mammals to the dormant state. During estivation the heart beats very slowly, with a comparable decrease in the rate of blood flow, and hence any mechanism to prevent the formation of a thrombosis during the inactive state would be of considerable survival value to the animal. In the active state a hemophilic condition of the blood would, on the contrary, be a distinct detriment, for any slight injury involving bleeding could lead to death. In estivation, since the animals are buried underground in their nests, injuries are very unlikely.

The various factors involved in this prolongation of clotting time are being studied.

References

1. CARTLAND, F. F., and KOCH, F. C. *Am. J. Physiol.*, **85**, 540 (1928).
2. KEITH, N. M., ROWNTREE, L. G., and GERAGHTY, J. T. *Arch. Internal Med.*, **16**, 547 (1915).

Reversed Phase Partition Chromatography of Steroids on Silicone-treated Paper[1]

Theodore H. Kritchevsky[2] and Arne Tiselius

Biokemiska Institutionen, Uppsala, Sweden

The conventional procedure of paper partition chromatography, in which the paper holds water as a stationary phase, is not satisfactory when applied to a class of compounds such as the steroids, which have limited water solubility. This difficulty can be circumvented in a number of ways.

One solution would consist of preparing derivatives having greater water solubility. Zaffaroni and co-workers (*1*) prepared the Girard T derivatives of a large number of steroid ketones, and determined their R_F values on filter paper using a water-butanol partition system. Their procedure, although ingenious, does not give satisfactory separations. Other difficulties involve the instability of the Girard T derivatives and also the inconvenience entailed in their preparation.

A second approach would concern itself with a modification of the partition coefficient in favor of the polar, stationary phase, by incorporating a polar phase other than water in the paper support.

An example of this solution can be found in another method developed by Zaffaroni and co-workers (*2*) for the separation of the more water-soluble adrenal cortical steroids on paper. These investigators impregnated filter paper with either formamide or propylene glycol, which served as the stationary, polar phase, and used benzene or toluene, respectively, as the mobile, nonpolar phase.

A third approach, which has been suggested for the chromatography of compounds whose partition coefficients are greatly in favor of the mobile, nonpolar phase, consists of reversing the two phases. In other words, the supporting substance, paper, silica, kieselguhr, or glass, is treated in such a manner as to hold the nonpolar phase stationary. The more polar phase is then employed as the moving one. Within the past few years a variety of procedures has been described for the production of reversed phase systems and for application of the reversed phase technique (*3–7*).

The partition chromatogram is most effective when the compounds to be separated have R_F values in the range 0.3–0.5. We believed that the application of the reversed phase technique to the problem of separating the less polar steroids would aid in the realization of these conditions.

In this report we wish to present a procedure by which we have been able to separate steroid mixtures by application of reversed phase partition chromatography on filter paper. A paper manufactured in Sweden (Munktell 20, 150 G) was found to be most satisfactory. Strips 8 × 48 cm were drawn through a 5% (by vol) solution of Dow Corning Silicone No. 1107 in cyclohexane. These strips were then blotted between sheets of an adsorbent paper to remove excess solution and placed in an oven at 110° C for 1 hr. The paper was rendered hydrophobic by this treatment and readily adsorbed vapors of nonpolar substances such as chloroform. The properties of the treated paper are not changed by washing with organic solvents.

The most satisfactory partition system employed for the separation of the group of steroids used was prepared by mixing 6 vol water, 10 vol absolute ethanol, and 10 vol reagent grade chloroform. The two phases separated in a few minutes but were allowed to stand at room temperature for 1 hr before use, the upper phase being the more polar.

Usually 3 mixtures were analyzed on one paper. The 3 mixtures in chloroform solution were applied at 3 different spots at 2-cm intervals along a line 6 cm from one end of the paper. The mixtures to be analyzed contained 10–25 γ of each component. The paper was then placed in an airtight glass cylinder, the atmosphere of which was kept saturated with vapors of the nonpolar phase. After allowing 1 hr for the incorporation of a stationary phase in the paper by adsorption of the vapors, the chromatogram was developed with the more polar alcohol-water phase in the conventional descending manner. When the moving solvent had run the desired distance (35–40 cm) the paper was removed and dried at 110° C for 10–15 min. It was then sprayed with a freshly prepared mixture of equal volumes of a 2% solution of *m*-dinitrobenzene in absolute ethanol and a 2.5 *N* solution of potassium hydroxide in absolute ethanol. In order to achieve maximum development of color, the paper was placed in a 110° oven for 30–60 sec. The distance traveled by a compound was measured from the origin to the center of the spot.

Because of the sensitivity of the R_F values to small differences in the amount of silicone incorporated in the paper, and also because of the many other factors (*8*) which affect the reproducibility of R_F values, we

[1] Aided in part by a grant from the Swedish Natural Science Research Council.

[2] Sloan Foundation fellow, Sept. 1950–Sept. 1951. Present address: Sloan-Kettering Institute for Cancer Research, New York.

