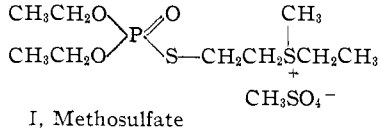
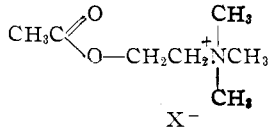
methosulfate, $n^{29}D$ 1.5023, a thick viscous liquid, could not be induced to crystallize and was purified by repeated precipitations by dissolving it in chloroform and adding to a 10-fold excess of ether; calculated for $C_{10}H_{25}O_7PS_3$; P, 8.1; found, P, 8.3. The infrared spectrum[4] in chloroform solution showed strong absorptions for the P=O and P–O–C moieties at 1250 and 1015 cm.$^{-1}$, respectively, and a weak shoulder for the P–O–CH_2CH_3 moiety at 1160 cm.$^{-1}$.

The formation of the sulfonium salt increased the anticholinesterase activity by a factor of about one hundred fold: from a 50% fly-brain cholinesterase inhibition concentration of 3×10^{-6} M for the O,O-diethyl S-2-mercaptoethyl phosphorothiolate to a 3.3×10^{-8} M for the methosulfate. It is of interest to note the similarity in structure of the sulfonium ion (I) to the natural cholinesterase enzyme substrate acetylcholine (II).



I, Methosulfate



II, Acetylcholine

The product is extremely soluble in water and showed excellent systemic activity in the cotton plant. For the determination of the systemic action, the bases of young cotton plants (6-leaf stage) were treated with 5λ of the methosulfate. After various intervals leaves were picked, and contact toxicity in Munger cells and house fly-head cholinesterase inhibition by the leaf homogenates were determined.[5] The results are shown in Table I.

TABLE I

SYSTEMIC ACTIVITY OF O,O-DIETHYL S-2-ETHYLMERCAPTOETHYL PHOSPHOROTHIOLATE METHOSULFATE

Time after treatment	House fly-head cholinestearase % inhibition 1	0.1[a]	0.01[a]	Per cent. mortality in Munger cells after 48 hours *Heliothrips haemorrhoidalis*	*Tetranychus bimaculatus*	*Aphis gossypii*
24 hr.	71	51		0	90	100
48 hr.	96	93				90
1 week	100	100	93	95	90	100
2 weeks	100	100	100	80	70	100
3 weeks	100	100	100	20	90	100
6 weeks	97	97	48	5	80	100

[a] Leaf homogenate diluted 1 to 10 and 1 to 100 with water.

Evaluation of contact toxicity of the methosulfate to the house fly, thrips, and mosquito larvae, gave LC_{50} values of 0.4%, 0.01%, 0.001%, respectively. The intraperitoneal LD_{50} to the mouse was between 1 and 5 mg. per kg.

DEPARTMENT OF ENTOMOLOGY
UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

T. R. FUKUTO
R. L. METCALF
R. B. MARCH
M. MAXON

RECEIVED MAY 9, 1955

(4) A Perkin–Elmer model 21 self-recording infrareds pectrophotometer was used in this work.

(5) R. L. Metcalf, R. B. March, T. R. Fukuto and Marion Maxon, *J. Econ. Ent.*, **47**, 1045 (1954).

FRACTIONATION OF DEOXYRIBONUCLEIC ACID (DNA) BY ION EXCHANGE[1]

Sir:

Recent evidence[2–6] clearly indicates that the total DNA of the cell is heterogeneous. Further exploration of this finding has been aided by the development of new fractionation procedures which afford greater resolution and are applicable to small quantities of material.

Highly polymerized calf thymus DNA[7] (*ca.* 1 mg./ml., *p*H 7) was applied to columns of ion-exchangers. The adsorbed DNA could not be eluted with concentrated NaCl solution at neutral *p*H from the strong-base anion-exchanger Dowex-1 Cl^-. It was eluted from the weak-base anion-exchanger Amberlite IR-4B OH^- with stepwise increase in NaCl concentration (0.1–3.0 M) yielding some 16–20 fractions, but this resin had several disadvantages. Of the substituted-cellulose derivatives[8] tested, the weak-acid cation-exchanger CM-cellulose had no affinity for DNA, but the weak-base anion-exchangers DEAE-cellulose[8] and ECTEOLA-cellulose[9] adsorbed it quantitatively from solution.

