labile reddish pigment within the rabbit ova as the sole manifestation of succinic dehydrogenase may signify the presence of a localized lesser degree of activity of this enzyme than found in certain other components of the ovary.

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Manuscript received September 11, 1952.

Paper Chromatography of Corticosteroids

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The paper chromatography of corticosteroids has been reported by other workers (*1–3*) and has generally given rise to reliable methods for the identification of such of these steroids as are known compounds. The present communication relates to a procedure for the paper chromatography of corticosteroids, in which a number of the usual techniques employed are simpler and more rapid than those previously described.

Although the present procedure is applicable to both descending and ascending paper chromatography, it has been more frequently applied to the latter and will be described as such. A sheet of Whatman No. 1 filter paper (43 × 43 cm) is folded into a cylinder in the manner indicated by Wolfson *et al.* (*4*). The materials to be chromatographed are applied to the paper as droplets of the appropriate solutions at points about 2 cm apart on the starting line, the latter being 8 cm from the bottom fold of the paper, and the spots formed by the droplets being not greater than about 0.5 cm in diameter. The paper is then placed in a cylindrical glass jar 15 cm in diameter and 46 cm high (Fisher Scientific Co., New York) containing a one-phase solvent mixture of xylene (225 ml) and absolute methanol (75 ml), and the jar is subsequently closed by means of a gastight cover. The solvent is allowed to ascend on the paper to a distance of about 25 cm from the starting line—this occurs over a period of about 2.5 hr. The paper is then removed from the jar and air-dried. Visualization of the locations of the various steroids and their quantitative estimation may then be performed by any of the known procedures (*1–3, 5–8*).

The R_f values obtained with a number of authentic corticosteroids[1] are shown in Table 1. It will be observed that drying the paper prior to chromatography results in a change of the R_f values. In general the results obtained with predried paper were found to be more reproducible, predrying presumably minimizing partition phenomena involving the moisture that may otherwise be present on the paper.

Whenever the quantities of materials applied to the starting line on the paper are equivalent to about 5 μg of standard corticosteroid, there is obtained, after development and visualization of the location of substances, a number of discrete spots corresponding to the various steroids present. Approximate dimensions of the various spots obtained are included in Table 1.

Advantages of the above procedure over those reported in the literature are as follows: A one-phase solvent system is used throughout, rendering unnecessary the equilibration of the paper in the solvent vapors prior to development; pretreatment of the paper with any solvent is unnecessary; lateral diffusion of steroid spots during development is quite limited, thus rendering unnecessary the precutting of the paper into a pattern of separated strips; the development period required for the resolution of mixtures of corticosteroids is not greater than 2–3 hr; air-drying of the paper afterwards is completed in less than 30 min.

TABLE 1

Corticosteroid		Paper dried 15 min at 100° C prior to development		Paper not dried prior to development	
Chemical Name	Letter Designation	R_f	Spot dimensions (horizontal × vertical diameter) cm	R_f	Spot dimensions (horizontal × vertical diameter) cm
11-Desoxycorticosterone	Q (Reichstein)	0.921	0.9 × 1.0	0.826	0.8 × 1.7
11-Desoxy-17α-hydroxycorticosterone	S "	.698	.7 × 5.5	.495	.9 × 3.8
Corticosterone	B (Kendall)	.653	.6 × 4.7	.491	.9 × 2.9
11-Dehydro-17α-hydroxycorticosterone	E "	.501	.8 × 3.5	.394	0.7 × 0.9
17α-Hydroxycorticosterone	F "	0.362	0.7 × 3.5	0.312	1.1 × 1.3

As in the case of the previously reported procedures for the paper chromatography of corticosteroids, the R_f values obtained by the present procedure are relative rather than absolute. Further, it is considered essential to base the final identification of an unknown corticosteroid on a direct comparison with authentic specimens of known substances, employing such criteria as specific color reactions (*6, 7*) and absorption spectra of sulfuric acid chromogens (*8*).

[1] Samples of corticosterone and 17α-hydroxycorticosterone were generously donated by Carl Djerassi and Gregory Pincus, and were kindly obtained for us by B. J. Brent.

The above-described procedure has also been successfully employed on a smaller scale using, most frequently, a 500-ml measuring cylinder of conventional type as the chromatographic vessel. Such a system was found to provide a convenient means of performing quick checks on the identity and state of purity of corticosteroids and related substances.

