A PHYSICO-CHEMICAL STUDY OF BARBITURATES IN AQUEOUS SOLUTION

Ionization and Hypnotic action

by

VATTARMADOM RAMA KRISHNAN
B.Sc.Hons. ('44), M.A. ('46), Annamalai University

Submitted to the Department of Chemistry and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy

August 1953

Acknowledgment

For guidance in carrying out this work I am indebted to Dr.Robert Taft who, in teaching and research, has provided me a high ideal to emulate. I am grateful to Dr.Joseph Burkhalter, Dr.Jacob Kleinberg, Dr.A.W.Davidson and Dr. R.Q.Brewster for their constant encouragement. To my teachers and colleagues in the departments of Chemistry and Pharmacy I am thankful for their valuable association and help. The University of Kansas which provided me financial assistance and the people of Kansas who extended their warm friendship and hospitality to me during my residence here will ever be high in my esteem.

CONTENTS

Introduction

Physico-Chemical Properties and Activity of Drugs

To say that the pharmacological actions of drugs could be interpreted solely in terms of simple physico-chemical principles, would entail the acceptance of a mechanistic concept of life processes, and be an underestimation of the complexity of the living organism. Nevertheless, attempts at translating biological and related processes in terms of known forces at play in the behavior of inanimate systems have led to successes of varying degree and have yielded useful generalizations. Workers in the field have sought an understanding of the mechanism of distribution of drugs in the body and their method of disposition at the site of action as well as to establish what properties are necessary or sufficient for any particular type of activity.

In view of the colloidal nature of the tissues involved and the enzymatic character of the processes therein on the one hand and considering the common constitutional dependence of the physical as well as chemical properties of substances, on the other, the separation of the factors, physical and chemical, may be more formal than real. However, the dominant role of physical properties in the function of drugs would justify a separate approach to their study (1 , 2)

In the following discussion, no comprehensive review of the physico-chemical approaches to the study of the pharma-

cological action of drugs has been attempted, but what is sought is only to present such aspects as are of interest in relation to the activity of the barbiturates, and to hypnosis in particular.

The multifunctional character of the barbiturates as sedatives, hypnotics and general anesthetics, all of them related, would render it logical to draw, where possible, on on the gains hitherto made in the field of general anesthesia, a field which has been studied from the physico-chemical angle probably more than any other pharmacological action (3).

The Meyer and Overton theory of lipid solubility resulted from attempts at correlating the anesthetic activity of certain substances with their distribution coefficient between olive oil (octyl alcohol has been preferred later) and water (4, 5). Solubility in lipids would be a measure of the tendency of the drugs to pass into the lipoids of the centers of their action and this has been shown to parallel, qualitatively, the general anesthetic activity of a wide series of compounds and more quantitatively, amidst closely related members of any single group. Thus, for the barbiturates, the product of the anesthetic doses and the distribution coefficients, determined on the acid form, lie in a very close range (6, 7, 8).

Meyer and Hemmi consider that narcosis is effected when a definite concentration of the narcotic is reached in the cell lipoid - a concentration, more a function of the cell

affected rather than of the substance acting; in a limited series which implies common functional groupings (9).

Lipid solubility has also been shown to be a determining factor in the penetration of substances into cells (10).

It is probable, with equal validity of the relations observed, that the site of action of the drug lies beyond the lipoid phase. Water-soluble substances penetrate the capillaries of the nervous system very slowly, while the lipoid soluble ones penetrate more rapidly. The small, but noticeable interval before the onset of anesthetic action of the intravenously administered barbiturates could be explained as the time required for the penetration of the drugs into or through the lipoid layer (11).

Reference might be made in this connection to more recent studies in the nature of the lipoid in nerve cells in relation to the proteins present (12) and also to a suggestion that anesthetics may displace the lipoid from a state of solution in proteins (13).

An equilibrium between the affected phase in the cell and the external phase with which it is in contact is the basis of the postulate by Fergusson that the thermodynamic potential of the drug in the affected phase, whatever its nature, would be equal to its potential in any known phase in equilibrium at the site which lends itself to quantitative investigation (14).

The partial molar free energy given by the relation

$$\bar{F} = F_o + RT \ln a$$

where F_o is the molar free energy in the standard state and a the mole fraction activity; the pure substances are taken as the standard state in each case. The activity has been measured by Fergusson by evaluating the ratio of the partial pressure of the respective component over the solution at the concentration considered and the vapor pressure of the pure substance at the same temperature. An alternative method employed is a determination of the solubility of the active substance, the ratio between the actual concentration of the substance and its solubility representing the activity.

The concentrations at which different substances are equally effective vary considerably, although their thermodynamic activities at equitoxic level lie remarkably close together. The success of the concept over a series of diverse chemical structures has lent support to this approach (15). The relation of active dose level to the aqueous solubility is evident and may be traced back to Richet's rule as early as 1893 (16).

The importance of surface tension in affecting the distribution of drugs have been long realized particularly in the case of surface active compounds (17). Traube's work nn surface tension of hypnotics shows decrease of surface tension to parallel an increase of activity (18). Surface activity of some monoalkyl and dialkyl barbituric acids investi-

gated by Giacalone confirms the law of Traube in general and reveals in addition, a close inverse relation between surface activity and solubility (19).

The principle is quite evident that the greater a given compound depresses surface tension, the more it tends to accumulate at the surface and thus facilitates absorption by the phase with which it is in contact. In the study of local anesthetic action of Nupercaine, the enhanced activity of the free base over the salt has been shown to be in conformity with this principle. However, the general validity has only been considered qualitative at best.

Adsorption may be expected to play a significant role in the distribution and activity of drugs in the body (19, 20). The adsorptive capacity of finely ground activated charcoal for methylene blue is decreased by narcotics and the reduction in the activity of enzymes by narcotics has been attributed to the same mechanism (21). Tabern and Schelberg's studies in the adsorption of barbiturates from solution by activated charcoal show a qualitative relation between adsorptive ability and efficiency of the hypnotic action (7).

In biological systems, there is a relatively steady voltage gradient of considerable magnitude between any two points, characteristic of the species, and to some extent of the individual, which is altered only by changes in the fundamental biology of the organism. The potential difference has been described as the sum of the intermediate boundary

potentials and as an index of stored energy (22). Studies have been made in the electrolyte balance, in ionic transport, etc., in an attempt to explain such potentials through known initial differences in electrolyte concentration and ionic mobilities across phase boundaries, but the actual causes remain still obscure (23). Attention has been directed to the importance of ionization equilibria in the body as affecting the phase boundary potentials (24).

Regarding the part that drugs may play in this respect, Beutner and Barnes have attributed the stronger cholinergic properties of acetylcholine to the imparting of a greater negative potential to the lipoid surface at the site of nerve contact by an increasing ion concentration (24). The existence of characteristic potential curve patterns in the course of barbiturate hypnosis points to analogous effect of hypnotics on boundary potentials (23).

The ready reversibility of the action of the majority of drugs, the barbiturates included, seems to indicate that ionic or short range molecular forces are probably involved in the binding of drugs to the receptor tissues rather than covalent combinations, since the latter are of considerable strength and hence less reversibly made or broken.

The ionization of drugs and the factors affecting ionization *in vivo* have received attention in recent years. The charge on the ionic form of the drug would influence the ease and rate of adsorption on charged receptor surfaces. In the

penetration of the substance through membranes, retardation may be caused by ionic groups being held by unlike charges on the outer surface of membranes, and repelled by like charges (10).

The drug-receptor combination considered as an ionic equilibrium would be governed by the law of mass action and would be influenced by the ionization of the drug itself. Studies through quantitatively controlled variation of the extent of ionization of drugs, in several examples studied, have shown the dependence of activity on the ionization and indirectly, on the hydrogen ion concentration, if acid or base ionization is involved.

Vermast noticed in 1921 the tendency of weak acids to exhibit their maximum activity at the pH where they were least ionized (26). All members of a series of thirty barbiturates examined by Clowes, Keltch and Krahl have been found to enter the eggs and larvae of the sea urchin Arbacia exclusively as molecules and to cause depression in cell division and respiration (27). On the other hand, among the members of a series of acridines, greater activity corresponds to increased ionization (28), and a plot of antibacterial concentrations of the acridine cations reveals a direct competition of these with hydrogen ions.

There are intermediate cases, comprising many weak acids and bases, where undissociated molecules as well as their ions have been shown to be active in relatively different degrees (29).

Clowes and Keltch (30) have shown the increased efficiency of neutral molecules as compared to their ions in the narcotising action of barbituric acids on the worm Arenicola.

