The remaining series of monomers are so incomplete that the data are presented (Figs. 13-15) without any attempt at correlation.

Summary

1.   The ultraviolet absorption of the monomers and polymers of a number of substituted vinyl aromatic compounds are reported for the range 250-300 mμ.

2.   The ultraviolet absorption method has been successfully applied to the determination of the monomer ratio in copolymers of butadiene with many of these substituted vinyl aromatic compounds.

3.   The relative intensities of absorption for various substituted polystyrenes have been compared with predictions based on a theory by Sklar.

URBANA, ILLINOIS   RECEIVED MARCH 20, 1947

The Ultraviolet Absorption Spectra of Simple Unsaturated Compounds.   I.   Mono- and p-Disubstituted Benzene Derivatives

BY LEONARD DOUB AND J. M. VANDENBELT

Benzene exhibits absorption in two regions of the accessible ultraviolet. One is a highly resolved band of low intensity (ϵ max. = ca. 200) at 240-60 mμ. The other is an intense band (ϵ max. = 7,400) in the neighborhood of 200 mμ. The majority of studies reported in the literature have been concerned with the longer wave length absorption and have assumed that absorption of benzene derivatives in this region is best explained by modification of this band. That the shorter wave length high intensity absorption might be displaced in derivatives to account for spectra near 250 mμ has been recognized in at least one instance.[1] Surprisingly, however, there appears to have been no systematic study of the fate of this band in simple benzene derivatives although numerous isolated reports of high intensity short wave length absorption are on record.[2-8] Clearly it is of importance for the rationalization of the effect of substitution on benzene absorption to relate the maxima of a given derivative to one or the other of these parent bands and to discriminate from effects following the introduction of new chromophoric groups.

This study of the effect of substitution on the short wave length (ca. 200 mμ) absorption of benzene in general corroborates and extends the concept that this band is capable of displacement to and beyond 250 mμ. In addition, it would appear to provide a basis for a more logical interpretation of benzenoid absorption than has heretofore appeared.

Methods and Materials.—A Beckman quartz spectrophotometer with a hydrogen discharge tube source was used for all measurements. Extinction coefficients were calculated by dividing the observed densities by the molar cell concentration. Dilutions were prepared such that the peak absorption of each band fell on the density scale betwen 0.4 and 0.8. Readings in the difficulty accessible region below 220 mμ were obtained so far as possible at a slit width of 1.0 mm.; if necessary the check switch position at 0.1 was used.

Calibration of the instrument with a mercury vapor arc indicated that the wave length scale in the ultraviolet region was correct to a few tenths of a millimicron. Reported wave lengths of band peaks are therefore taken directly from the highest instrument readings as the peak was read through in small increments; in the case of a broad band the center was estimated by symmetry from its graph.

Liquids were redistilled before use. Solid compounds were recrystallized from a suitable solvent. Boiling and melting points were checked with the literature values as a confirmation of purity. Liquid samples such as benzene and toluene were weighed into stoppered flasks and dissolved in methanol. This solution was diluted into water and a blank containing the same amount of methanol prepared for instrument reading. The methanol concentration was never greater than 2%.

General Absorption Characteristics.—Figure 1 gives curves of representative monosubstituted and p-disubstituted derivatives.[9]   The curves

(1)   Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944, p. 151.

(2)   Dede and Rosenberg, Ber., **67**, 147 (1934).

(3)   Flexser, Hammett and Dingwall, THIS JOURNAL, **57**, 2103 (1935).

(4)   Morton and McGookin, J. Chem. Soc., 901 (1934).

(5)   Conrad-Billroth, Z. physik. Chem., B19, 76 (1932); **B20**, 222 (1933); **B25**, 139 (1934); **B25**, 217 (1934).

(6)   Kumler and Strait, THIS JOURNAL, **65**, 2349 (1943).

(7)   Kumler, ibid., **68**, 1184 (1946).

(8)   The above are a few representative studies on short wave length benzenoid absorption. The following sources may be consulted for others: "International Critical Tables," Vol. V, McGraw-Hill Book Co., Inc., New York, N. Y., 1929; Morton, "Absorption Spectra," 2nd ed., Adam Hilger, London, 1942; Brode, "Chemical Spectroscopy," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1943.

(9)   The absorption spectra of the majority of the compounds contained in this study have been reported previously at least in part. These have been repeated and recorded here because most of the earlier curves are unreliable in the range below 220-230 mμ or are not reported at all in this region. In addition, the diversity of solvents used in these previous studies renders comparison extremely difficult. In this study the solvent is water, except in those few cases where a trace of methanol was used to facilitate solution of the compound. Water was chosen as the solvent because the conditions

have been arranged in order of increasing wave length of the more intense band. Where two intense bands occur the more displaced band is used. The values of $\lambda_{max.}$, $\epsilon_{max.}$, and $\Delta\lambda$ (displacement of the primary band relative to the 203.5 mμ band of benzene) of all the compounds included in this study are contained in Tables I and II. The most displaced intense bands are designated in the tables as the first primary bands; where second intense bands occur, they are referred to as second primary bands.[10] The low intensity bands (exemplified by the 280 mμ band of aniline) when present (absent in sulfanilamide, nitrobenzene and others) are referred to as secondary absorption. The values assigned $\lambda_{max.}$ and $\epsilon_{max.}$ are those of the highest points of the broad regions of absorption, no cognizance being taken of resolution within these regions.

With the compounds presented here, primary absorption is greater than $\epsilon = 6 \times 10^3$, while secondary absorption is never more than $\epsilon = 2.6 \times 10^3$, and usually much less. Thus the two types are easily differentiated. Obviously, where a compound is capable of existing simultaneously in two or more distinct molecular forms, this criterion for secondary absorption must be applied with care. Thus aniline, whose cation is transparent at 230 mμ, gives only feeble absorption at this point when measured at low pH ranges, although the molecular species gives intense absorption in this region.

The monosubstituted derivatives (Table I) exhibit primary absorption over the whole range from that of benzene at 203.5 mμ to that of nitrobenzene at 268.5 mμ, with a rather general increase of intensity with displacement. Secondary absorption in general is displaced approximately in proportion to the primary band and varies considerably in intensity, although there is some tendency to increase in intensity with displacement. The resolution exhibited markedly in the secondary, and slightly in the primary bands of benzene itself decreases with displacement of the bands.

The disubstituted compounds (Table II) show primary absorption starting well in the region common to the monosubstituted derivatives and extending to the edge of the visible region. When present, the secondary absorption tends to increase in intensity with displacement in similar fashion to that of the monosubstituted compounds, although it seems somewhat more erratic. Frequently, secondary absorption appears to be absent. However, asymmetry of the single band in some of these instances suggests that the secondary may still be present fused with the first primary band although no distinct maximum can be identified. The primary band is distinctly greater than in the monosubstituted compounds, and, as

governing ionization of acids and bases are best known and most easily controlled in this medium. As a consequence, the absorption of molecular and ionic species can be assigned with considerable accuracy.