Because of its favorable capacity, low shedding blank, the essential reproducibility of elution pattern and high recovery of DNA obtained with neutral eluting solutions, ECTEOLA-cellulose appears to be the most promising chromatographic medium. Most of the calf thymus DNA adsorbed on it was eluted by either discontinuous or continuous change of concentration of sodium chloride solution (Figs. 1 and 2). DNA from pneumococcus possessing transforming activities[10] gave patterns similar to Fig. 2. The fractionation appears to depend, in part, on the molecular size or state of aggregation of the polynucleotide components of the nucleic acids. A mixture of mono-deoxyribonucleotides was completely eluted with 0.01 M phosphate, *p*H 7, devoid of sodium chloride, whereas a deoxyribonuclease digest of calf thymus DNA containing a large proportion of oligonucleotides required increases in sodium chloride concentration up to 0.22 M for quantitative elution (*cf.* Fig. 2). A highly polymeric ribonucleic acid (RNA) from pneumococcus exhibited the same heterogeneous type of behavior as did DNA. After treatment with ribonuclease, the remaining RNA fragments were also

(1) This investigation was supported by grants from the United States Public Health Service, and from the Aromic Energy Commission, Contract No. AT(30-1)-910.

(2) A. Bendich, *Exp. Cell. Res.*, **3**, suppl. 2, 181 (1952).

(3) A. Bendich, P. J. Russell, Jr., and G. B. Brown, *J. Biol. Chem.* **203**, 305 (1953).

(4) E. Chargaff, C. F. Crampton and R. Lipshitz, *Nature*, **172**, 289 (1953).

(5) G. L. Brown and M. Watson, *Nature*, **172**, 339 (1953).

(6) J. A. Lucy and J. A. V. Butler, *ibid.*, **174**, 32 (1954).

(7) H. Schwander and R. Signer, *Helv. Chim. Acta*, **33**, 1521 (1950).

(8) H. A. Sober and E. A. Peterson, THIS JOURNAL, **76**, 1711 (1954).

(9) Peterson and Sober have prepared ECTEOLA-cellulose by treating alkaline cellulose with epichlorohydrin and triethanolamine (unpublished). The batch of exchanger used in these studies had a capacity of 7.2 mg. DNA per g. We are indebted to Drs. Peterson and Sober for their generous gifts of these exchangers, and to Dr. G. B. Brown for bringing their previously unpublished observations to our attention.

(10) R. D. Hotchkiss and J. Marmur, *Proc. Nat. Acad. Sci.*, **40**, 55 (1954).

