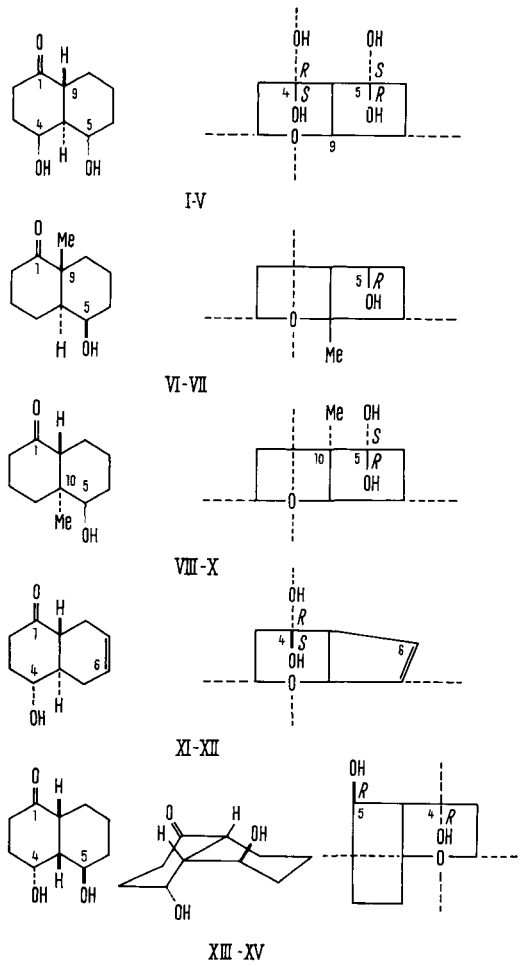

Note: In order to save space, each basic formula shows *all* relevant substituents. The compounds examined did *not* contain more than one hydroxyl group each.

that calculated from DJERASSI's[8,9] values; Δa (VI–I) = + 67. (iv) The differences Δa (IX–IV) and Δa (X–V) are for an axial methyl group in the 3-position relative to a cyclohexanone carbonyl (– 20, – 22). These values for Δa '3-axial-methyl' are similar to that found by DJERASSI, LUND, and AKHREM[11] for Δa '3-equatorial-methyl', viz: – 25. (v) The amplitudes of the Δ^6-octalones (XI, XII) are *less* than those of the corresponding decalones. This is in accordance with the octant projection for the Δ^6-octalones, in which ring B is flattened towards the horizontal symmetry plane. (vi) The negative amplitudes for the *cis*-decalones XIII, XIV, and XV show that for these compounds the 'steroidlike' conformation is preferred (DJERASSI and STAUNTON[8]). (vii) The contributions of the hydroxyl groups in the *cis*-decalone series are zero and positive as expected[15].

Zusammenfassung. Die Rotationsdispersionskurven einiger optisch aktiver Dekalonderivate sind gemessen worden. Ihre Amplituden (a) und die Amplitudenbeiträge (Δa) der Substituenten werden im Rahmen der Oktantenregel diskutiert.

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Department of Chemistry, Westfield College, Hampstead, London (England), May 28, 1962.

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[15] *Acknowledgment.* We are greatly indebted to Professor V. PRELOG and Dr. W. ACKLIN, ETH Zürich, for generously supplying the materials. We are grateful to the Department of Scientific and Industrial Research for a grant and to the U.S. Army Research and Development Group (Frankfurt am Main) for a contract.

Liver Catalysis in the Association of Estrogen to Protein

RIEGEL and MUELLER[1] have demonstrated the presence in rat liver of an enzyme which catalyzes the formation of a protein-bound metabolite of C^{14}-estradiol. In a series of *in vitro* experiments SZEGO and ROBERTS[2] obtained results which indicated that rat liver promotes the binding of C^{14}-estrone or its metabolites to the proteins of their serum incubation medium. On the other hand, SANDBERG et al.[3] obtained evidence from a similar study which suggested that the binding of C^{14}-estrone occurred with a serum protein or a serum soluble protein originating from the liver. This communication describes (a) the results of a comparison of the action of rat liver slices and rat liver homogenates in promoting the binding of both C^{14}-estrone and estradiol 17β-acetate to protein and (b) the progress made in the attempts to characterize the factors concerned with the formation of 'estroprotein' through the fractionation of rat liver homogenates.