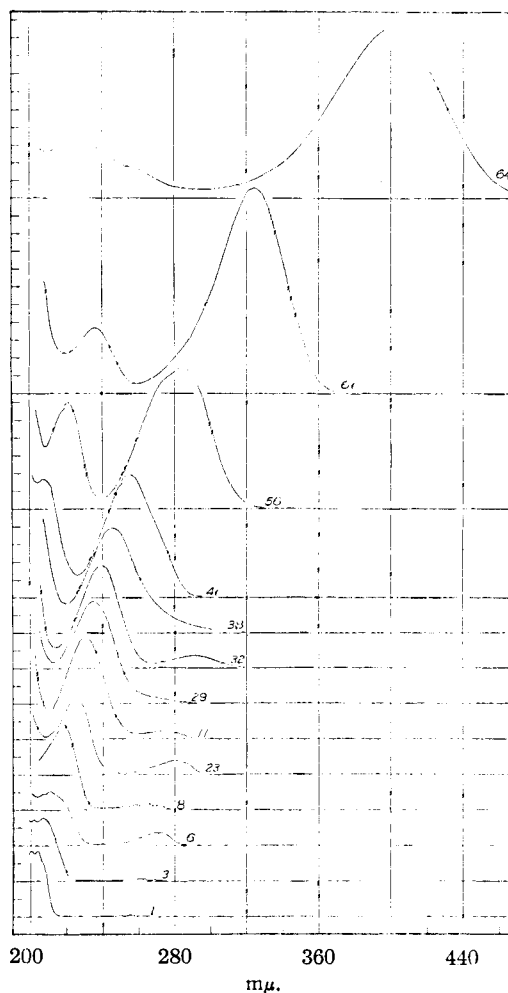(10) Attention has previously been called to the existence of these bands by Kulmer; he referred to them as X' bands.'

Fig. 1.—Ultraviolet absorption spectra of representative mono- and p-disubstituted benzene derivatives arranged to show progression of the bands. Each unit on the ordinate axis represents an extinction coefficient ϵ of 2000. The numbers refer to compounds listed in Tables I and II.

the wave length increases, the intensity of absorption rises coincident with decreasing monochromatism of the band. The secondary absorption, on the other hand, retains the same magnitude in the two types. It is interesting to note that with derivatives exhibiting more displaced absorption, a second band of relatively intense absorption appears in the shorter wave length region. For consistency, it has been designated in this paper as the second primary band. Its ratios with the primary band (lower part of column 10, Table II) are very similar to those found by Kumler.[10] The occurrence of the second primary band apparently is a general property of benzenoid absorption. Although the instrument used in this investigation does not allow measurement at the low wave lengths necessary to confirm this, it appears reasonable to assume that this band is present in most if not all simple benzenoid compounds.

<div align="center">

TABLE I

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF MONOSUBSTITUTED BENZENE DERIVATIVES

</div>

Compd. no.	Compound	Primary band $\lambda_{max.}$, $m\mu$	$\epsilon_{max.}$	Secondary band $\lambda_{max.}$, $m\mu$	$\epsilon_{max.}$	$\lambda_{sec.}/\lambda_{pri.}$	$\Delta\lambda$ ($\lambda_{pri.}$ −203.5)	$\Delta\lambda_{calcd.}$ ($\delta\lambda_0$ −23.5)
1	Benzene	203.5	7,400	254	204	1.25	0	0.55
2	Aniline cation	203	7,500	254	160	1.25	−0.5	2.1
3	Toluene	206.5	7,000	261	225	1.25	3.0	6.1
4	Chlorobenzene	209.5	7,400	263.5	190	1.25	6.0	5.8
5	Bromobenzene	210	7,900	261	192	1.24	6.5	8.0
6	Phenol	210.5	6,200	270	1450	1.28	7.0	12.6
7	Anisole	217	6,400	269	1480	1.24	13.5	13.3
8	Benzenesulfonamide	217.5	9,700	264.5	740	1.22	14.0	(14.0)
9	Benzonitrile	224	13,000	271	1000	1.21	20.5	20.5
10	Benzoic acid anion	224	8,700	268	560	1.20	20.5	21.2
11	Benzoic acid	230	11,600	273	970	1.19	26.5	(26.5)
12	Aniline	230	8,600	280	1430	1.22	26.5	(26.5)
13	Phenol anion	235	9,400	287	2600	1.22	31.5	31.5
14	Acetophenone	245.5	9,800	...	..	..	42.0	39.8
15	Benzaldehyde	249.5	11,400	...	..	..	46.0	45.4
16	Nitrobenzene	268.5	7,800	...	..	.,	65.0	68.1

Limiting attention to the more displaced (*i. e.*, first) primary band, a progression of effect is obvious (Fig. 1). In contiguous compounds the appearance and order of intensity of the bands are similar, regardless of the diversity of substituent groups, and it would appear reasonable to interpret them all as being due to displacement of the 203.5 $m\mu$ band of benzene. Since this point of view has not generally been recognized, and since in some instances primary absorption might be assigned to unique chromophores formed by the substituent groups, further arguments for this concept will be considered in detail.

That absorption of this magnitude in the region under consideration cannot be assigned logically to the substituent groups themselves is borne out from a consideration of these groups (*e. g.*, —Br,

Fig. 2.—Ultraviolet absorption spectra of *n*-heptyl bromide (‒ ‒ ‒) compared with bromobenzene (——), and acetic acid (- - - -) compared with benzoic acid (——).

—OH, —NH₂, —COOH) when combined with alkyl chains. Even when these groups show absorption above 200 $m\mu$, the intensity is inadequate to account for that of the corresponding benzene derivative. Figure 2 gives the absorption curves of *n*-heptyl bromide compared with bromobenzene, and acetic acid compared with benzoic acid. In addition to being displaced, the absorption of these alkyl compounds would have to be increased about 40 and 300 times respectively to equal that of the benzene derivatives.[11]

The regularity in amount of displacement of both the primary and secondary bands in the derivatives relative to those in benzene is apparent from the $\lambda_{sec.}/\lambda_{pri.}$ ratios shown in Tables I and II. The ratios are calculated only for those compounds in which primary and secondary absorptions are separated widely enough in the spectrum to permit precise assignment of the position of the secondary maximum. When the secondary absorption is highly resolved, the wave length of the strongest resolved band is used. This similarity makes it appear extremely probable that both secondary and primary absorptions arise from the same chromophoric groups, rather than that the benzene substituents are acting independently as chromophores. There would be no reason to expect a uniform correspondence between two absorbing groups whose chromophoric properties are determined independently, and since the secondary bands have been associated with benzene absorption,[4,5] it is reasonable to infer that the primary band also is benzenoid in origin.