The acidic or basic ionization constant, as the case may be, of the drugs and the pH of the medium determine the ratio of acid or base to the salt (i.e., the ions) in the various tissues. At a given pH this ratio depends on the pK value and for a given compound the pH is the determining factor (31).

Kindler has shown that the speed with which barbiturates produce sleep decreases with increasing acidity of the acids, which corresponds to an increasing proportion of their ionic form at the pH of blood, 7.8 (32). A tenfold decrease in activity in the narcotizing ability of barbiturates has been demonstrated by an increase of pH from 7 to 9 in the medium (30). Both these results lead to the same conclusion that the barbiturates are more active in the un-ionized form.

Attention may be drawn to the conformity of the results with the theory of lipoid solubility.

Metabolism and activity of barbiturates

There is here presented a brief discussion of the absorption, distribution and metabolic fate of a group of barbiturates which were selected for experimental study. They are treated from the standpoint of structure, clinical use as hypnotics and sedatives, and variation in duration of action. Their rapidity of action will be viewed in relation to the extent of their ionization.

In general, the barbiturates are orally administered as sodium salts in an adult dose of 0.1 to 0.3 gm (33, 34). They are usually ingested in a resting stomach which contains on the average, 50 ml of gastric juice at pH 1.6 to 1.7. These compounds may therefore be expected to be present solely in the acid form and, since their ionization constants are in the neighborhood of 1×10^{-8}, they would be practically un-ionized in the acid medium. Absorption occurs readily from the stomach and the gastro-intestinal tract. It has been shown that narcosis can follow intragastric administration to guinea pigs with ligature around the pylorus (35). Considering that the time taken for a test feed of water only to pass into the intestines is about half hour (33), and as the barbiturates take their effect within a maximum period of half hour and as a minimum much less, the absorption may be expected to take place in the main, through the stomach wall, by a process of diffusion, the acids being insoluble (36).

After absorption, barbiturates enter a wide variety of

cells as shown by distribution studies, but undergo considerable localization in the central nervous system, especially in the thalamus which is of particular significance in the production of sleep (37). There are discrepancies, however, in the reports on the concentration of barbiturates in the cerebrospinal fluid (38). Localization has been observed to some extent also in the liver, kidney and the spleen (39).

The barbiturates show variation in the onset and duration of action, as presented in the table below (40).

Class	Onset of action, hrs.	Duration of activity, hrs.	Compounds under study
Long	0.5 - 1	10 - 12	Barbital & Phenobarbital
Moderate to Long	.5 - 1	8 - 10	Probarbital
Moderate	.25 - .5	6 - 8	Amobarbital
Short to Moderate	.25 - .5	4 - 6	Pentobarbital
Short	.25	3 - 4	Secobarbital
Ultrashort		.5 - 1	

Doubt has been expressed as to whether the differences between the blood levels of the drugs are appreciable in the hypnotic state and the recovery stage. A fall from 8.4% to 7.6 has been noted for barbital, while for amobarbital no difference was noticeable (level 2.9mg. per cent)(41).

There is a general trend in the direction of fall of blood level required for a given activity on passing from the

long acting towards the shorter acting barbiturates (38).

The hypnotic activity of barbiturates is closely associated with the interference of respiratory activity of the nervous system and its oxygen uptake (42).

The detoxification of the barbiturates takes place through the kidney and alternatively in the body, mainly in the liver. All barbiturates with long duration of effect are appreciably excreted in the urine unchanged, barbital excretion in this manner being 65-90% and phenobarbital about 25%. The excretion of moderately acting barbiturates in the urine is less than 3%, and short acting ones are observable only in traces. The short and ultrashort acting compounds have their duration of action parallel to their time of destruction in the liver (43). It has been shown that in case of injury to the liver even the short acting drugs become long acting (44), and renal damage causes a distinct retardation of barbital excretion with little change in the course of depression due to destructible barbiturates, while animals with severe kidney damage do not recover from the effects of any long acting barbiturate (45). Rabbit liver slices have been found to degrade secobarbital, amobarbital and pentobarbital but not probarbital, phenobarbital, and barbital under similar conditions (46). Thus there is direct correlation between *in vivo* stability of barbiturates and their duration of action. Conversely, duration of action could be a test of *in vivo* stability (38).

The time differences in the onset of action of the barbiturates can scarcely be accounted for on the basis of their metabolic fates and the explanation of the onset of action must be traced elsewhere. The stomach wall may be selective for the diffusion of different compounds but in this case the selectivity cannot account for the time factor, as in the same variations are observed also in the intravenous administration of these compounds (38).

On the basis of studies in the distribution of hypnotics between ether and aqueous sodium carbonate, Klimesch has observed that the barbiturates which cause immediate hypnosis are more extensively hydrolyzed in solution than the slow acting ones(46). The extent of hydrolysis of the barbiturate ions would parallel the concentration of the corresponding un-ionized acid at any given pH. Thus a direct correlation between the rapidity of onset of action of the barbiturates and the relative abundance of their free acid in plasma has been suggested.

Busch sought to obtain the values of free acid to salt ratio of the barbiturates at the pH of blood by deriving an apparent ionization constant of the acids through the measurement of the pH of solutions containing known amount of the acid and the corresponding salt (47). The results are at variance with those of Klimesch in that long acting barbital and medium acting amobarbital are both shown to have the same acid to salt ratio in blood, while the other compounds examined

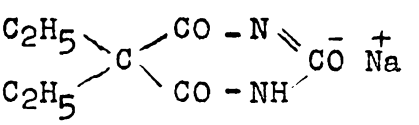
are in order.

A more direct determination of the acid ionization constants of several barbiturates may be helpful in evaluating the function of undissociated acid with regard to the time of onset of action of the barbiturates.

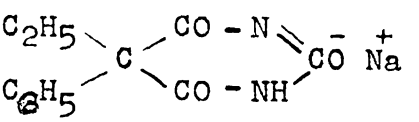
The experimental work which follows consists in an extended study of many physical properties of aqueous solutions of a group of barbiturates (see p. 14) and their corresponding free acids. Included among the properties of the salts studied are electrical conductance, viscosity, density, refractive index, solubility, and surface tension. These measurements in general have been carried out at 25°C and over a wide range of concentrations. For the free acids the ionization constants have been established by methods to be described. This extended study provides many data now completely lacking and, as discussed later, may throw some light on the clinical behavior in this class of drugs.

The substituted barbiturates employed in this study were obtained from the following sources: the monosodium salt of barbital from Merck & Co.; of phenobarbital from Abbot Laboratories; of probarbital from Squibb & Sons; and the sodium compounds of pentobarbital, amobarbital and secobarbital from Eli Lilly & Co.* They are identified below by their chemical names and structural formulae. Their trade names which are also in common use are given in parentheses.

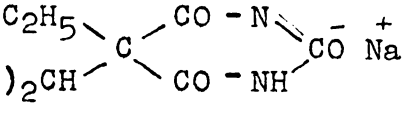
Barbital Sodium (Veronal Sodium) — Sodium 5, 5 diethyl barbiturate



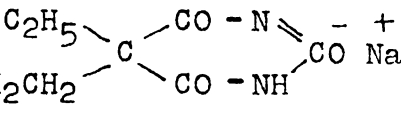
Phenobarbital Sodium (Luminal Sodium) — Sodium 5-phenyl-5-ethyl barbiturate



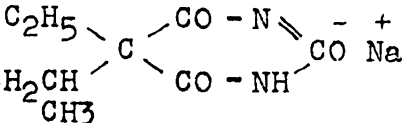
Probarbital Sodium (Ipral Sodium) — Sodium 5-ethyl-5-isopropyl barbiturate



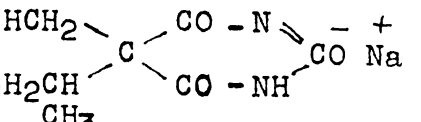
Amobarbital Sodium (Amytal Sodium) — Sodium 5-isoamyl-5-ethyl barbiturate



Pentobarbital Sodium (Nembutal Sodium) — Sodium 5-ethyl-5-(1-methyl butyl) barbiturate



Secobarbital Sodium (Seconal Sodium) — Sodium 5-allyl-5-(1-methyl butyl) barbiturate



*The generic names have been preferred in subsequent pages for the sake of brevity and ease of expression.