Materials and Methods. Techniques involving the incubation of steroid and rat liver slices in serum were essentially the same as those described earlier by SZEGO[4].

Homogenates of rat liver were prepared in 0.1 M potassium phosphate buffer (pH 7.4) according to the procedure described by BUCHER and MC GARRAHAN[5]. Prior to fractionation, glutathione and Versene were introduced to give final concentrations of 0.01 M and 0.001 M, respectively. All preparative steps were carried out at 0°. The crude homogenate was first centrifuged at 5.000 × g for 10 min. The supernatant fluid, containing only microsomes and soluble cell constituents, termed 'microsol', was then fractionated as follows. The major part of the microsol fraction was centrifuged at 105.000 × g (1 h) and the supernatant fluid, S_1, decanted. The sediment was resuspended in buffer and washed once by centrifug-

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ing at 105.000 × *g* (1 h) to yield the major fraction of microsomes, M_1. A part of S_1 was centrifuged at 197.000 × *g* (3 h) and the supernatant, S_2, decanted. The residual pellet, consisting mainly of small microsomes[6], M_2, was rinsed with buffer and finally resuspended in fresh buffer. Incubation vessels contained 5–40 μg of either 16-C^{14}-estrone or 4-C^{14}-estradiol 17 β-acetate and DPN (0.008 *M*) together with the specified liver fraction and were adjusted to a final volume of 2.2 ml.

The vessels were incubated at 37° in a Dubnoff metabolic incubator for 4 h under oxygen and the vessel contents were then centrifuged in the cold. Samples (20 μl) were then subjected to paper electrophoresis using the horizontal pressure-plate procedure[7]. Paper strips derived either from liver tissue and buffer or in the case of the homogenate afforded only a weak stain with bromphenol blue at the origin. The dried strips were divided into 2.0 cm segments and then were eluted twice with 1.0 ml of 0.25% polyvinylpyrrolidone (PVP) in 0.01 *N* NaOH. Each eluate was plated in a stainless steel planchet to give a residue (5.0 mg PVP) of constant thickness and distribution which was then analyzed for radioactivity in an automatic gas flow counter.

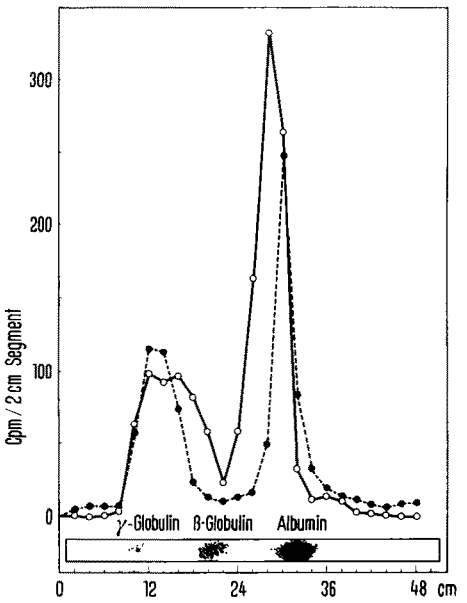

Fig. 1. The electrophoretic separation of protein-bound radioactivity after incubation of 16-C^{14}-estrone (o—o) and 4-C^{14}-estradiol 17β-acetate (•---•) in the presence of surviving rat liver tissue in a homologous serum medium. The separation of serum proteins, which are stained by bromphenol blue, is indicated below the curves. See the text for conditions of incubation, paper electrophoresis and isotopic analysis.