TABLE 1

Compound	Relative R_F
Androstanedione 3,17	0.25
Δ^4Androstenedione 3,17	0.385
Androsterone	0.420
Isoandrosterone	0.527
Dehydroisoandrosterone	0.60
Testosterone	0.64

have not attributed great significance to their absolute magnitudes. Instead we have calculated the R_F values of the steroids relative to the slowest moving steroid in our series, androstanedione 3, 17. Since this compound has an average R_F in the neighborhood of 0.25, we have fixed this value as a "standard." Every mixture analyzed contained androstanedione 3, 17. The R_F values of the other components of the mixture were calculated relative to the distance that the "standard" substance had moved. At least 5 different determinations of the relative R_F values were made with each steroid in the series. The average relative R_F values are recorded in Table 1. Variation of the relative R_F values from the average for any one particular compound did not exceed ±4%.

Efforts are now being made to investigate the quantitative aspects of this method.

References

1. ZAFFARONI, A., BURTON, R., and KEUTMAN, E. *J. Biol. Chem.*, **177**, 109 (1949).
2. ———. *Science*, **111**, 6 (1950).
3. BOSCOTT, R. J. *Nature*, **159**, 342 (1947).
4. BOLDINGH. *Experientia*, **4**, 270 (1948).
5. HOWARD, G., and MARTIN, A. J. P. *Biochem. J.*, **46**, 532 (1950).
6. PARTRIDGE, S. M., and SWAIN, T. *Nature*, **166**, 272 (1950).
7. KRITCHEVSKY, D., and CALVIN, M. *J. Am. Chem. Soc.*, **72**, 4330 (1950).
8. CONSDEN, R., GORDON, A., and MARTIN, A. J. P. *Biochem. J.*, **38**, 224 (1944).

The Alarm Reaction and the Hibernating Gland

Paavo Suomalainen and Anna-Maija Herlevi

Zoological Laboratory,
Helsinki University, Helsinki, Finland

The alarm reaction (*1*) appears, among other things, as an enlargement of the adrenal cortex, rapid increase of the adrenocorticotropic hormone secretion of the pituitary gland and corticosterone secretion of the adrenals, etc. Selye and Timiras (*2*) have found that in rats, coincident with the lipid discharge of the adrenal cortex, reduetion in the sudanophile element of the brown fat tissue takes place. The alarm reaction was brought about by keeping piebald female rats in hunger and cold for 16 hr. Reduction in the lipids of the adrenal cortex and peptic ulcer showed that the alarm reaction had really taken place in them.

In animals that hibernate a special adipose tissue, the brown fat tissue, is found, which is called the hibernating gland (*3*). It is a bilateral formation which extends over the neck, axillary region, and anterior part of the back. Its lobes extend to the mediastinum and diaphragm and surround the large blood vessels of the thoracic cavity.

Using the method of Selye and Timiras (from information in a personal communication from P. S. Timiras, Montreal), we have investigated the hibernating gland of the hedgehog (*Erinaceus europaeus*) in the summer (5 animals, July), during hibernation (5 animals, January, after 3 months' sleep), and in animals spontaneously awakened in captivity from hibernation in May (5 animals, killed immediately after awakening). Immediately after killing the animal, the brown fat tissue was fixed in Bouin's fluid for at least 48 hr. After this it was sectioned with a freezing microtome, and the sections stained with Sudan IV and put into glycerol, where they were examined with the microscope.

The hibernating gland was most intensely stained with Sudan in the summer hedgehog; staining was weakest in the just awakened hedgehog. In hibernating hedgehogs the stainability lay approximately halfway between the two extremes. The stronger the stainability, the more sudanophile substance occurs in the hibernating gland.

The size of the sudanophile particles in the hibernating gland was greatest in the summer hedgehog, smallest in the just awakened animal. In the hibernating hedgehog it was again intermediate between the two.

We find that in the hedgehog in hibernation, and especially in the animal awakened from it, one of the phenomena typical of the alarm reaction described by Selye and Timiras has taken place: reduction in number and size of the sudanophile particles in the brown fat tissue. Judging from this, the awakening of the hedgehog from hibernation (*4*) is such a great physiological strain that it induces an alarm reaction.

References

1. SELYE, H. *Textbook of Endocrinology.* Montreal: A. W. T. Franks (1947).
2. SELYE, H., and TIMIRAS, P. S. *Nature*, **164**, 745 (1949).
3. RASMUSSEN, A. T. *J. Morphol.*, **38**, 147 (1923).
4. SUOMALAINEN, P. *Sitzber. finn. Akad. Wiss.*, **1943**, 163 (1944).

A Method for Dissection and Electrical Study *in Vitro* of Mammalian Central Nervous Tissue[1]

Donald O. Rudin and George Eisenman

Anesthesia Laboratory, Harvard Medical School,
Massachusetts General Hospital, Boston

Dissection of the spinal cord in the living cat has been found to be a simple and reproducible procedure if the piarachnoid membrane is first carefully re-

[1] This study was made possible by grants from the USPHS, RG 1941, and from the U. S. Army, W-49-007-MD-371 OI No. 323–46.