The salts were purified by the following procedure: Barbital Sodium was dissolved in the minimum amount of warm water and filtered to remove undissolved impurities. To this solution, an equal volume of absolute alcohol was added according to the method of Manov, Shulte and Kirk (48). After cooling the mixture in an ice bath, the solid that separated was filtered off and dried in an oven at 100° C. The yield amounted to 45%. The pH of a 0.02 molal solution of the salt was 9.78, in agreement with the value quoted and used as a criterion of purity by the same authors. The electrical conductance of a 0.1 normal solution showed no change after a second precipitation process.

The same method applied to phenobarbital sodium gave very low yields. The addition of ether to an alcoholic solution of the salt gave slightly better result. A third sample obtained by shaking the salt with ether successively to remove ether soluble free-acid impurities was found not to differ in conductance from the previous two samples. The last of these methods was employed for the purification in bulk of the other homologous salts, they being similar in their solubility characteristics to phenobarbital sodium.

In each case the identity of the compound was confirmed by conversion to the respective acid following the procedure for assay described in the U. S. Pharmacopeia and the New and Nonofficial Remedies, 1952.

The method adopted for the preparation of the acids

consisted in acidifying aqueous solutions of the salts by the addition of pure hydrochloric acid, and extracting the organic acid that was deposited by redistilled chloroform (ether in the case of phenobarbital). The extract was washed several times with water until free from any trace of chloride ion, filtered and the solvent removed by evaporation. The residue was kept for four hours at a temperature of 100° C.

The melting points of the acids are given below:

	Observed	Literature (49)
Barbital	190° C.	191° C.
Phenobarbital	174-175° C.	174° C.
Probarbital	202° C.	203° C.
Pentobarbital	130° C.	130° C.
Amobarbital	155-156° C.	156, 158° C.
Secobarbital	78-80° C.	81°, 82° C.

The absence of chloride and sulfate impurities was confirmed for both the salts and the acids.

The lack of stability of the aqueous solutions of these salts, barbital sodium excepted, was a reason for their purification from non-aqueous media. Decomposition, which gave a water insoluble deposit on standing overnight, was observed in the case of probarbital, pentobarbital, amobarbital and secobarbital sodium and was more evident when the solutions were concentrated. Although there was no easy method of ensuring the absolute stability of solutions during

measurement, the non-variance of properties such as conductance and refractive index for the first eight hours, after solution, was considered sufficient for the purpose of the measurements; all measurements were made within this eight-hour period.

All these compounds are light powders and are white and odorless. They are hygroscopic to varying degrees and must be kept in tightly stoppered containers. For weighing out, the substances were heated in the weighing bottle itself and maintained at 100° C. in an incubator before cooling in a dessicator and weighing.

The aqueous solutions were made up individually at each desired concentration, calibrated volumetric vessels, at 25° C., being employed for the purpose. Concentrations ranged from 0.01 to 1.0 normal. Conductivity water, obtained from a 'Barnstead' still and stored in closed pyrex vessels, was employed throughout the experiments.

All work was carried out at 25° C. except where otherwise stated. For this purpose a thermostat was employed whose temperature fluctuation did not exceed 0.01° C.

Density

A precise determination of the densities of the solutions of the sodium salts of the barbiturates was desired in order to calculate the molal concentrations of the solutes as well as to investigate the density - composition relations, which are of interest in view of the comparatively heavy organic anions involved.

A pyknometric method of measurement of density was employed, a Parker and Parker type pyknometer being used for the purpose (50). The capillary graduations were calibrated for volume by the use of a mercury thread and the differences in levels of liquids in the two limbs taken into account by corrections for the corresponding volume. All fillings of the pyknometer were done in the thermostat and the temperature of weighing was only 3-4° lower, obviating any correction on that account. Correction for buoyancy was incorporated in the calculation of the densities by the relation (51)

$$d = \frac{W_1 - W}{W_W - W} d_W + \sigma\left(1 - \frac{W_1 - W}{W_W - W}\right)$$

W = mass of pyknometer
W_W = mass of pyknometer filled with water
W_1 = mass of pyknometer with the solution
d_W = density of water in gm. per ml. at 25° C
σ = density of air

Stationary conditions of the pyknometer surface were assured by wiping the surface initially with damp cloth followed with

a dry linen and letting the pyknometer stand for a half hour in the balance case before weighing.

The densities of the solutions have been tabulated in Table I and plotted against concentration in molality (52).

The variation of density may be expressed as a function of molality of the solute by the general expression,

$$D_m = D_o + am + bm^2 + cm^3$$

D_m = Density at molality $\underline{m}$ at 25°
D_o = Density of water
a, b, c, = Constants

The constants have been evaluated by setting up equations in the three unknowns for each electrolyte and solving them by the use of determinants. The values of the coefficients are given in Table II.

The relations express the concentration dependence of density with a deviation no greater than 1/10000. The change is continuous and follows a closely similar pattern for all the salts.

TABLE I

Concentration Moles/liter	Density in gms. per ml. at 25°C of aqueous solutions of sodium salts: Barbital	Phenobarbital	Probarbital	Amobarbital	Pentobarbital	Secobarbital
0.05	1.0011_9	1.0013_8	1.0012_1	1.0003_5	1.0007_0	1.0006_8
0.10	1.0046_6	1.0055_9	1.0046_6	1.0039_4	1.0042_6	1.0044_2
0.20	1.0121_7	1.0139_8	1.0121_8	1.0106_8	1.0113_0	1.0118_1
0.40	1.0267_2	1.0305_7	1.0270_5	1.0241_5	1.0252_7	1.0262_7
0.60	1.0412_7	1.0471_4	1.0415_7	1.0374_7	1.0389_8	1.0403_0
0.80	1.0554_0	1.0636_1	1.0561_4	1.0500_6	1.0520_5	1.0538_4

Concentrations:

Molar	Molal					
0.05	.05046	.05057	.05050	.05060	.05059	.05062
0.10	.10162	.10202	.10177	.10213	.10210	.10221
0.20	.2060	.2076	.2066	.2081	.2080	.2084
0.40	.4236	.4306	.4260	.4325	.4320	.4338
0.60	.6539	.6707	.6598	.6753	.6741	.6786
0.80	.8978	.9300	.9090	.9397	.9374	.9460

TABLE II

Density as a function of Molality (Temperature 25° C)

$$D_m = D_o + am + bm^2 + cm^3$$

$$D_o = 0.99707$$

	a	b	c
Sodium Barbital	0.076408	-0.01708	0.00503
Sodium Phenobarbital	0.085643	-0.021505	0.00764
Sodium Probarbital	0.075993	-0.01413	0.00222
Sodium Amobarbital	0.068937	-0.01664	0.00503
Sodium Pentobarbital	0.072235	-0.01865	0.00535
Sodium Secobarbital	0.07332	-0.01380	-0.00012