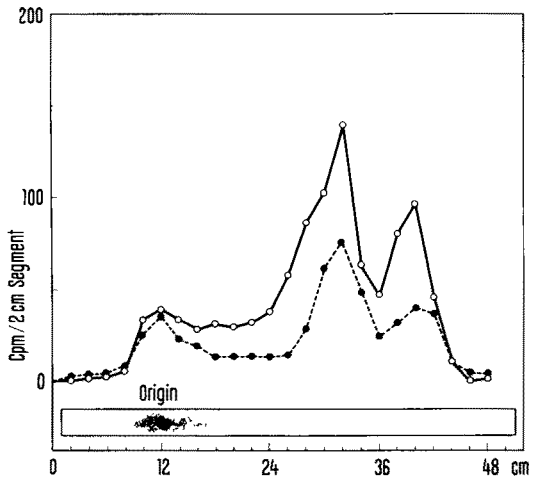

Fig. 2. An experiment similar to that shown in Figure 1 with the exception that phosphate buffer (pH 7.4) was substituted for the homologous serum medium.

Results. It was observed in accord with the report of SZEGO and ROBERTS[2] that a significant portion of the radioactivity appeared in the albumin area of the serum proteins (Figure 1) when the incubation of either 16-C^{14}-estrone or 4-C^{14}-estradiol 17 β-acetate was carried out in serum and in the presence of surviving rat liver tissue. In addition, a considerable part (15-19%) of the original radioactivity remained with liver tissue as was also observed by SANDBERG et al.[3]. The latter authors suggested that a significant part of the so-called estroprotein may not represent binding of the steroid with serum protein but merely be the infiltration of the serum by a protein from the liver with a greater affinity for the binding of estrogens. However, it remains to be established whether such binding is the result of an intra- or extra-cellular event.

The incubation of these same steroids in the presence of rat liver slices in buffer (without serum) again gave rise to a prominent band of radioactivity in the region corresponding to albumin in Figure 1. Furthermore, there appeared a previously undescribed band of radioactivity at 37–48 cm (see Figure 2), ahead of the major peak corresponding to Figure 1.

In an effort to characterize the factors responsible for the two radioactive peaks (Figure 2) incubation studies were performed with each of the several fractions derived from rat liver homogenate. A fraction approaching a microsome-free supernatant (S_2) and four additional fractions were obtained by differential centrifugation. The results derived from incubation of these fractions with 4-C^{14}-estradiol 17 β-acetate are compiled in the Table.

Microsomes alone, whether large (M_1) or small (M_2), are incapable of promoting the association of radioactivity to liver protein. Incubation of either the microsol or supernatant fraction, S_1, resulted in a distribution of

Distribution of radioactivity (4-C^{14}-estradiol 17β-acetate)[a] on electrophoretic strip following incubation of fractions of liver homogenate

Fraction[b]	Added radioactivity cpm/20 μl	% Radioactivity in 12 cm segments[c] 0–12	13–24	25–36	37–48	Total %
Microsol	1293	15	10	37	11	73
S_1	1212	10	4	36	11	61
M_1	1144	75	4	4	4	87
S_2	1206	5	4	39	28	76
M_2	1206	66	2	2	2	72
$S_2 + M_2$	1143	10	5	37	11	66

[a] Specific activity 7000 cpm/μg
[b] See *Materials and Methods* for origin of these fractions
[c] See Figure 1 for proteins corresponding to segments

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radioactivity in which the major portion was present as a single peak in the area (25–36 cm) corresponding to albumin. However, in both of these cases a small amount of radioactivity (ca 11%) was detected in the region (37–48 cm) corresponding to the second peak of Figure 2. Finally, it is apparent from the Table that the radioactivity in the second region is greatly increased when the incubation was performed with S_2. This result must be related, directly or indirectly, to the paucity of microsomes in this fraction since, on recombination of this fraction with microsomes ($S_2 + M_2$) the second peak of radioactivity is again low.

Efforts are now being directed toward the identificatino of the metabolite(s) in each of the areas of radioactivity[8].

Zusammenfassung. Die Inkubation von 16-C^{14}-Oestron und 4-C^{14}-Oestradiol-β-acetat in Puffer (pH 7.4) bei Gegenwart von Rattenleber ergibt elektrophoretisch zwei deutliche radioaktive Bänder. Das erste Band entsteht im Albuminbereich und stimmt mit den früheren Beobachtungen von SZEGO und ROBERTS[2] überein. Das zweite, früher nicht beschriebene Band entsteht deutlich ausserhalb des Albuminbereiches. Beide Kurvengipfel werden mit wenig homogenisierter Rattenleber, die möglichst frei von Mikrosomen ist, gewonnen. Der zweite Gipfelpunkt wird in diesem Fall der obenauf schwimmenden Phase zugeschrieben.