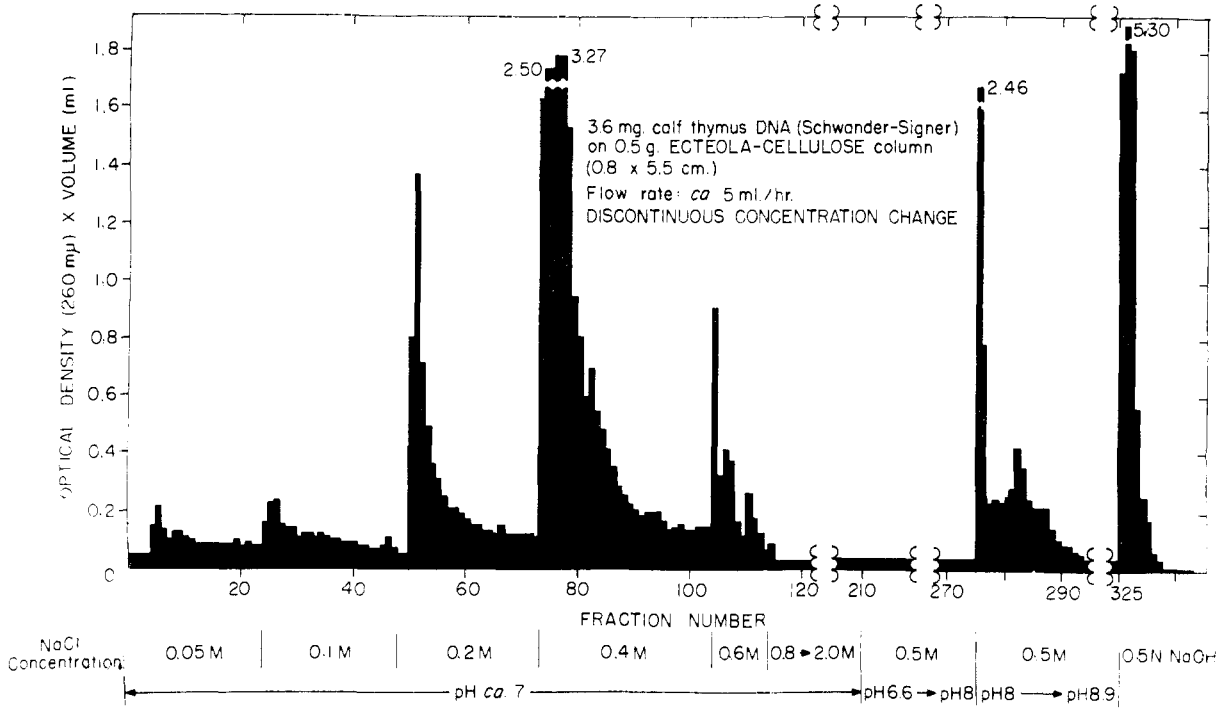


Fig. 1.—Column of ECTEOLA-cellulose saturated with DNA, discontinuous elution (recovery 90 to 100%).

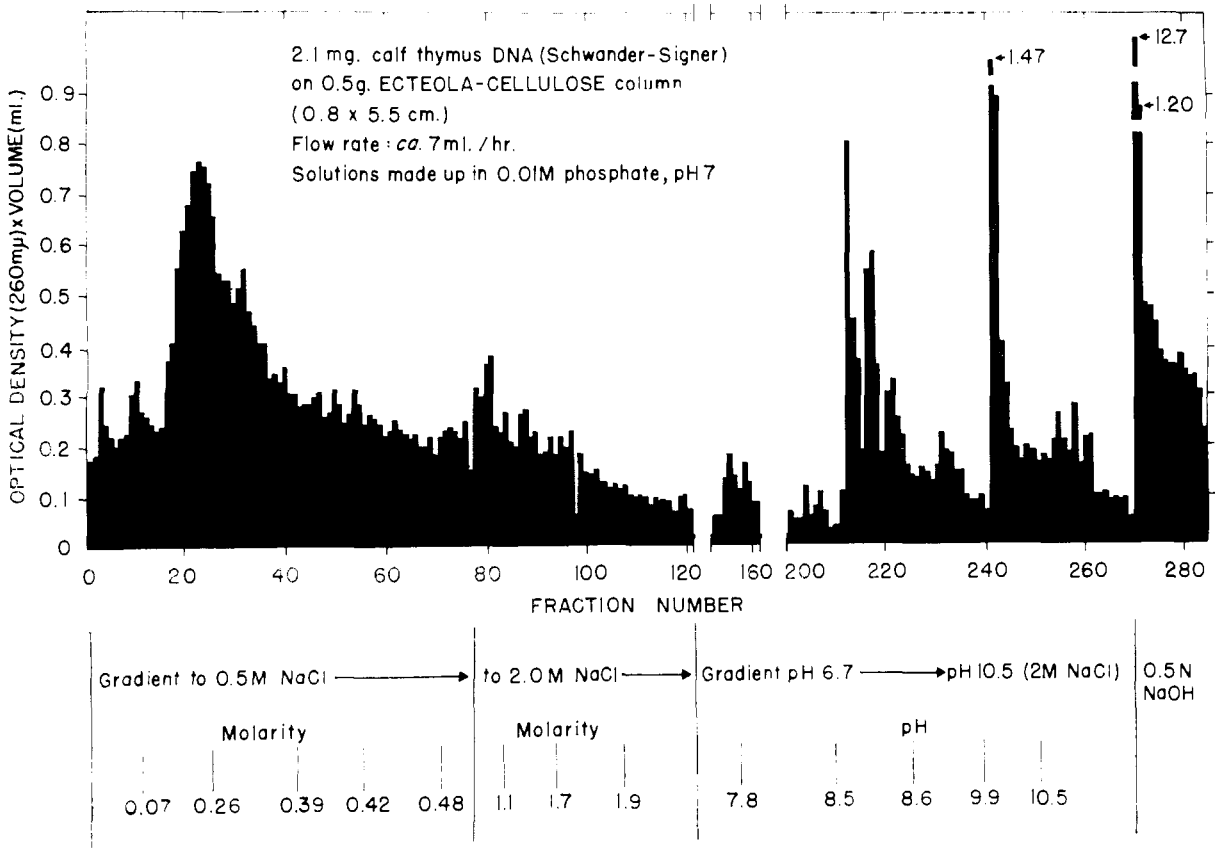


Fig. 2.—Column of ECTEOLA-cellulose about one-half saturated with DNA, gradient elution (recovery 90 to 100%).

completely removed from the column before 0.22 *M* sodium chloride was reached.