Legend for facing graph

A. ——— --- ——— Amobarbital Sodium

B. — — — — — — — — Barbital Sodium

C. ---------- Pentobarbital Sodium

D. —— —— —— —— Phenobarbital Sodium

E. ———————— Probarbital Sodium

F ——— - ——— - — Secobarbital Sodium

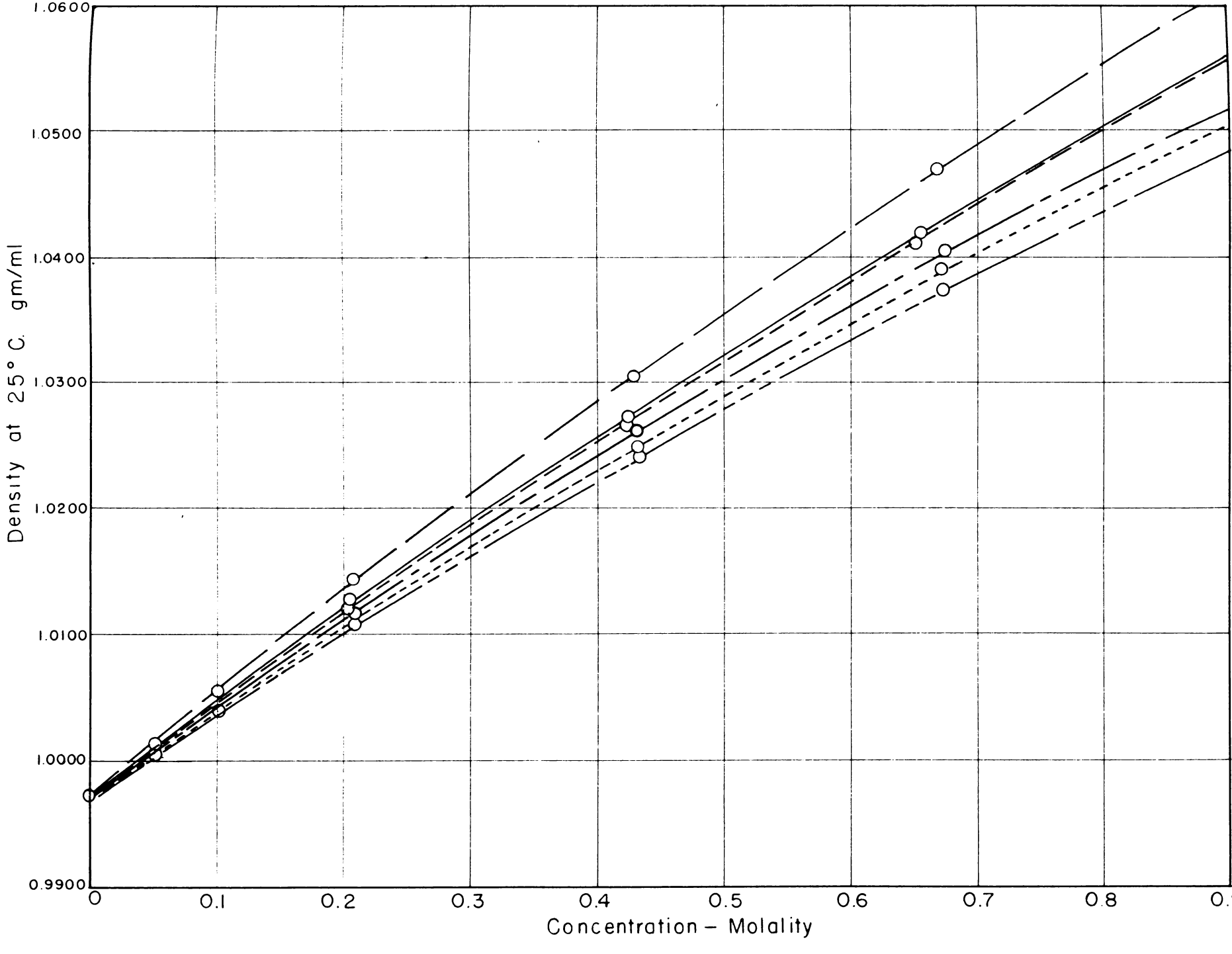

1.0600
1.0500
1.0400
1.0300
1.0200
1.0100
1.0000
0.9900
Density at 25° C. gm/ml
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Concentration – Molality

Fig. 1

Apparent Molar Volumes

Masson (52) has proposed the following relation for the apparent molar volume $\phi(V_2)$, defined as $V - V_0$ where V_0 and V are the volumes of the solvent and of the solution containing 1 mole of the electrolyte.

$$\phi(V_2) = \phi^{o}(V_2) + a\sqrt{c}$$

$\phi^{o}(V_2)$ is a constant as also is a, and c is the concentration of the solute in moles per liter.

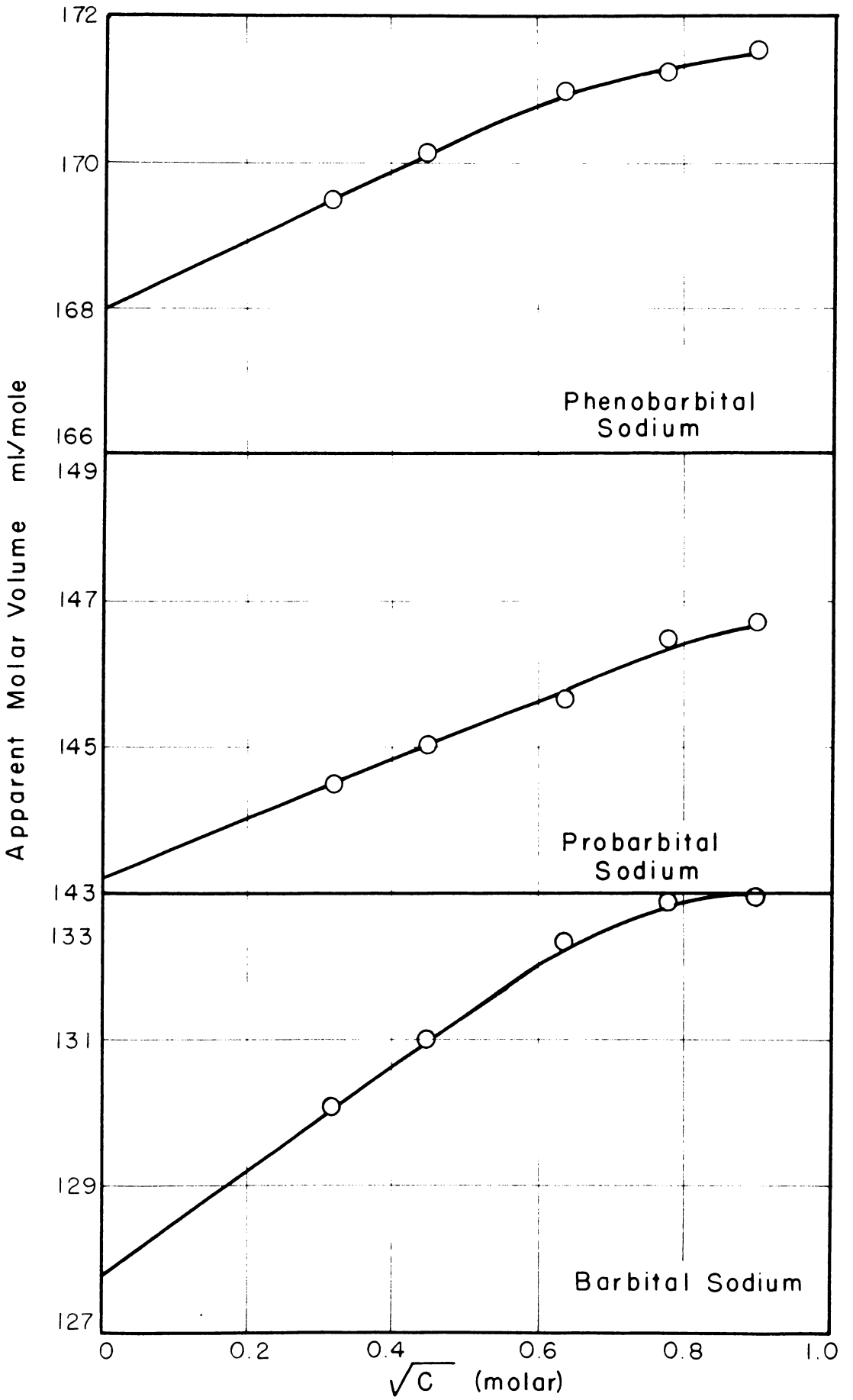
A theoretical treatment of the partial molar volumes of electrolytes in solution by means of the Debye-Hückel concept has been carried out by Redlich and Rosenfeld (53) and has been extended to the more readily determinable apparent molar volumes (54) which takes the form given by Masson.