(11) It can be predicted that benzene derivatives whose substituent groups are themselves capable of intense absorption in this region may exhibit anomalous behavior. In this paper, a deliberate attempt was made to exclude compounds of this type. However, the nitro derivatives may conceivably fall in this class. Since the alkyl nitrates absorb in the 220 $m\mu$ region, while nitrobenzene absorbs at 268.5 $m\mu$, a displacement of almost 50 $m\mu$, it was felt that the two bands could be discriminated. Nevertheless, the exceptional behavior of the nitro compounds in some of the later developments in this paper may be a result of this feature of their absorption.
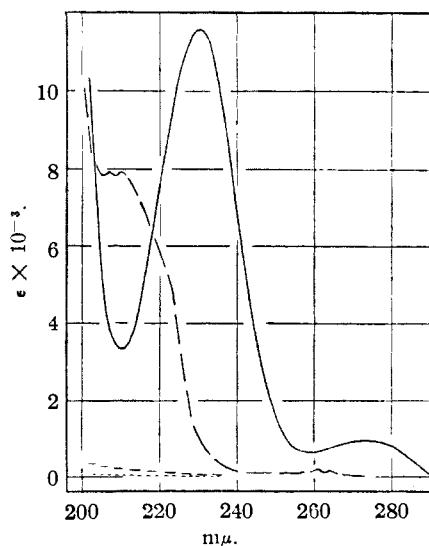
TABLE II

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF p-DISUBSTITUTED BENZENE DERIVATIVES

Compd.	Compound	Solvent	First primary band $\lambda_{max.,}$ mμ	$\epsilon_{max.}$	Second primary band $\lambda_{max.,}$ mμ	$\epsilon_{max.}$	Secondary band $\lambda_{max.,}$ mμ	$\epsilon_{max.}$	λsec./ λ1st. pri.	$\Delta\lambda$ (λ1st. pri. -203.5)
17	p-Toluidine cation	0.5 N HCl	207.5	7,900	...	....	260	320	1.25	4.0
18	p-Chloroaniline cation	2 N HCl	215.5	9,200	...	....	263	360	1.22	12.0
19	Sulfanilamide cation	2 N HCl	217.5	10,700	...	....	264	700	1.21	14.0
20	p-Bromoaniline cation	0.1 N HCl	218.5	8,860	...	....	263	220	1.20	15.0
21	p-Aminophenol cation	pH 3	218.5	6,240	...	....	262.5	1450	1.20	15.0
22	p-Aminobenzonitrile cation	2 N HCl	223	14,300	...	....	270	940	1.21	19.5
23	p-Chlorophenol	0.1 N HCl	225	8,900	...	....	279.5	1600	1.24	21.5
24	p-Toluenesulfonamide	.1 N HCl	226	13,200	...	....	262.5	540	..	22.5
25	p-Aminobenzoic acid cation	2 N HCl	226.5	12,300	...	....	270	970	1.19	23.0
26	p-Toluidine	pH 11	232	8,900	...	....	286	1600	1.23	28.5
27	p-Tolunitrile	0.1 N NaOH	234	17,200	...	....	268	750	..	30.5
28	p-Chlorobenzoic acid anion	pH 11	235	13,600	...	....	...	..	..	31.5
29	p-Toluic acid anion	1 N NaOH	235	11,600	...	....	...	..	..	31.5
30	p-Chlorobenzonitrile	0.1 N NaOH	237.5	19,100	...	....	269.5	820	..	34.0
31	p-Bromobenzoic acid anion	1 N NaOH	239	14,100	...	....	...	..	..	35.5
32	p-Chloroaniline	0.1 N NaOH	239	11,700	...	....	290	1500	1.21	35.5
33	p-Bromoaniline	.1 N NaOH	239.5	12,800	...	....	290	1340	1.21	36.0
34	p-Aminoacetophenone cation	2 N HCl	240	13,000	...	....	280	1050	..	36.5
35	p-Chlorobenzoic acid	0.1 N HCl	241	16,300	...	....	...	..	..	37.5
36	p-Toluic acid	1 N HCl	241.5	14,400	...	....	...	..	..	38.0
37	p-Chlorophenol anion	1 N NaOH	244	11,700	...	....	298	2600	1.22	40.5
38	p-Hydroxybenzoic acid (1st) anion	pH 8	245	11,900	...	....	...	..	λ 1st. Pri.	41.5
39	p-Bromobenzoic acid	0.1 N HCl	245.5	16,400	...	....	...	..	λ 2nd. Pri.	42.0
40	p-Anisic acid anion	.1 N NaOH	247	14,000	...	....	...	..	..	43.5
41	p-Hydroxybenzoic acid	.1 N HCl	255	13,900	207.5	13,400	...	..	1.23	51.5
42	p-Methylacetophenone	pH 5	256	14,400	...	....	...	..	..	52.5
43	p-Anisic acid	0.1 N HCl	256.5	15,900	208	14,750	...	..	1.23	53.0
44	Sulfanilamide	pH 7	258	16,600	...	....	...	..	..	54.5
45	p-Nitroaniline cation	2 N HCl	258	8,700	208	7,800	...	..	1.24	54.5
46	p-Bromoacetophenone	pH 6	258.5	17,500	...	....	...	..	..	55.0
47	p-Nitrobenzoic acid	0.1 N HCl	264.5	12,400	...	....	...	..	..	61.0
48	p-Aminobenzoic acid anion	.1 N NaOH	265	14,900	...	....	...	..	..	61.5
49	p-Dinitrobenzene	.1 N NaOH	266	14,500	...	....	...	..	..	62.5
50	p-Aminobenzonitrile	1 N NaOH	270	19,800	212	13,800	...	..	1.27	66.5
51	p-Nitrobenzoic acid anion	1 N NaOH	274	10,600	...	....	...	..	..	70.5
52	p-Hydroxyacetophenone	0.1 N HCl	275	14,300	218.5	10,700	...	..	1.26	71.5
53	p-Methoxyacetophenone	.1 N HCl	276.5	15,500	219.5	10,600	...	..	1.26	73.0
54	p-Hydroxybenzoic acid (2nd) anion	1 N NaOH	280	16,300	...	....	...	..	..	76.5
55	p-Nitrochlorobenzene	pH 6	280	10,300	217	7,150	...	..	1.29	76.5
56	p-Hydroxybenzaldehyde	0.1 N HCl	283.5	16,000	221	12,000	...	..	1.28	80.0
57	p-Aminobenzoic acid	pH 3.75	284	14,000	219.5	9,900	...	..	1.29	80.5
58	p-Nitrotoluene	pH 6	285	9,250	217	6,700	...	..	1.31	81.5
59	p-Aminoacetophenone	0.1 N NaOH	311.5	17,100	231	6,950	...	...	1.35	108.0
60	p-Nitrophenol	pH 3	317.5	10,000	225.5	6,900	...	..	1.41	114.0
61	p-Hydroxyacetophenone anion	0.1 N NaOH	324.5	23,100	236.5	7,500	...	..	1.38	121.0
62	p-Hydroxybenzaldehyde anion	.1 N NaOH	330	27,900	238	7,500	...	..	1.39	126.5
63	p-Nitroaniline	1 N NaOH	381	13,500	226	6,700	...	..	1.69	177.5
64	p-Nitrophenol anion	1 N NaOH	402.5	19,200	226	6,500	...	..	1.78	199.0

