density along the c axis is given, in which carbon and oxygen atoms are fairly well resolved.

At this stage of the research, from the positions of the electron density maxima, we could deduce that: (1) the benzene carbon atoms have fixed positions and are all placed at the same distance (2.25 ± 0.05 Å.) from the chromium atom. (2) The plane in which the oxygen atoms are contained is parallel within experimental errors to the benzene ring. (3) The C and O atoms of each C=O group are collinear with the chromium atom. (4) The angles OC–Cr–CO are equal and very near to 90° (exp. 89°). The O–Cr distances are 2.95 ± 0.05 Å. (to compare with the value 3.08 Å. quoted for chromium hexacarbonyl).[3]

It seems to us that the model of the molecule thus established (see Fig. 2) favors the hypothesis

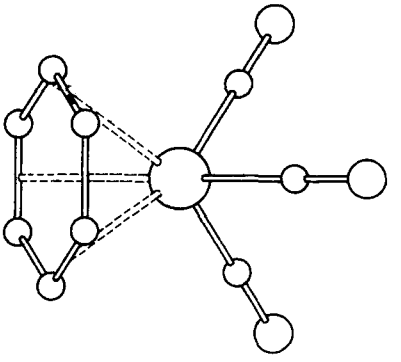

Fig. 2.—Model of the molecule corresponding to the Fourier projection of Fig. 1.

of a d^2sp^3 hybridization of the chromium atom in tricarbonylchromium-arenes.[4]

We acknowledge the helpful suggestions of Prof. G. Natta and Prof. R. Ercoli, who also supplied us the sample.

(3) L. O. Brockway, R. V. G. Ewens and M. W. Lister, *Trans. Faraday Soc.*, **34**, 1350 (1938).

(4) E. O. Fischer, K. Öfele, H. Essler, W. Fröhlich, J. P. Mortensen and W. Semmlinger, *Chem. Ber.*, **91**, 2763 (1958).

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RECEIVED MARCH 10, 1959

COUNTERCURRENT DISTRIBUTION STUDIES WITH ADULT HUMAN HEMOGLOBIN

Sir:

A few experiments with hemoglobin have been made as part of a general study of the application of countercurrent distribution to proteins and peptides. In view of the ease with which the heme is dissociated from the protein, the latter was first studied and found to be resolved into more than one component.

Globin (about 150 mg.) prepared from carbonmonoxyhemglobin[1] gave the distribution pattern of Fig. 1 in 2-butanol–1% aqueous dichloroacetic acid (1:1) at 82 transfers. Plots of partition ratios across the bands and experimental curves wider than the calculated indicated neither band to be entirely homogeneous at this stage.

(1) M. L. Anson and A. E. Mirsky, *J. Gen. Physiol.*, **13**, 469 (1930).

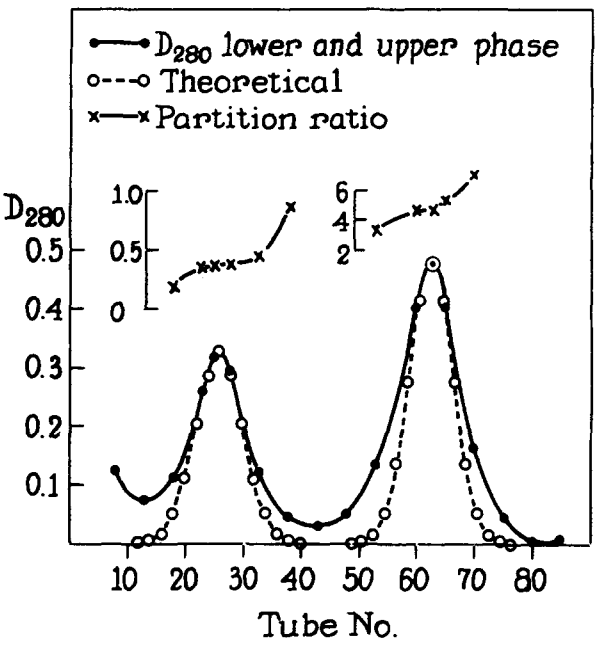

Fig. 1.—Countercurrent distribution pattern of the globin from hemoglobin.