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Manuscript received September 2, 1952.

Microanatomical Study of DDT-moribund *Anopheles quadrimaculatus* Say

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Histological studies following administration of DDT have been made principally on the roach (*Periplaneta americana* L. [*1, 2*]), on the honeybee (*Apis mellifera* L.[*1*]), and on the housefly (*Musca domestica* L. [*3*]). For the most part these studies were made on one stage only and did not include examinations of all the tissues of these insects.

Since the action of DDT still remains obscure, it seemed advisable to make intensive histological studies of *all* the tissues of an insect from representative periods during its life span. The common malaria mosquito of the Southeastern United States, *Anopheles quadrimaculatus* Say, was selected for these studies.

Fourth-instar larvae, pupae, and adults of both sexes were exposed topically to massive doses of pure *p,p'*-DDT, and when moribund (about 2–8 hr) were studied by examination of dissected living specimens and by examination of sectioned fixed specimens. All test animals were acutely poisoned and showed every sign of extreme intoxication. Twenty-five test animals from each stadium were used with equivalent numbers of controls.

Dissections were made in insect Ringer's and were either examined while fresh under the microscope with and without phase contrast, or were examined after fixation with alcohol or formalin in whole mounts prepared of the various tissues.

Complete transverse, sagittal, and frontal serial sections at 6–15 μ were made from alcoholic–formalin-, Bouin-, or Carnoy-fixed material. Sections stained with Delafield's and Haidenhain's iron hematoxylin and counterstained with eosin were examined at 100 and at 1000 diameters.[1]

Every effort was made to study all the representative tissues of these insects and to determine by comparison with controls whether visible changes in the structure and staining reactions had occurred. Detailed cytological, histological, and histochemical studies of normal *A. quadrimaculatus* are in preparation for publication at a later date.

Tissues examined included: (1) hypodermis; (2) tracheae; (3) thoracic molting glands in larvae; (4) micro- and macro-oenocytes; (5) imaginal discs of undifferentiated and differentiated thoracic and abdominal muscles; (6) imaginal discs of eyes, antennae, legs, wings, and halteres; (7) anal papillae of larvae; (8) neurocytes and neuropile of frontal, supraesophageal, thoracic, and abdominal ganglia; (9) neurosecretory cells in supraesophageal ganglion; (10) corpora allata–cardiaca complex; (11) immature, differentiating, and mature gonads and associated structures (mucus gland, atrium, spermatheca of female and accessory glands of male); (12) dorsal vessel; (13) alary muscles; (14) micro- and macro-pericardial cells; (15) thoracic ventral nephrocytes in larvae; (16) ventral diaphragm of adult; (17) internal and external fat body; (18) buccal cavity; (19) salivary glands; (20) esophagus; (21) gastric caecae of larvae; (22) dorsal and ventral diverticula of adult (23) midgut; (24) Malpighian tubules; (25) hind-gut; and (26) rectal papillae of adult.

No microanatomical changes in cell or nuclear structure (e.g., cytoplasmic vacuoles, hypertrophy, atrophy, surface irregularities, karyorrhexis) or abnormal reactions to hematoxylin and eosin (e.g., overstaining, diffuse staining, blotching, or failure to stain) were encountered in any of the tissues of any of the stages that were examined. These findings indicate that DDT does not operate to produce visible structural damage to cells during representative periods of the life span of *A. quadrimaculatus*.

The present observations confirm and extend those of Richards and Cutkomp (*2*), who found absence of definite pathology in DDT-poisoned roaches. No comparison can be made between the present observations and those of Chang (*1*), who reported breakdown of Golgi bodies in the ganglia of DDT-poisoned roaches and honeybees, since the special methods required for their demonstration were not used in the present studies.

Absence of definite pathology in the central nervous system of the mosquito and the roach following DDT administration is not peculiar to insects, for similar findings have been reported by Globus (*4*) in monkeys, dogs, cats, and rats acutely poisoned with DDT.

A few investigators have reported cellular changes in insects (*1, 3*) and in vertebrates following DDT

[1] Grateful acknowledgment is made to Ralph D. Lillie and James H. Peers, Laboratory of Pathology and Pharmacology, for extending me the courtesy of their laboratory facilities. I am indebted to Joseph Woodard of their laboratory for having cut and stained most of the sections.