It would appear, then, that the absorption of many if not all simple mono- and p-disubstituted benzene compounds can be pictured as a progression of at least three bands which, regardless of the degree of displacement, maintain a relatively regular wave length relationship. The most accessible and perhaps most typical of those would seem to be the first primary band.
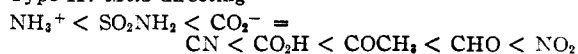
Interaction of Groups in Band Displacement. —If the substituent groups are divided into electron contributing (ortho–para directing) and electron attracting (meta directing) types, and arranged in order of increasing $\Delta\lambda$ values of the primary band from Table I, the following series are obtained

Type I: Ortho–para directing

CH$_3$ < Cl < Br < OH < OCH$_3$ < NH$_2$ < Ō

Type II: Meta directing

NH$_3^+$ < SO$_2$NH$_2$ < CO$_2^-$ =
$\qquad$ CN < CO$_2$H < COCH$_3$ < CHO < NO$_2$
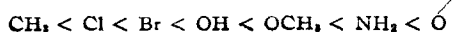
It is interesting to note the effect of various combinations of these groups in p-disubstituted compounds on the displacement of the first primary bands. Where two groups of the same type are opposed this band is displaced but slightly compared to that of the most displaced monosubstituted compound. This is illustrated in p-nitrobenzoic acid. Here where each group alone is capable of considerable displacement of the primary benzene band ($\Delta\lambda -$ COOH = 26.5, $\Delta\lambda$ —NO$_2$ = 65.0), the combination gives approximately the same displacement ($\Delta\lambda$ = 61) as that ascribable to the nitro group alone. In contrast, combination of two groups of different types in the para positions gives a marked displacement

relative to the most displaced monosubstituted
compound. This effect is least with groups sepa-
rately giving the least displacement and greatest
with those groups with the greatest effect individ-
ually. The first primary band of p-nitroaniline
has a $\Delta\lambda$ value of 177.5 which represents three to
six times the effect of the single groups on the
primary benzene band. Illustrative of the action
of weakly displacing groups is toluenesulfonamide.
Here the displacement of the disubstituted com-
pound ($\Delta\lambda = 22.5$) represents only a small in-
crease over the displacement of benzenesulfona-
mide ($\Delta\lambda = 14.0$). Intermediate between these
extremes is p-nitrophenol where the displacement
($\Delta\lambda = 114$) is slightly less than twice that of the
nitro group ($\Delta\lambda = 65$).

This situation suggests that the displacement
of the primary band of benzene by substitution is
due to an electronic interaction of the groups with
the benzene nucleus which results in a lessening of
the constraint or force holding the electrons re-
sponsible for the absorption of light. This dis-
placement is greatest for those groups capable of
greatest electronic interaction across the ring (or-
tho-para vs. meta orienting) and least for those
groups capable of the least interaction across the
ring (ortho-para vs. ortho-para; meta vs. meta).

If consideration is limited to those p-disubsti-
tuted compounds containing opposite types of
groups in the two positions the relation shown in
Fig. 3 can be obtained. Here the $\Delta\lambda$ values of the
disubstituted compounds are plotted against the
product of the $\Delta\lambda$ values of the monosubstituted
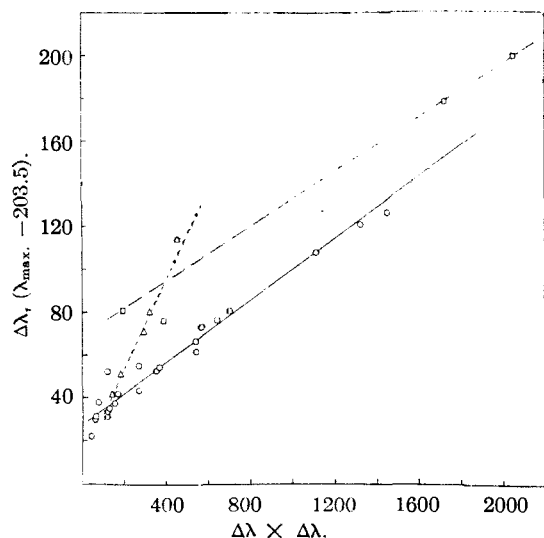compounds containing these groups.

Fig. 3.—Correlation between observed displacement
values ($\Delta\lambda$) of p-disubstituted compounds and the product
of the respective $\Delta\lambda$ values of monosubstituted compounds.
— —, nitro compounds (squares); - - -, hydroxy
compounds (triangles); ———, main trend (circles).
Only those derivatives containing ortho-para vs. meta
directing groups are used.

It is apparent from inspection that the distri-
bution of points allows three separate trends to be
identified. The majority of the compounds fall
in the series represented by the solid line. The
agreement, while far from exact, is close enough to
suggest that with these compounds the tendency
of a substituent group to displace benzene absorp-
tion separately in the monosubstituted compounds
measures semiquantitatively its effect in p-disub-
stituted compounds. The points in Fig. 3 which
constitute deviations from this general trend can
be divided into two families each of which seems to
be fairly consistent within itself. These families
are shown by the two dotted lines, one of which
represents all of the compounds containing the
hydroxyl group, the other representing all the
compounds containing the nitro group. It would
appear that within these separate families, too, the
displacement of the p-disubstituted compound is
a function of the displacement caused separately
in the monosubstituted compounds, although the
evidence is not entirely convincing in the case of
the nitro compounds. This is in line with the
generally recognized interaction of groups in the
para positions and points toward a reinforcement
of the existing electronic effect of one group by the
complementary action of another.

This approach to a quantitative relationship
would seem to justify further examination and
redefinition of concepts with the object of develop-
ing a more comprehensive formulation of the situ-
ation.

From the data so far presented it may be tenta-
tively assumed that the $\Delta\lambda$ value of a substituent
group is to some extent a measure of the electron
attracting or contributing power of that group.
It is conceivable that some of the groups used in
Fig. 3 may have had a different electronic relation
to the ring in the p-disubstituted compounds than
in the monosubstituted compounds whose $\Delta\lambda$
value was used in the formulation. An example
of how different effects may be ascribed to the
same group is that of the carboxyl group in p-
aminobenzoic acid and p-nitrobenzoic acid (men-
tioned above).