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The Rollin H. Stevens Memorial Laboratory of the Detroit Institute of Cancer Research, Detroit (Michigan U.S.A.), May 30, 1962.

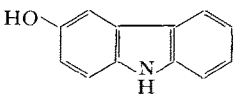
[8] *Acknowledgment.* This investigation was supported in part by research grant, CY-4519(C3) from the National Cancer Institute of the United States Public Health Service and in part by an institutional grant from the United Foundation of Greater Detroit trough the Michigan Cancer Foundation.

Metabolism of Carbazole[1]

Previous studies[2,3] in these laboratories of the metabolism of ergometrine and lysergic acid diethylamide indicated that these indole derivatives were metabolised in the rat by hydroxylation in the aromatic ring of the indole skeleton. Paper chromatographic evidence showed that the major metabolite of ergometrine was the glucuronide of 12-hydroxyergometrine.

To ascertain the position of hydroxylation in the aromatic ring of indole derivatives the metabolic fate of carbazole has been investigated in the rat and rabbit.

After an intraperitoneal dose of 4 mg/kg of carbazole in propylene glycol, the urine of rats contained a conjugated hydroxycarbazole as the major metabolite. This metabolite was purified by paper chromatography using several systems and then hydrolysed with 0.5*N* hydrochloric acid. Comparative chromatography of the phenol so obtained with the four possible monohydroxycarbazoles on paper and thin-layer chromatograms (Table) indicated it to be 3-hydroxycarbazole (I).



(I)

Compound	Rf, system 1[a]	Rf, system 2[b]	Colour[c]
1-Hydroxycarbazole	0.62	0.57	red
2-Hydroxycarbazole	0.39	0.26	orange
3-Hydroxycarbazole	0.57	0.31	purple
4-Hydroxycarbazole	0.81	0.45	pink
Hydrolysed metabolite	0.57	0.31	purple

[a] Chloroform, benzene, ethyl acetate, water (6, 2, 2, 5) on Silica-gel thin-layer chromatograms.
[b] Toluene, iso-octane, methanol, water (15, 5, 16, 4) on Whatman No. 4 paper.
[c] Reagent: Diazotised sulphanilamide.

A two-fold increase in urinary glucuronide content, as determined by the method of FISHMAN and GREEN[4] was observed after oral dosing of carbazole (1 g) in acacia suspension to a rabbit. The glucuronide was separated by the method of SMITH and WILLIAMS[5] as a colourless gum which after hydrolysis with a β-glucuronidase preparation[6] at 36° in acetate buffer[7] afforded a phenolic product identical on both paper and thin-layer chromatograms, with the hydrolysed metabolite from rat urine and with 3-hydroxycarbazole.

Carbazole-C^{14} (6.0×10^5 d.p.m./mg) was prepared from aniline-C^{14} sulphate (Radiochemical Centre, Amersham) by diazotisation and reduction to phenylhydrazine-C^{14} hydrochloride, condensation with cyclohexanone to 1,2,3,4-tetrahydrocarbazole-C^{14} and dehydrogenation with palladium charcoal (10%) in mesitylene. After intraperitoneal injection of carbazole-C^{14} (2.48×10^6 d.p.m.) to rats the 48-h urine contained 1.61×10^6 d.p.m. (65% of original dose). Hydrolysis of the urine with 0.5*N* hydrochloric acid and ether extraction gave an extract containing 1.36×10^6 d.p.m. (55% of original dose). The ether extract was chromatographed on paper using solvent system 2 and the 3-hydroxycarbazole (detected by both diazotised sulphanilamide reagent and by radioactive scan) after elution with methanol and purification possessed 0.82×10^6 d.p.m. (33% of original counts). The remaining counts (0.50×10^6 d.p.m.) in the ether extract were present in a more polar band which remained on the starting line of the chromatogram. This more polar band was separated using the solvent system ethyl acetate, pyridine, water (3:1:1) into two phenolic bands which were possibly di- or polyhydroxylated carbazoles.

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