N. Y., during the summer and fall of 1951. The predominant genera of mammals inhabiting these forested areas are *Peromyscus, Clethrionomys, Blarina,* and *Sorex,* which may be considered as a biological unit designated as "small mammals."

The population was reduced by the rapid removal of individual animals from the study areas by operating groups of trap lines. Each trap line consisted of 20 stations 50 feet apart, with 3 traps at each station. The several trap lines in each location were distributed so that they formed a rough rectangle. In each case the distribution was such that the population resident on 40–80 acres was reduced nearly 60% during the first 3 days of trapping. On the Maine location, 8 trap lines were used, making a total of 480 traps set each night. The term "trap night" will be used to indicate one trap set one night. On the Maine location trapping was run for 15 consecutive days with a terminal 3-day period of trapping following a 5-day interval. On the New York location, four trap lines were set during the first 10 days. Two were placed in the form of a cross on each of the opposite corners of a quarter-section. From the 11th through the 33rd day, 5.3 trap lines within and at the other two corners of the quarter-section were added. Although this procedure increased the area of sampling,

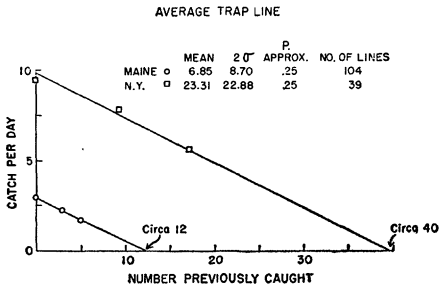

FIG. 1. Average trap lines for Maine and New York. Data for Maine cover 104 trap lines run during 1949, 1950, and 1951. Data for New York cover 39 trap lines run during 1951. Were the approximate probability of capture ($P = .25$) to continue on later days without invasion, the resident population exposed to an average trap line would be indicated by the intersection of the abscissa by the lines drawn through the first 3 days' captures.

it did not appear to alter the trend of the results.

The study on the Maine location was conducted by A. Dexter Hinckley under the supervision of John B. Calhoun. The study on the New York location was conducted by William L. Webb and Earl F. Patric.

In both locations large numbers of trap lines have been run at other sites within the same general habitat, and the resultant data (Fig. 1) reveal the general effect that this method of trapping has upon the resident population. The population at the Maine location was less than one third that in New York. The t test of the significance of the difference between the mean population densities of the two areas has a probability of less than .001. In both areas, the trend of decrease through the first 3 days was such as to indicate an approximate probability of capture of 0.25—i.e., on the initial day 25% of the population is removed, and on each succeeding day 25% of those remaining is removed. Were there no invasion

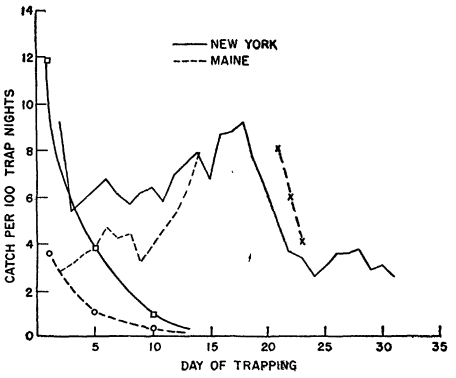

FIG. 2. Observed and expected sequences of captures in Maine and New York arising from continuous trapping beyond 3 days. The left-hand exponential curves are approximated expected curves had there been no invasion of the trapped area, where the catch for the first 3 days is 58% of the residents, and where the probability of capture is 0.25. The observed curves are 3-day moving averages, with the exception of the last 3 days for Maine, which are the observed captures. There was no trapping in Maine on days 16 through 20. The 425 animals taken in Maine and the 855 taken in New York are shown in terms of catch per 100 trap nights for the sake of a better comparison.