Central cuts from each band were concentrated on a rotary evaporator to remove the butanol, then dialyzed against 0.1 N acetic acid and lyophilized.

A 30 mg. sample of each was converted to the dinitrophenyl derivative at pH 9 with 2,4-dinitrofluorobenzene (1 hr. at 40°). The recovered derivative was hydrolyzed in 6 N HCl for 15 min.[2] at 110°. The yellow ether-soluble material was distributed to 120 transfers in the system benzene–

TABLE I

AMINO ACID COMPOSITION OF 22-HOUR HYDROLYSATES OF α AND β COMPONENTS

Values are given as g. of amino acid per 100 g. protein (a correction of 13% bound DCA has been assumed).

Amino acid	α	β	Average α and β	Lit.[4,5] for normal white hemoglobin A
Aspartic	9.35	9.84	9.60	9.64
Threonine	5.78	4.61	5.20	5.13
Serine	6.03	2.82	4.43	4.05
Glutamic	4.42	8.77	6.60	6.55
Proline	4.83	4.82	4.83	5.02
Glycine	2.93	5.52	4.23	4.32
Alanine	10.60	7.37	8.99	9.15
Valine	8.37	12.22	10.30	10.36
Methionine	1.63	0.75[a]	1.19	1.25
Leucine	13.35	14.62	13.99	13.94
Tyrosine	2.84	2.94	2.89	3.05
Phenylalanine	6.67	7.85	7.26	7.33
Lysine	9.75	9.50	9.63	9.28
Histidine	9.45	8.17	8.81	8.32
Arginine	3.20	3.38	3.29	2.82
Cysteine	0.74[a]	1.32[a]	1.03	0.73–1.10
Tryptophan[b]	1.27	2.48	1.88	2.03

[a] Determined as cysteic acid or methionine sulfone after oxidation with performic acid. [b] Values calculated from absorption spectrum studies on intact protein.

(2) H. S. Rhinesmith, W. A. Schroeder and N. Martin, THIS JOURNAL, **80**, 3358 (1958).

glacial acetic acid–0.1 *N* HCl (2,2,1).[3] Material from the left band of Fig. 1 gave a good yield of a DNP-peptide identified by hydrolysis, partition ratios and chromatography as DNP-Val-Leu. That from the right gave none of this material. From the work of Rhinesmith, Schroeder and Martin,[2] it appears that the left band of Fig. 1 contains protein corresponding to their α chain while the right, lacking this terminal sequence, probably contains the terminal Val-His-Leu sequence of their β chain.

The analytical data in the table show wide differences for most of the amino acid residues.

The globins from horse hemoglobin recently have been resolved.[6]

The over-all results reported in this communication are interesting in connection with those reported by Singer and Itano.[7]

(3) W. Hausmann, J. R. Weisiger and L. C. Craig, THIS JOURNAL, **77**, 723 (1955).

(4) H. Stein, H. G. Kunkel, R. D. Cole, D. H. Spackman and S. Moore, *Biochem. Biophys. Acta*, **24**, 640 (1957).

(5) R. D. Cole, W. H. Stein and S. Moore, *J. Biol. Chem.*, **233**, 1359 (1958).

(6) S. W. Wilson and D. B. Smith, *Can. J. Biochem. Physicl.*, **37**, 405 (1959).

(7) S. J. Singer and H. A. Itano, *Proc. Nat. Acad. Sci.*, **45**, 174 (1959).

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RECEIVED MARCH 19, 1957

OXIDATIVE METABOLISM OF ESTROGENS[1]

Sir:

The major path of metabolism of the estrogenic hormones in man is oxidative. Estrone and estradiol are interconvertible *in vivo* and this study was initiated to determine whether estrone or estradiol served as the immediate precursor of the more oxygenated metabolites. A mixture of estradiol 6,7-H^3 and estrone-16-C^{14} was injected rapidly and intravenously in human subjects. Urine collections were obtained at frequent intervals and a blood sample was obtained at 30 minutes after injection. Estrone, estradiol-17β, estriol (16α,17β), epiestriol (16β,17β) and 2-methoxyestrone were isolated and purified to radiochemical homogeneity. These were analyzed for C^{14} and H^3 and the ratio (C^{14}/H^3) of the two isotopes[2] measured. Three studies with concordant results were made on different patients using differing weight ratios of estrone and estradiol. A representative experiment is shown in Table I.