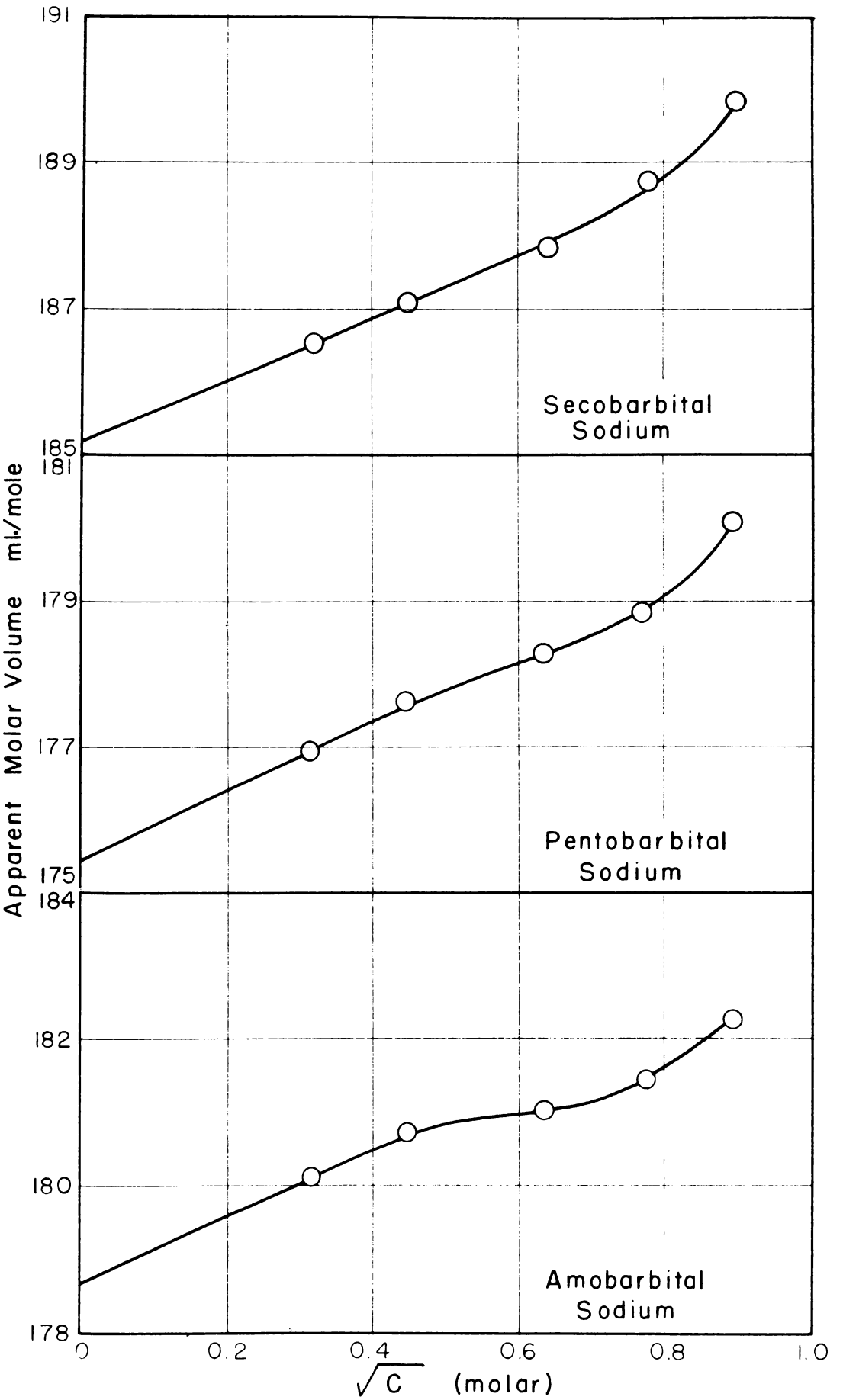
From the experimental density data of the solutions given in Table I, the apparent molar volumes of the solutes at various concentrations have been calculated and expressed in terms of ml. per mole in Table III. The values plotted against the square root of the respective molar concentration in Figure 2 show, in general, a linear rise in the range 0.1 to 0.5 molar, giving rise to a gradual flat at higher concentration for barbital, probarbital and phenobarbital sodium while a steep rise is indicated for pentobarbital, amobarbital and secobarbital sodium. A possible significance of this rise will be considered in a subsequent section.

The values at high dilution are susceptible, even to the extent of several-fold, to small errors in the volume make-up

TABLE III

Solute	Apparent molar volumes in ml. per mole at the concentration in moles per liter				
	0.1	0.2	0.4	0.6	0.8
Barbital Sodium	130.08	131.03	132.41	132.89	132.93
Probarbital Na	144.48	145.08	145.68	146.48	146.68
Phenobarbital Na	169.60	170.15	170.98	171.21	171.54
Pentobarbital Na	176.92	177.62	178.28	178.84	180.04
Amobarbital Na	180.13	180.73	181.09	181.47	182.27
Secobarbital Na	186.55	187.10	187.83	188.78	189.87


172
170
168
166
Phenobarbital Sodium
149
147
145
143
Probarbital Sodium
133
131
129
127
Barbital Sodium
Apparent Molar Volume ml/mole
0
0.2
0.4
0.6
0.8
1.0
√C (molar)


Apparent Molar Volume ml./mole
191
189
187
185
Secobarbital Sodium
181
179
177
175
Pentobarbital Sodium
184
182
180
178
Amobarbital Sodium
0
0.2
0.4
0.6
0.8
1.0
√C (molar)

of the solutions and in their densities; concentrations less than 0.1 molar have therefore been left out as beyond the range of precision of the values here reported.

Refractive Index

The refractive indices of the solutions of the barbiturates at different concentrations have been measured by the use of an Abbe refractometer whose prisms were maintained at 25° C by circulating water from the thermostat. Readings were taken with white illumination but with the usual compensation (i.e., to the D line).

The usual precautions in the maintenance of clean prism surfaces were observed and sufficient time allowed for the establishment of thermal equilibrium before each reading.

The observed values have been given in Table IV.

Concentration and refractive index:

The index of refraction has often been employed as a measure of the concentration of solutions by interpolation from empirical calibration curves. The nature of the curves would depend on the manner in which concentrations are expressed; in general, molarity may be expected to give rise to the nearest linear relationship (55). In the case of barbital, probarbital and phenobarbital sodium, the plot of the refractive indices of the barbiturates in solution against their molarity in Figure 3, shows very close linearity, up to the highest concentration investigated (0.8 molar). Amobarbital, pentobarbital and secobarbital sodium are observed to deviate from linearity at concentrations beyond 0.5 molar.

The plots have been used as calibration curves for the solubility and adsorption studies to follow.

TABLE IV

Concentration moles/liter	Refractive indices of Sodium salts in aqueous solution at 25° C.					
	Barbital	Phenobarbital	Probarbital	Amobarbital	Pentobarbital	Secobarbital
0.00	1.3325	1.3325	1.3325	1.3325	1.3325	1.3325
0.05	1.3344	1.3351	1.3345	1.3347	1.3350	1.3349
0.10	1.3364	1.3377	1.3367	1.3370	1.3372	1.3377
0.20	1.3400	1.3429	1.3409	1.3417	1.3419	1.3428
0.40	1.3478	1.3536	1.3488	1.3507	1.3510	1.3533
0.60	1.3548	1.3642	1.3569	1.3596	1.3602	1.3628