In Fig. 3 only those compounds containing
ortho-para vs. meta directing groups were selected.
It was assumed that the $\Delta\lambda$ values of the mono-
substituted compounds measured the electron
contributing influence for the ortho-para directing
groups and the electron attracting effect for the
meta directing groups. Since the orienting in-
fluence of a group in chemical substitution may
depend on polarizability factors called into opera-
tion upon the approach of a reactive center, there
is no assurance that the result is a measure of
the electronic situation in the "resting" molecule.
The halogens have been considered an example of
this kind of relation.[12]

(12) Remick, "Electronic Interpretations of Organic Chemistry,"
John Wiley & Sons, Inc., New York, N. Y., 1943; Branch and Cal-
vin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New
York, N. Y., 1945.

Similarly, in Fig. 3 it is possible, for example, that a $\Delta\lambda$ value for a monosubstituted compound which was actually the result of an electron attraction influence may have been used to describe a disubstituted compound in which the group exerted an electron contributing influence by virtue of the effect of a more powerfully electron attracting group and *vice versa*. In the monosubstituted compounds the action of the group toward the ring is essentially determined by its relation to the hydrogen atom which occupies the *para* position. It would thus appear that the presentation in Fig. 3 could be improved by deriving the band displacement values ($\Delta\lambda$) of some of the groups from those *p*-disubstituted compounds where the directional character of the electronic interaction is more clearly defined than in the corresponding monosubstituted compounds. Because of the relatively weak effect of hydrogen, this procedure would have the added advantage that the values so derived could be expected to be more representative of the group.

Another shortcoming of the presentation of data in Fig. 3 is apparent from inspection. If the trend represented by the solid line be continued it appears to intersect the abscissa at some point negative to the zero point. This would seem to indicate that the fundamental chromophore originates at shorter wave length than the benzene primary band and the hydrogen should be assigned a displacing effect with respect to this hypothetical chromophore. Hydrogen then can take its place with the other substituent groups and should be assigned a $\Delta\lambda$ value; the effect of the other groups when substituted for hydrogen is generally to displace the fundamental band still farther.[13]

Using this concept and modifying the presentation in Fig. 3 along the lines suggested above, a more comprehensive description of the relation of substitution to the displacement of the primary band can be achieved. The formulation employed, as outlined below, is one of several which could be used, and no fundamental significance should be attached to the selection of this particular approach. Assume that the primary band of benzene has its hypothetical origin, as discussed above, at a point λ_0, and define the displacement produced on this band by substitution as $\Delta\lambda_0$. If $203.5 - \lambda_0 = A$, then the observed $\Delta\lambda$ values can be converted to the new system by the relation $\Delta\lambda_0 = \Delta\lambda + A$. From Fig. 3 it can be assumed that the plot of the $\Delta\lambda$ product of the substituent groups *versus* the $\Delta\lambda$ observed for the disubstituted compound approximates a straight line. From

(13) Introduction of this concept allows consideration of a class of compounds which could not be presented in Fig. 3. The aniline cation shows a primary band at 203 mμ which would give a negative $\Delta\lambda$ value for the amine cation. Since negative $\Delta\lambda$ product values were meaningless in Fig. 3, compounds containing the amine cation were not included. They could be included, however, if their absorption was considered to originate from a hypothetical point at a shorter wave length than that of the benzene primary band itself.

the assumption of λ_0 this line must pass through the origin. This gives the equation

$$\delta\lambda_0' \times \delta\lambda_0'' = k\Delta\lambda_0 \qquad (1)$$

where $\delta\lambda_0$ is a derived value for the displacing effect of each group in the *p*-disubstituted compounds and is analogous to the monosubstituted $\Delta\lambda$ value of Fig. 3 but implies that hydrogen is treated as a substituent group and itself carries a $\delta\lambda_0$ value. Since equation (1) is arbitrary it probably can have no absolute solution, consequently no attempt was made to evaluate the constants on this basis. However, a working solution can be obtained by an appropriate selection of compounds in which the $\delta\lambda_0$ values of the groups in the compounds are identified with the $\Delta\lambda_0$ of the compound itself. Of course, due to the approximate nature of the relationship, the values derived in this manner for equation (1) would vary depending on the choice of compounds.

For the purpose of this evaluation, compounds containing the amino, carboxyl and sulfonamide groups were selected. It seems fairly certain that these groups in *p*-aminobenzoic acid and in sulfanilamide preserve the same electronic relation to the benzene ring as shown individually in their respective monosubstituted compounds. The $\Delta\lambda_0$ values (*i. e.*, $\Delta\lambda + A$) of the three corresponding monosubstituted compounds were arbitrarily equated to $\delta\lambda_0'$ and $\delta\lambda_0''$ of the groups and combined in equation (1) for sulfanilamide and *p*-aminobenzoic acid as follows: $(\Delta\lambda' + A)(\Delta\lambda'' + A) = k(\Delta\lambda + A)$, where $\Delta\lambda'$ and $\Delta\lambda''$ correspond to the displacement values of the monosubstituted compounds involved.

Evaluation of the constants in equation (1) by solution of simultaneous equations leads to

$$\delta\lambda_0' \times \delta\lambda_0'' = 24.05\ (\Delta\lambda_0) \qquad (2)$$

where $\lambda_0 = 180$ mμ and $A = 23.5$.

Using these values for the amino, carboxyl and sulfonamide groups as standard it is possible to calculate from combinations with other substituent groups the values for these groups which correspond to equation (2). A consequence of this evaluation is that hydrogen as a substituent automatically possesses the same value when opposed to both *ortho–para* and *meta* directing groups. Since it is unlikely that hydrogen would have identical values in these instances, this may be considered an inherent weakness of the treatment.

Limiting attention to those compounds containing *ortho–para* directing *vs.* *meta* directing groups the relationship shown in Fig. 4 is obtained. Here the observed $\Delta\lambda_0$ values of the disubstituted compounds are plotted against the product of the $\delta\lambda_0$ values derived for the separate groups according to equation (2). The solid points represent the compounds which were used to derive values for their respective groups and no significance can, of course, be attached to the fact that they fall exactly on the line representing

equation (2) (the solid line Fig. 4). They are included in the plot, however, since they represent real points and are part of the data presented.[14]
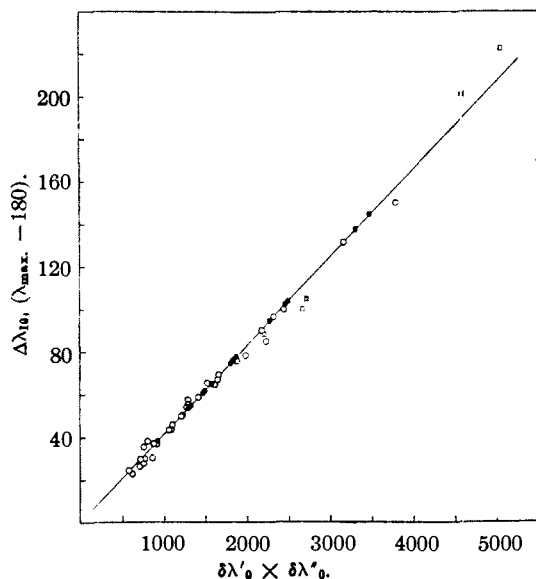

Fig. 4.—Correlation between observed displacement values ($\Delta\lambda_0$) of p-disubstituted compounds and the product of the derived $\delta\lambda_0$ values for the respective substituent groups: solid points, standard compounds; squares, nitro compounds.