The values obtained show that estrone approaches C^{14}/H^3 of the injected mixture more rapidly than does estradiol. Earlier in the experiment when these values for estrone and estradiol are different, the ratios for estriol, epiestriol and 2-methoxyestrone agree with the estrone ratio. The

(1) We express our appreciation to our colleagues Drs. Leon Hellman and Barnett Zumoff, who made possible the studies with the patients, and for the support of grants from the American Cancer Society and from the National Cancer Institute (CY-3207), United States Public Health Service.

(2) The ratio is obtained from the counts per minute measured for C^{14} and H^3 in a Packard "Tri-Carb" Scintillation Spectrometer, Model 314, and is therefore an arbitrary value. Portions of the injection solution were counted concurrently with the metabolites to insure that standard conditions were observed.

TABLE I

ISOTOPE RATIO OF URINARY AND BLOOD METABOLITES

Dose: Estrone-16-C^{14} 10.9 mg., 29.5 μc. Estradiol-6,7-H^3 1.5 mg., 150 μc. Measured isotope ratio,[2] C^{14}/H^3 = 0.92. *Insufficient metabolite for analysis.

Time of urine collection, minutes	C^{14}/H^3				
	Estrone	Estradiol	Estriol	Epi-estriol	2-Methoxy-estrone
0–30	1.15	0.08	*	*	*
30–60	0.84	.39	0.72	*	0.82
60–120	.81	.62	.77	0.77	.82
120–180	.83	.66	.81	.76	.87
240–300	.85	.74	.80		.91
360–540	.87	.81	.86		.85
Blood sample at 30 minutes					
"Free"	4.00	0.04	*		
"Conjugated"	0.85	0.48	*		

free and conjugated blood steroids show different C^{14}/H^3; the conjugates mirrored the urinary values found from 30 to 60 minutes later.

It can be concluded that (1) estrone serves as the principal, if not the exclusive, substrate for hydroxylation at C-16 to give both the α and β hydroxy compounds. (2) Estrone similarly serves as the principal substrate for hydroxylation in ring A. (3) The combined rate of all reactions in the body by which estradiol is oxidized to estrone is greater than the similar processes reducing estrone to estradiol. (4) Virtually only estrone is available for metabolic transformation, *i.e.*, conclusions 1 and 2 may well be a consequence of conclusion 3. (5) While estradiol may be the hormone produced by the ovaries, peripheral hormone action may be effected largely through estrone, which formally is a metabolite.

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RECEIVED MARCH 12, 1959

REACTION PRODUCTS OF GROUP VIB METAL CARBONYL COMPOUNDS WITH ORGANIC COMPOUNDS OF TRIVALENT GROUP VA ELEMENTS

Sir:

We wish to report the preparation of compounds formed by the reaction of trivalent Group VA compounds (such as tertiary phosphines and phosphites) with carbonyl compounds of Group VIB metals (such as chromium hexacarbonyl and benzene chromium tricarbonyl).

The reactions of metal carbonyls with trivalent phosphorus compounds to give derivatives of carbonyls of the first series of the Group VIII metals (*e.g.*, bis-(triphenylphosphine)-nickel dicarbonyl)[1] and of Group VII metals (*e.g.*, triphenylphosphine-manganese tetracarbonyl)[2] have previously been described. No reactions between the hexacarbonyls of the Group VIB metals and trivalent phosphorus compounds have been reported, although reactions of the hexacarbonyls with ammonia,[3,4]

(1) W. Reppe and W. J. Sweckendiek, *Ann.*, **560**, 104 (1948).

(2) W. Hieber and G. Wagner, *Z. Naturforsch.*, **12b**, 478 (1957).

(3) W. Hieber and W. Abeck, *ibid.*, **7b**, 320 (1952).

(4) W. Hieber, W. Abeck and H. K. Platzer, *Z. anorg. allgem. Chem.*, **280**, 252 (1955).