Legend for facing graph

A. ——— --- ——— Amobarbital Sodium

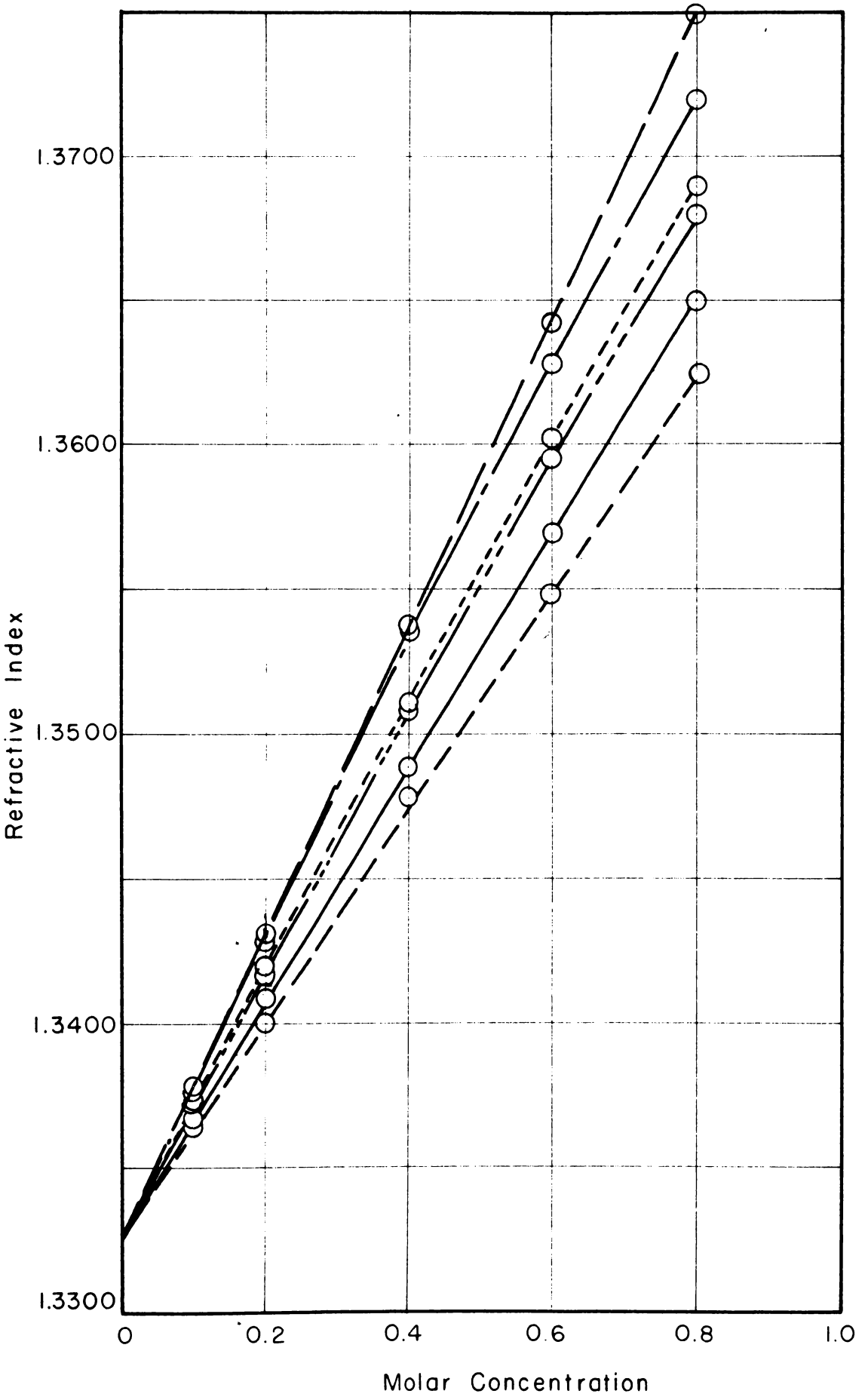
B. — — — — — — — — Barbital Sodium

C. ---------- Pentobarbital Sodium

D. —— —— —— —— Phenobarbital Sodium

E. ———————— Probarbital Sodium

F ——— - ——— - — Secobarbital Sodium


1.3700
1.3600
1.3500
1.3400
1.3300
Refractive Index
0
0.2
0.4
0.6
0.8
1.0
Molar Concentration

Apparent molar refraction

The quantity, apparent molar refraction, has been defined as

$$R_{app.} = R - X_o W_o$$

where R is the total refraction of a solution containing one mole of solute and $X_o W_o$ is the refraction which the amount W gms. of the solvent present in the solution would have in the pure state.

Substitution of Lorentz and Lorenz expression for R gives

$$R_{app.} = \frac{n^2-1}{n^2+2} \cdot \frac{M_1+W_o}{d} - \frac{n_o^2-1}{n_o^2+1} \cdot \frac{W_o}{d_o}$$

where n and n_o refer to the specific refractive index of the solution and the solvent respectively, d and d_o their densities and M_1 the molecular weight of the solute.

In terms of molality m, the expression becomes

$$R_{app.} = \frac{n^2-1}{n^2+2} \cdot \frac{1}{d} \cdot \frac{1000 + mM_1}{m} - \frac{n_o^2-1}{n_o^2+2} \cdot \frac{1}{d_o} \cdot \frac{1000}{m}$$

The work of Kohner (56), Geffcken (57) and Kruis (58) in aqueous solution has shown the small linear variation of the apparent molar refraction with concentration. Extrapolation to zero concentration yields a value for the molecular refraction of the solute which may differ from its true value only to the extent of any refractometric effect arising from the change of state of the substance; and this effect has been shown to be very small (55).

The direct determination of the refractive index of the solid barbiturates being difficult, an evaluation of $R_{app.}$ by the above method would allow an estimation of the molecular refraction of these substances. The values of $R_{app.}$ have accordingly been obtained (Table V) and plotted against molar concentration in Figure 4.

The molar refraction values of the barbiturates obtained by extrapolation of the plot to zero concentration have been given below and are compared with the values calculated on the basis of refractive additivity. In these calculations, the experimental value of barbital sodium (from $R_{app.}$) is taken as the standard, to which the further contributions by the additional atoms or structures present in the other substances have been added, with due allowances for double bonds and conjugation* where present (59).

	From $R_{app.}$	Calculated
Barbital Sodium	49.9	..
Probarbital Sodium	54.5	54.5
Pentobarbital Sodium	63.6	63.7
Amobarbital Sodium	63.4	63.7
Phenobarbital Sodium	64.9	64.8
Secobarbital Sodium	68.7	68.0

It is observed that the law of additivity of refraction

*Conjugation and ring structure contributions nearly cancel each other in the case of benzene.

TABLE V

Solute	Apparent molar refraction at the concentrations (molar) 0.1	0.2	0.4	0.6	0.8
Barbital Sodium	49.74	49.21	48.80	48.15	48.40
Probarbital Sodium	54.33	53.82	53.28	52.87	52.82
Phenobarbital Sodium	65.01	64.52	64.75	64.66	64.73
Amobarbital Sodium	63.10	63.37	62.82	62.57	62.22
Pentobarbital Sodium	63.73	63.24	62.66	62.69	62.51
Secobarbital Sodium	68.66	67.74	67.94	66.98	66.45

Legend for facing graph

A. ——— --- ——— Amobarbital Sodium

B. — — — — — — — — Barbital Sodium

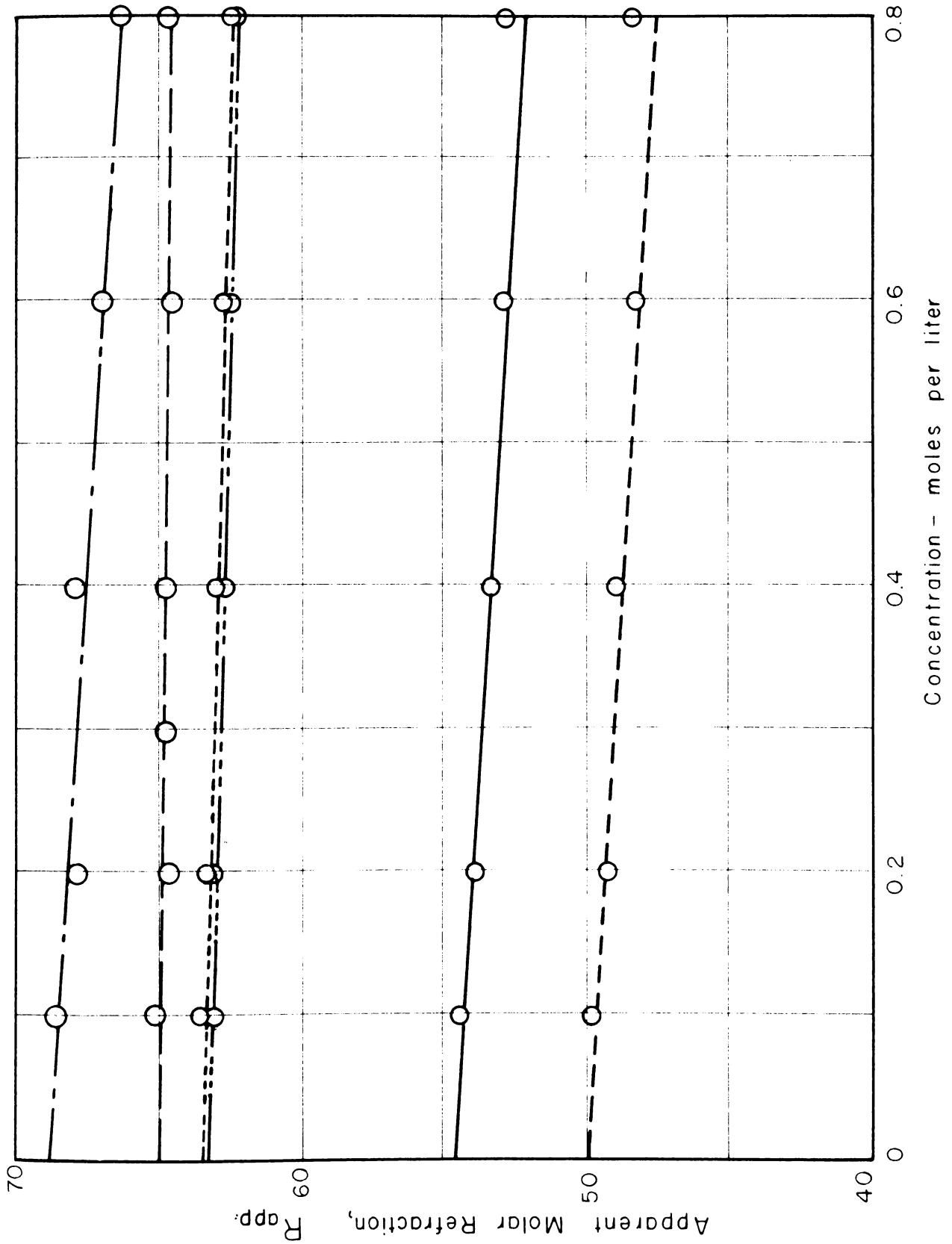
C. ---------- Pentobarbital Sodium

D. —— —— —— —— Phenobarbital Sodium

E. ———————— Probarbital Sodium

F ——— - ——— - — Secobarbital Sodium

Fig.4


Apparent Molar Refraction, $R_{app.}$
70
60
50
40
0
0.2
0.4
0.6
0.8
Concentration - moles per liter

holds here within limits of experimental error.