In Fig. 4, in contrast to the formulation of Fig. 3, hydrogen is considered as a substituent and the amine cation is included. The inclusion of mono-substituted derivatives is the only exception to the limitation that compounds included in Fig. 4 contain *ortho–para* directing *vs. meta* directing groups.

A list of the $\delta\lambda_0$ values derived from equation (2) and used in Fig. 4 is given in Table III for each group along with reference to the p-disubstituted compound used for its evaluation. These values have been converted to quantities comparable with the observed $\Delta\lambda$ values in monosubstituted compounds by subtracting 23.5, and are included in Table I as $\Delta\lambda_{calcd}$. adjacent to the column of observed $\Delta\lambda$ values to facilitate comparison.

Examination of Fig. 4 shows unquestionably that a correlation is established. The family of hydroxyl compounds which formed a separate

(14) In deriving the $\delta\lambda_0$ values for the groups used in Fig. 4, only compounds in which the group was combined with a strongly effective group of the opposite type were used. For example, the value for the —OH group was calculated in the compound p-hydroxybenzoic acid, using the same value for —COOH originally employed in equation (2). The —CHO group was then calculated in the compound p-hydroxybenzaldehyde, using the value obtained for —OH as the reference standard. Similarly, —CH₃, —Cl and —Br were determined against —COOH. In a few cases where alternative standard groups could be used, that one giving the more representative $\delta\lambda_0$ value was chosen. Use of the more strongly displacing groups insured that the $\delta\lambda_0$ value of the group was decisively due to the kind of electronic interaction represented by its type. It also contributed to accuracy, since the larger displacements reduced the relative error in assignment of $\Delta\lambda$ values.

TABLE III

$\delta\lambda_0$ VALUES FROM EQUATION (2)

Substituent group	Compd. no. (Table II) used for calcn.	$\delta\lambda_0$	Substituent group	Compd. no. Table II) used for calcn.	$\delta\lambda_0$
—H	11, 12	24.05	—CN	27	43.9
—NH₃⁺	21	25.6	—CO₂⁻	29	44.7
—CH₃	36	29.6	—CO₂H	57	50.0
—Cl	35	29.3	—NH₂	44, 57	50.0
—Br	39	31.5	—O⁻	61	55.0
—OH	41	36.1	—COCH₃	52	63.3
—OCH₃	43	36.8	—CHO	56	68.9
—SO₂NH₂	44	37.5	—NO₂	60	91.6

pattern in Fig. 3 now follows the general trend quite satisfactorily. The nitro compounds (the squares in Fig. 4) which were also exceptional in Fig. 3 conform much better here. However, this last class of compounds contains the majority of the outstanding deviations. If the nitro compounds are included in the general trend, then of the 37 non-standard compounds in Fig. 4, none of the $\Delta\lambda_0$ values deviates more than 14 mμ from the derived line and only 8 deviate more than 5 mμ. If the nitro compounds are excluded, the maximum deviation is cut to 8 mμ, and only 4 deviate more than 5 mμ from the line.

This accuracy is surprising and perhaps even fortuitous in view of the inherent error involved in assignment of the wave length maxima in some instances, where extremely broad, asymmetric, or partially resolved bands occur. The wave length selection is difficult in the lower wave length region, where there may be fusion of the band with still shorter wave length absorption. Uncertainty in this region is especially contributive to error since a small variation in the observed $\Delta\lambda$ of a group represents a relatively large part of the displacement value.

Despite the limitations pointed out previously, the correlation of Fig. 4 is impressive, and justifies the assumption that the primary band, is a common denominator of benzenoid absorption and identifies it as such even where it extends into the region where the original secondary absorption of benzene itself appears. This explanation receives added support when it is remembered that the relation shown in Fig. 4 can be developed without reference to controversial electronic effects, the differentiation of groups employed being essentially based on the classical separation into *ortho–para* directing and *meta* directing in chemical substitution reactions.[15]

(15) A logical extension of the electronic interpretations of absorption preceding presentation of Fig. 4 is that a group should be capable of exhibiting either an electron contributing or an electron attracting effect on the displacement of the primary band, depending upon the relative demand placed upon it by the other group in the para position. This suggests that alternate values could be assigned the groups which would measure in equation (2) their displacing tendency when exhibiting opposite electronic effects. Unfortunately, not enough compounds of the requisite type were available to the authors to investigate this possibility systematically.

Ionization Effects in Band Displacement.—

As outlined above the displacement of the benzene band can be identified with either electron contributing or electron attracting influences in the substituent groups. As a corollary of this position any influence which clearly increases these tendencies should result in increased displacement of the band while influences diminishing these effects should lessen the displacement of the band by the substituent group. Acidic ionization is an ideal test of these influences; here ionization is invariably accompanied by the loss of a single proton and the group is left with an isolated negative charge. A residual charge of this sort placed on an electron attracting group would clearly diminish its attracting influence while, when placed on an electron contributing group, the effect should be that of increasing the already existing action of the group.

Ionization of benzoic acid, p-toluic acid, p-aminobenzoic aicd and sulfanilamide,[16] for example, is accompanied by a shift (6–8 mμ) to shorter wave length of the primary band coincident with lessening of the electron attracting effect of the group. Ionization of phenol, p-chlorophenol and p-hydroxybenzaldehyde is accompanied by a shift (19–47 mμ) of the primary band to longer wave length coincident with strengthening of the electron contributing effect of the group. Figure 5 gives the absorption curves of the various ionic species of p-hydroxybenzoic acid. Here as the pH is increased to higher values the ionization of the carboxyl group with shift to shorter wave length occurs first, and is then followed by ionization of the hydroxyl group with shift to longer wave length. Thus the general ionization characteristics can be identified with the groups themselves and appear regularly even in the presence of one another.