Evidence that the DNA fractions desorbed from the exchanger at neutral *p*H largely maintain their integrity is furnished by the following observations. Fractions are still essentially non-dialy-

sable and are precipitable by alcohol. On re-chromatography, the fractions studied still possess their original chromatographic properties. Individual fractions from pneumococcal transforming DNA[11] show considerable biological activity, comparable to that present in the original preparation.

These chromatographic analyses provide further evidence for the heterogeneous nature of DNA from single sources, and the method appears applicable to the fractionation of RNA. It furnishes a technique for an experimental approach to the study of the metabolism and the character of biologically active nucleic acids.[12]

THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK 21, NEW YORK
AARON BENDICH
JACQUES R. FRESCO[13]
HERBERT S. ROSENKRANZ

DEPARTMENT OF MICROBIOLOGY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY, NEW YORK 32, N. Y.
SAM M. BEISER

RECEIVED MAY 6, 1955

(11) Unpublished observations, in collaboration with R. D. Hotchkiss, D. J. Hutchison and M. T. Dowling, on pneumococcal DNA possessing transforming activity.

(12) We gratefully acknowledge the valuable advice and support of Dr. G. B. Brown.

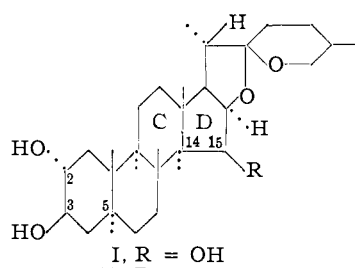
(13) Fellow of the United States Public Health Service, 1952–1954. Present address, Department of Pharmacology, New York University College of Medicine, New York 16, N. Y.

CORRELATION OF DIGITOGENIN WITH PROGESTERONE

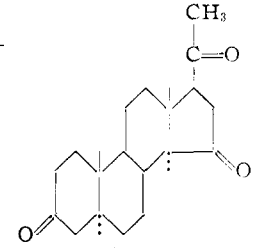
Sir:

Although it has now been demonstrated[1,2] that digitogenin is a 2α,3β,15-trihydroxy-5α-spirostan derivative, the available evidence does not permit an unequivocal assignment to the stereochemistry of the C/D ring juncture. Thus, digitogenin (I), as the 2,3-diacetate[3] or 2,3-dicathylate[4] can be oxidized to the corresponding 15-ketone which is very readily inverted at C-14 by base. From the course of the Wolff–Kishner reduction[3] of both isomers, which proceeded in poor yield to furnish gitogenin (II), it was suggested tentatively that digitogenin has the 14β(C/D *cis*) configuration, while the opposite conclusion might be reached on the basis of the results of desulfurization studies.[4] We have now been able to arrive at a rigorous solution of this problem, which also has an important bearing on the relative stability of fused hydrindanone systems.