The variation of apparent molar refraction of the solute with increase of concentration of the solution represents departures from exact composition additivity in the aqueous mixtures.

Solubility

To determine the solubility of the salts, each, in estimated excess, was mixed with water in a solubility flask and kept stirred for 24 hours in the thermostat. The mixture was filtered through a sintered filter under suction and the filtrate analyzed for the solute by measurement of its refractive index as well as by a process of titration against standard acid (60). Since the barbituric acids have their ionization constants in the neighborhood of 1×10^{-8}, their salts are extensively hydrolyzed in solution which permits estimation by titration with hydrochloric acid. In each case a control was run against a standard solution of the respective salt and the actual amount of solute in the saturated solution determined by reference to this standard. The saturated solutions were diluted several-fold in standard volumetric vessels and their strengths brought close to the concentration of the standard solution each time.

For the refractive index method, the composition - refractive index plots of Figure 3 were used as calibration curves (by extrapolation where necessary).

Both methods gave closely consistent values for barbital, probarbital and phenobarbital sodium. The solutions of amobarbital, pentobarbital and secobarbital sodium had a considerably high viscosity making filtration slow and the filtrates gave analyses which varied with the relative amounts of water and the salts used in the mixture.

The results (at 25° C.) for barbital, probarbital and phenobarbital sodium are given below.

	By titration	By refractive index	
Barbital sodium	0.884	0.883	moles/liter of saturated solution
Probarbital sodium	1.22	1.20	"
Phenobarbital sodium	2.22	2.17	"

Surface Tension

In order to investigate the direction and extent of variation of the surface tension of the barbiturate solutions with change of concentration, measurements were made with the use of a torsion balance after Lecomte du Nouy (61). The temperature of measurement was 22 ± 1° C. Special precautions were taken to ensure the cleanliness of the vessels and the platinum loop employed. Measurements were always made 10 minutes after the preparation of the solutions to obviate an observable time effect on the surface tension value.

The values of surface tension of the solutions tabulated (Table VI) and plotted against concentration (Figure 5) show a marked depression ~~and~~ indicating positive adsorption of the solute on the surface, which is particularly high for amobarbital, pentobarbital and secobarbital sodium even at low concentrations. These, in addition, show a notable minimum in their surface tension plots at concentrations 0.4 to 0.6 molar.

TABLE VI

Surface Tension

Concentration eq/lit.	Barbital	Phenobarbital	Probarbital	Amobarbital	Pentobarbital	Secobarbital
	Surface tension in dynes per cm. at 22° C.					
0.05	69.5	69.0	67.5	62.4	61.2	56.5
0.1	66.7	65.9	64.2	55.7	53.3	47.1
0.2	62.6	62.2	60.1	51.0	49.4	43.9
0.4	61.8	55.7	57.4	47.1	43.6	42.1
0.6	60.4	49.7	54.1	43.9	42.1	43.6
0.8	59.3	47.4	50.2	45.5	42.4	44.9

Legend for facing graph

A. ——— --- ——— Amobarbital Sodium

B. — — — — — — — — Barbital Sodium

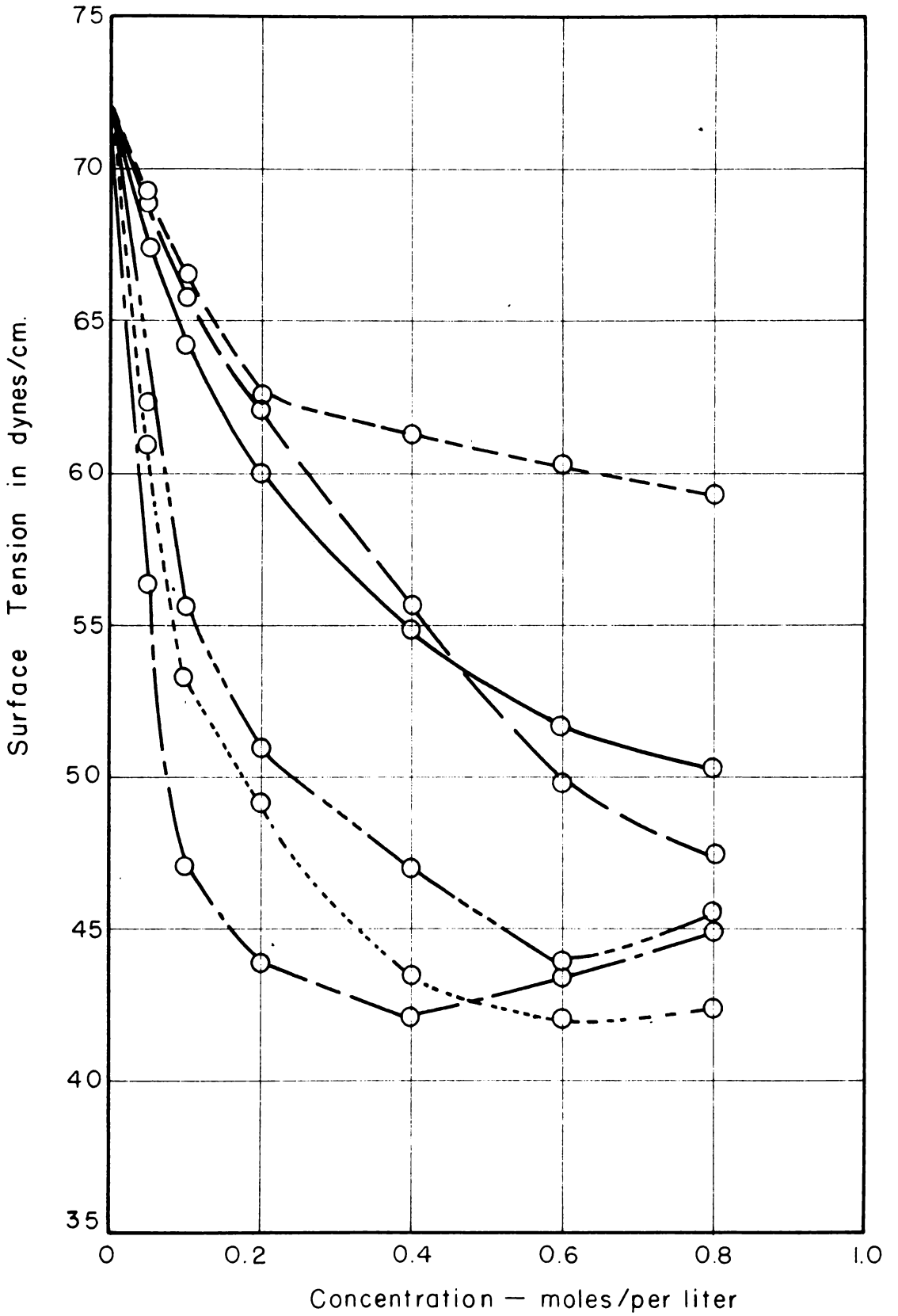
C. ---------- Pentobarbital Sodium

D. —— —— —— —— Phenobarbital Sodium

E. ———————— Probarbital Sodium

F ——— - ——— - — Secobarbital Sodium

Fig. 5


75
70
65
60
55
50
45
40
35
Surface Tension in dynes/cm.
0
0.2
0.4
0.6
0.8
1.0
Concentration — moles/per liter

Adsorption

Adsorption of the barbiturates from dilute aqueous solution on charcoal was studied using activated vegetable carbon 'Nuchar', grade C-190-N, as the adsorbant. The carbon was heated to 110° C for one hour and cooled in a dessicator. 50 cc. of 0.1 molar solutions of the barbiturates were added to 2 gms. of the activated charcoal in a stoppered vessel and placed in the thermostat for two hours with occasional stirring. Equilibrium was found to be rapidly established. The contents were rapidly filtered through a cotton plug and the filtrate analysed by both the refractive index and titration procedures described under solubility studies.

Control analyses were run with the solutions prior to adsorption and the percentage of adsorption referred to these solutions.