In the development of the relation shown in Fig. 4 it was convenient to assume that a group may change electronic character when opposed in the benzene ring by a more powerfully effective group of the same type. It might be expected that the usual ionization shifts would be reversed in such instances. The behavior of p-nitrobenzoic acid is illustrative of this situation. Ionization of the carboxyl group is usually accompanied by a shifting of the band to shorter wave length, but in this instance it is actually shifted to longer wave length, indicating that a weak contributing effect has been conferred upon the carboxyl group by the presence of a free negative charge.

A further confirmation of the validity of this interpretation of ionization effects and the function of the substituent groups on benzene absorption is found in the observation by Flexser, Hammett and Dingwall[3] that benzoic acid in strong sulfuric acid shows a shift of its absorption to longer wave length. Here ionization is probably accompanied by addition of a proton (positive

(16) Vandenbelt and Doub, THIS JOURNAL, 66, 1633 (1944).

charge) which would be expected to enhance the electron attracting powers of this group. As developed above, increase of the existing electron attracting power should be accompanied by a shift to longer wave length.
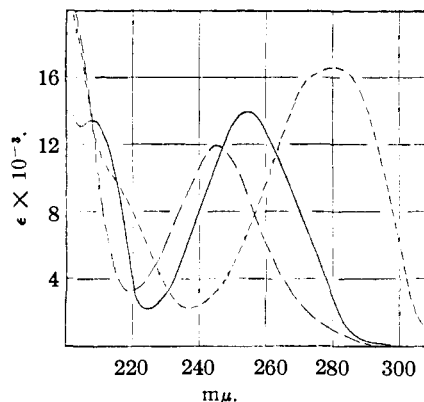

Fig. 5.—Ultraviolet absorption of p-hydroxybenzoic acid: pH 3.0 (———), pH 7.0 (— —), pH 11 (– – – –).

The following rule governing ionization and light absorption in mono- and *para* disubstituted compounds is tentatively proposed: Where ionization of a group attached to a benzene ring enhances the already existing tendency for electron transfer to or from the ring, the maximum of the primary band is shifted to longer wave length; where ionization diminishes this tendency, a shift to shorter wave length results.[17]

General

The quantitative aspects of the relationships pointed out in this paper have been derived primarily to validate the assumptions involved. As was pointed out, a number of approximations were made and the equations are in any case arbitrary. However, they should have at least a limited applicability in a practical way to the identification and confirmation of structure of new benzenoid compounds. Repeatedly, in the collection of data for this paper, a prediction of the wave length of the absorption maximum of a compound was successful within a few millimicrons.

If a solvent other than water be employed, new $\delta\lambda_0$ values must be derived, and indeed applica-

(17) Since the above relation arises from transfer of a proton relative to the substituent group it might be expected that other modifications of the group would lead to changes in absorption spectra predictable on the same basis. While in some instances this may be the case, in general, caution must be exercised in such extension. This arises on one hand because the electrical effect of variation of a group (e. g., by methylation, acetylation, etc.) cannot be accurately predicted, and on the other hand because some modifications of groups actually introduce new double bonds into the system (e. g., acetylation) leading to entirely new relations to the benzene ring not predictable from the original group. In this connection the effect of ionization is clear in that the group added or removed is small (i. e., a proton) and that the electrical effect is entirely without ambiguity.

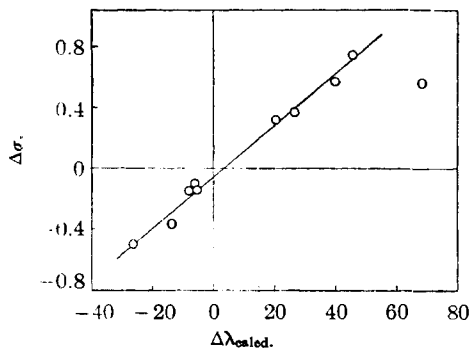bility of these empirical relationships should be investigated.[18]

Fig. 6.—Comparison of the $\Delta\sigma$ values of Price with the corresponding band displacement values ($\delta\lambda_0$ -24.05) relative to hydrogen.

Obviously, these generalizations need further investigation to determine more precisely their limitations and delineate their applicability. It would seem reasonable, for instance, that some compounds which contain groups themselves capable of absorption may show anomalous behavior. Further, the use of $\delta\lambda_0$ values as constants in the absolute sense is not justified. Substitution may be expected to cause some degree of variation in these values.

It is interesting from the theoretical point of view to speculate on the type of electronic interaction involved in the displacement of the absorption of benzene by substitution. In the body of the paper this was dealt with in a general sense, a directional or vector influence being assumed without any commitment as to the mechanism or mode of electronic interaction. Comparison of some of the extremes of the groups involved does, however, give some insight into this feature. The amine cation, which causes exclusive *meta* substitution in chemical reaction, causes little or no displacement of benzene absorption, while the nitro group, also highly *meta* directing, is the most effective displacing group observed. An important point of contrast between these two groups is the difference in their ability to participate in actual electron transfer. This would seem to im-

(18) An attempt was made to apply equation (1) for ethanol as solvent using the data on *p*-disubstituted compounds given by Kumler.[7] It was found on proceeding as in the derivation of Fig. 4 that the number of compounds remaining after using the appropriate standards was not sufficient to justify inclusion in this paper. But it was apparent that a slightly different line would have been obtained. Since the approximations involved would not seem to warrant this procedure, it is suggested that the same constants be used as in equation (2); the corresponding $\delta\lambda_0$ values may then be derived in the same manner as for Fig. 4.

The absorption of dimethylaniline and diethylaniline in ethanol was determined in order to attempt inclusion of monosubstituted compounds in this same transposition. It was noted that while the *p*-disubstituted compounds seemed to conform as in Fig. 4, these two monosubstituted compounds were somewhat anomalous. It is uncertain whether this discrepancy is fundamental with the compounds or lies in the inadequacy of the treatment of hydrogen as a substituent group.

ply that the effects which cause displacement of the primary band are similar to if not identical with the so-called mesomeric or resonance effects as contrasted with a coulombic displacement of the electron mass.

An obvious implication of this concept is that the order of groups determined by their displacement potentialities should parallel some other known arrangement in terms of other physical or chemical properties. Figure 6 shows the correlation obtaining between the band displacement values of the different groups and the differential polarizability ($\Delta\sigma$) values calculated by Price[19] from the σ values derived by Hammett[20] for these groups from considerations of chemical reactivity. In this plot $\delta\lambda_0$ — 24.05 has been used. This relates the displacement value of the groups to that of hydrogen, and makes them comparable to the $\Delta\sigma$ values, which also refer to hydrogen. The values of $\delta\lambda_0$ — 24.05 were given the same sign as $\Delta\sigma$. In this arrangement the displacement values for electron attracting groups are positive and for electron contributing groups are negative. Except for the nitro group, which seems exceptional here as previously, the correlation is fairly good, and at least indicates that the sequence of effect is the same in both instances.