Δ^2-5α,22a-Spirosten-15β-ol, readily obtainable[3] by sodium iodide treatment of digitogenin 2,3-dimesylate, was oxidized with perbenzoic acid to the 2α,3α-epoxide (m.p. 188–190°, $[\alpha]^{23}D$ −56° ($CHCl_3$); found: C, 75.31; H, 9.83) which was reduced with lithium aluminum hydride to 22a,5α-spirostane-3α,15β-diol (m.p. 238–240°, $[\alpha]_D$ −74° ($CHCl_3$); found: C, 75.23; H, 10.02). Side chain degradation[2] produced Δ^{16}-allopregnene-3α,15β-diol-20-one diacetate (m.p. 142–143°, $[\alpha]_D$ −152° ($CHCl_3$), λ_{max}^{EtOH} 231 mμ, log ϵ 4.00; found: C, 71.74; H, 8.70) which was hydrogenated (palladium–charcoal, ethyl acetate) and saponified[5] (2% methanolic potassium hydroxide, steam bath, 2 hours) to yield allopregnane-3α,15β-diol-20-one (III), (m.p. 239–241°, $[\alpha]_D$ +59° ($CHCl_3$), +84° (pyridine), no high selective ultraviolet absorption; found: C, 75.37; H, 10.30). Mild oxidation (15 min.) with chromium trioxidel ed to allopregnane-3,15,20-trione (IV) (m.p. 222–223°, $[\alpha]_D$ +137° ($CHCl_3$); found: C, 76.72; H, 8.79), which was also obtained from 15β-hydroxyprogesterone[6] by catalytic hydrogenation (palladium–$BaSO_4$, ethyl acetate) to allopregnan-15β-ol-3,20-dione (m.p. 256–258°, $[\alpha]^{23}D$ +93° ($CHCl_3$); found: C, 76.12; H, 9.70) followed by mild chromium trioxide oxidation, or by palladium hydrogenation of 15-ketoprogesterone[6] (m.p. 155–157°, $[\alpha]^{23}D$ +200° ($CHCl_3$), found: C, 76.90; H, 8.57). Identity of the 3,15,20-trione IV, prepared by all three routes was demonstrated by infrared comparison as well as by conversion at identical rates (mutarotation: +130° → +55°), to the 14β,17α-isomer (m.p. 186–189°, $[\alpha]^{23}D$ +60° ($CHCl_3$); found: C, 76.32; H, 8.91), when allowed to stand at room temperature in 0.02 *N* methanolic potassium hydroxide for 18 hours. This latter isomerization parallels that of 15-ketoprogesterone to its 14β,17α-isomer (m.p. 213–214°, $[\alpha]^{23}D$ +113° ($CHCl_3$); found: C, 76.53; H, 8.72).[6,7]



I, R = OH
II, R = H



IV

The above correlation of digitogenin (I) with a microbiological oxidation product of progesterone establishes the 14α-configuration for digitogenin, which can now be given the rigorous name 22a,25a,-5α-spirostane-2α,3β,15β-triol.[8] It is instructive to note that while 15-keto derivatives in the choles-

(1) F. L. Warren and P. A. S. Canham, *Chem. and Ind.*, 727 (1954).

(2) C. Djerassi and T. T. Grossnickle, *ibid.*, 728 (1954).

(3) C. Djerassi, T. T. Grossnickle and L. B. High, *ibid.*, 473 (1955).

(4) D. L. Klass, M. Fieser and L. F. Fieser, THIS JOURNAL, **77** in press (1955); we are grateful to these authors for an advance copy of their paper.

(5) That no inversion occurred at C-17 had already been demonstrated with the corresponding 2α,3β,15-triacetoxy-20-ketone (ref. 2), which was regenerated after saponification and reacetylation.

(6) The microbiological oxidation of progesterone to 15β-hydroxyprogesterone has been reported by J. Fried (AAAS Gordon Research Conference on Steroids, August, 1953), *cf.* J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman, *Recent Progress Hormone Research*, **11**, 157 (1955); J. Fried, R. W. Thoma, P. Grabowich and J. R. Gerke, *Chem. and Ind.*, in press (1955).

(7) The alkali-isomerized 3,15,20-triketones are formulated as 17α-pregnane derivatives since ring C/D *cis*-fused 20-ketosteroids possessing the 17β-configuration are epimerized at C-17 by alkali (*cf.* R. C. Elderfield, *J. Biol. Chem.*, **113**, 631 (1936); K. Meyer, *Helv. Chim. Acta*, **30**, 1976 (1947)), while those having the 17α-configuration are stable in that medium (*cf.* P. A. Plattner, H. Heusser and A. Segre, *ibid.*, **31**, 249 (1948)).

(8) The assignment of the β-configuration to the 15-hydroxyl group in III and therefore also in I is based on the following considerations: The molecular rotation values calculated for the 15α- and 15β-epimers of allopregnane-3α,15-diol-20-one from the values for 15α- and 15β-hydroxyprogesterone (*cf.* ref. 6) and the average value for the change Δ^4-3-ketone → allo-3α-ol (−261°) (*cf.* D. H. R. Barton and W. Klyne, *Chem. and Ind.*, 755 (1948)) are +461° and +237°, respectively. The latter value (15β) is in good agreement with the found value of +197°.