Another feature of the semiquantitative treatment of group interaction and band displacement is that the chromophore representing the primary benzene absorption appears to originate at a wave length shorter than the point of absorption of benzene itself. It must be emphasized that no significance can be attached to the particular point of origin found in Fig. 4, since the use of different compounds as standards would lead to somewhat different points of origin. But regardless of the choice of primary compounds, the origin would appear farther back than benzene itself. This benzene absorption thus may be considered to arise in the region ordinarily ascribable to the double bond alone, and by substitution can be displaced to the edge of the visible region. In contrast to the elaborately conjugated systems heretofore studied, this absorption is unique in that the displacement is not accompanied by any corresponding increase in the length or size of the absorbing chain.

Summary

The absorption of benzene and its mono- and *para* disubstituted derivatives can be represented as an approximately regular progression of at least three bands. These are designated as the secondary, first primary, and second primary bands, respectively, in order of decreasing wave length. The spectrum position of these bands is determined by the displacing value of substituent groups, both singly and in combination.

(19) Price, "Mechanisms of Reactions at Carbon–Carbon Double Bonds," Interscience Publishers, Inc., New York, N. Y., 1946.

(20) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Company, Inc., New York, N. Y., 1940.

The first primary band has been shown to be capable of displacement to the edge of the visible region; this would seem to clear up confusion in the literature where the more displaced bands have been identified solely with the secondary absorption.

An empirical semi-quantitative relationship has been developed involving the interaction of substituent groups in the displacement of the first primary band for *p*-disubstituted compounds where the groups are of complementary types— *i. e.*, *ortho–para vs. meta* directing.

The displacing effect ($\Delta\lambda$) of a substituent group on the primary band of benzene has been associated with a directional displacement of elec-

trons to or from the benzene ring, depending on the electron attracting or contributing character of the group. The effect of simple ionization on band displacement has been tentatively cited to corroborate this principle.

The kind of electronic interaction responsible for band displacement has been associated with mesomerism or resonance rather than with simple coulombic displacement effects.

It was noted that the order of substituent groups obtained from the magnitude of the derived displacement values ($\delta\lambda_0$) correlates well with that of the $\Delta\sigma$ values given by Price.

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Preparation and Properties of Serum and Plasma Proteins. XV. Some Factors Influencing the Quantitative Determination of Fibrinogen[1,2]

BY PETER R. MORRISON

Fibrinogen is the plasma protein which forms fibrin under the action of thrombin. Among the plasma proteins it readily may be identified not only by this, its physiological activity, but also by its very low coagulation temperature and its solubility characteristics which make it the most easily salted out of all the plasma proteins. Each of these three properties has been made the basis of various quantitative methods for its estimation in plasma, but all are subject to certain errors. These errors may lead to measurements which are too low due to incomplete precipitation of the fibrinogen, or too high due to the occlusion of other plasma components. Their magnitude can, therefore, only be evaluated by a study using purified components. The large-scale fractionation of human plasma[3] has both emphasized the need for a well-defined analytical method and provided quantities of stable concentrated fibrinogen and thrombin for its investigation. Many procedures have been proposed for the determination of fibrinogen.[4]

However, despite this great interest which has attached to fibrinogen and the many analytical techniques for it which have been described, the influence of the concentration of the reactants and of the presence of other plasma components has apparently never been systematically studied.

Since it is clear that the ultimate analysis must be based on the functional characteristic which defines fibrinogen—the ability to form fibrin under the action of thrombin—this study largely concerns itself with the yield of that reaction under various conditions. The effect of variation of fibrinogen and thrombin concentrations, and *p*H, and of the presence of contaminants, largely other proteins, is considered. These effects are of importance in determining fibrinogen both in solu-

(1) This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

(2) This paper is Number 61 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

(3) E. J. Cohn, *Chem. Rev.*, **28**, 395 (1941); *Science*, **101**, 51 (1945); *Am. Scientist*, **33**, 61 (1945).

(4) These techniques may be classified as follows:

(A) Separations by heat coagulation. Analysis by dry weight (Frederick, "Recherches sur la Coagulation du Sang," Gand. Paris and Leipzig, 1878. Cited by Gram, *J. Biol. Chem.*, **49**, 279 (1921). G. H. Whipple, *Am. J. Physiol.*, **33**, 50 (1914)).

(B) Separation by salting out. Analysis (a) by dry weight (W. Reyhe, "Nachweis und Bestimmung des Fibrinogens," Inaugural Dissertation, Strassburg (1898). Cited by Lewinski, *Arch. ges. Physiol.*, **100**, 611 (1903)); (b) by nitrogen (Lewinski, *ibid.*, P. N.

Howe, *J. Biol. Chem.*, **57**, 235 (1923); M. Florkin, *ibid.*; **87**, 629 (1930); W. L. Campbell and M. I. Hanna, *ibid.*, **119**, 15 (1937)).

(C) Separation by clotting (calcium chloride). Analysis (a) by dry weight (H. C. Gram, ref. 4A); D. P. Foster and G. H. Whipple, *Am. J. Physiol.*, **58**, 407 (1921); (b) by nitrogen (G. E. Cullen and D. D. Van Slyke, *J. Biol. Chem.*, **41**, 387 (1920); P. E. Howe, *ibid*, **49**, 93 (1921); T. B. Jones and H. P. Smith, *Am. J. Physiol.*, **94**., 144 (1930)); (c) by tyrosine (H. Wu, *J. Biol. Chem.*, **51**, 33 (1922); H. Wu and S. M. Ling, *Chinese J. Physiol.*, **1**, 161 (1927)); (d) by turbidity of the clot (K. Klinke and G. Elias, *Z. ges. exptl. Med.*, **77**, 717 (1913); K. K. Nygaard, R. Gathe and Th. Guthe, *Skand. Arch. physiol.*, **83**, 195 (1940); K. K. Nygaard, "Hemorrhagic Diseases. Photo-electric study of Blood Coagulability." C. B. Mosby Co., St. Louis, Mo., 1941).

(D) Separation by clotting (thrombin). Analysis by dry weight or nitrogen. This procedure has been used routinely in this Laboratory since 1941 when it became necessary to determine the fibrinogen content of various plasma fractions; K. Laki, *Studies Inst. Med. Chem. Univ. Szeged*, **2**, 27 (1942); L. Szecsenyi-Nagy, *Biochem. Z.*, **317**, 185 (1944).

(E) Separation by electrophoresis. (E. Stenhagen, *Biochem. J.*, **32**, 714 (1938); L. G. Longsworth, T. Shedlovsky, D. A. MacInnes, *J. Exptl. Med.*, **70**, 399 (1939); J. T. Edsall, R. M. Ferry and S. H. Armstrong, Jr., *J. Clin. Invest.*, **23**, 557 (1944); H. F. Deutsch and M. B. Goodloe, *J. Biol. Chem.*, **161**, 1 (1945)).