	% of solute adsorbed from 0.1 molar solutions	
Method:	Titration	Refractive index
Barbital Sodium	18.8	18.9
Probarbital Sodium	22.2	21.5
Phenobarbital Sodium	25.6	25.1
Pentobarbital Sodium	26.5	27.4
Amobarbital Sodium	26.4	26.7
Secobarbital Sodium	28.5	28.9

Viscosity

An Ostwald type capillary viscometer was employed for the measurement of the viscosities of the barbiturate solutions. The time of flow of the liquid through the capillary caused by a constant initial head of the liquid was registered by a stop watch reading to 0.2 sec. Due attention was paid to the cleanliness and the vertical allignment of the capillary. The instrument was calibrated with water and the viscosity of the solutions deduced from their respective times of flow for the same volumes of the liquids by the relation

$$\eta = \eta_w \frac{Dt}{D_w t_w}$$

where η is the absolute viscosity, D the density and t the time of flow of the liquid while these with the subscript w refer to the corresponding terms for water at the temperature of measurement, viz. 25° C

η_w for water is obtained from literature (62).

η', the relative viscosity is defined as η/η_w. The absolute and the relative viscosities of the barbiturates in solution at different concentrations have been listed in Table VII.

Extensive studies have been made on the viscosity of electrolytes in solution (63). One of the earliest but not too favored relations is that of Arrhenius

$$\eta' = A^c$$

where A is a constant and c is the concentration.

TABLE VII

Viscosity

Concentration mole/lit.	Barbital	Phenobarbital	Probarbital	Amobarbital	Pentobarbital	Secobarbital
	Viscosity in millipoises at 25° C. (Water=8.949)					
0.05	9.217	9.289	9.244	9.423	9.405	9.441
	*(1.030)	(1.038)	(1.033)	(1.053)	(1.051)	(1.055)
0.10	9.549	9.647	9.593	9.727	9.692	9.781
	(1.067)	(1.078)	(1.072)	(1.087)	(1.083)	(1.093)
0.20	10.02	10.41	10.26	10.61	10.60	10.65
	(1.120)	(1.163)	(1.146)	(1.186)	(1.184)	(1.190)
0.40	11.43	12.11	11.91	12.73	12.72	12.77
	(1.277)	(1.353)	(1.331)	(1.423)	(1.421)	(1.427)
0.60	13.23	14.32	13.84	15.48	15.46	15.66
	(1.478)	(1.600)	(1.547)	(1.730)	(1.728)	(1.750)
0.80	15.33	17.18	16.79	19.45	19.37	19.72
	(1.713)	(1.920)	(1.876)	(2.173)	(2.165)	(2.204)

* The numbers in parenthesis represent the relative viscosity.

Plot of log η' against concentration of the barbiturates has been found to yield rather close linearity in Figure 7. This result may be conpared with the variation of the relative viscosity with concentration in Figure 6, where there is a very rapid rise in viscosity with increase of concentration.

Onsager and Fuoss have deduced theoretically the relation (64)

$$\eta' = 1 + Ac^{\frac{1}{2}} + Bc + Dc \log c$$

for dilute solutions of electrolytes. Attempts to test this relation as also the simpler one

$$\eta' = 1 + Ac^{\frac{1}{2}} + Bc$$

showed no validity of these relations for the solutions of barbiturates. The closest fit was obtained by the use of the equation

$$\eta' = 1 + Ac^{\frac{1}{2}} + Bc + Dc^2$$

in the case of barbital, phenobarbital and probarbital sodium. Amobarbital, pentobarbital and secobarbital sodium were found to agree more closely with the equation

$$\eta' = 1 + Ac + Bc^2 + Dc^3$$

involving a cubic term.

The coefficients in the terms of these equations have been evaluated by the use of determinants and are given on page 46.

Relative Viscosity as

a Function of Molar Concentration

$$\eta' = 1 + AC^{\frac{1}{2}} + BC + DC^2$$

	A	B	C
Barbital Sodium	0.1167	0.2319	0.6899
Probarbital Sodium	0.01636	0.6240	0.4445
Phenobarbital Sodium	0.06434	0.50865	0.6808

$$\eta' = 1 + AC + BC^2 + DC^3$$

	A	B	C
Pentobarbital Sodium	0.7711	0.5504	0.3817
Amobarbital Sodium	0.9959	0.03415	0.56696
Secobarbital Sodium	0.9052	0.06157	0.86012

Legend for facing graph

A. ——— --- ——— Amobarbital Sodium

B. — — — — — — — — Barbital Sodium

C. ---------- Pentobarbital Sodium

D. —— —— —— —— Phenobarbital Sodium

E. ———————— Probarbital Sodium

F ——— - ——— - — Secobarbital Sodium

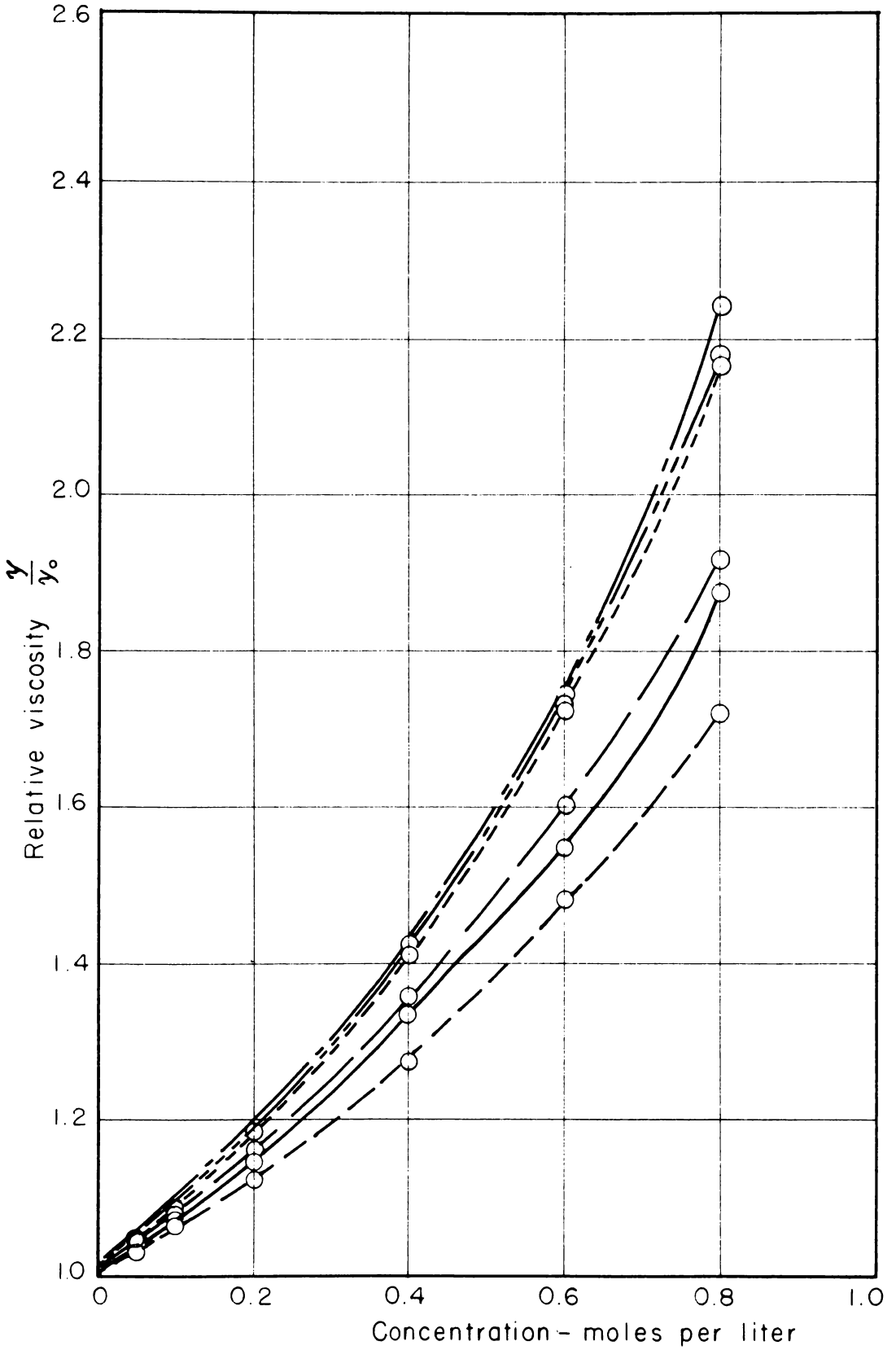
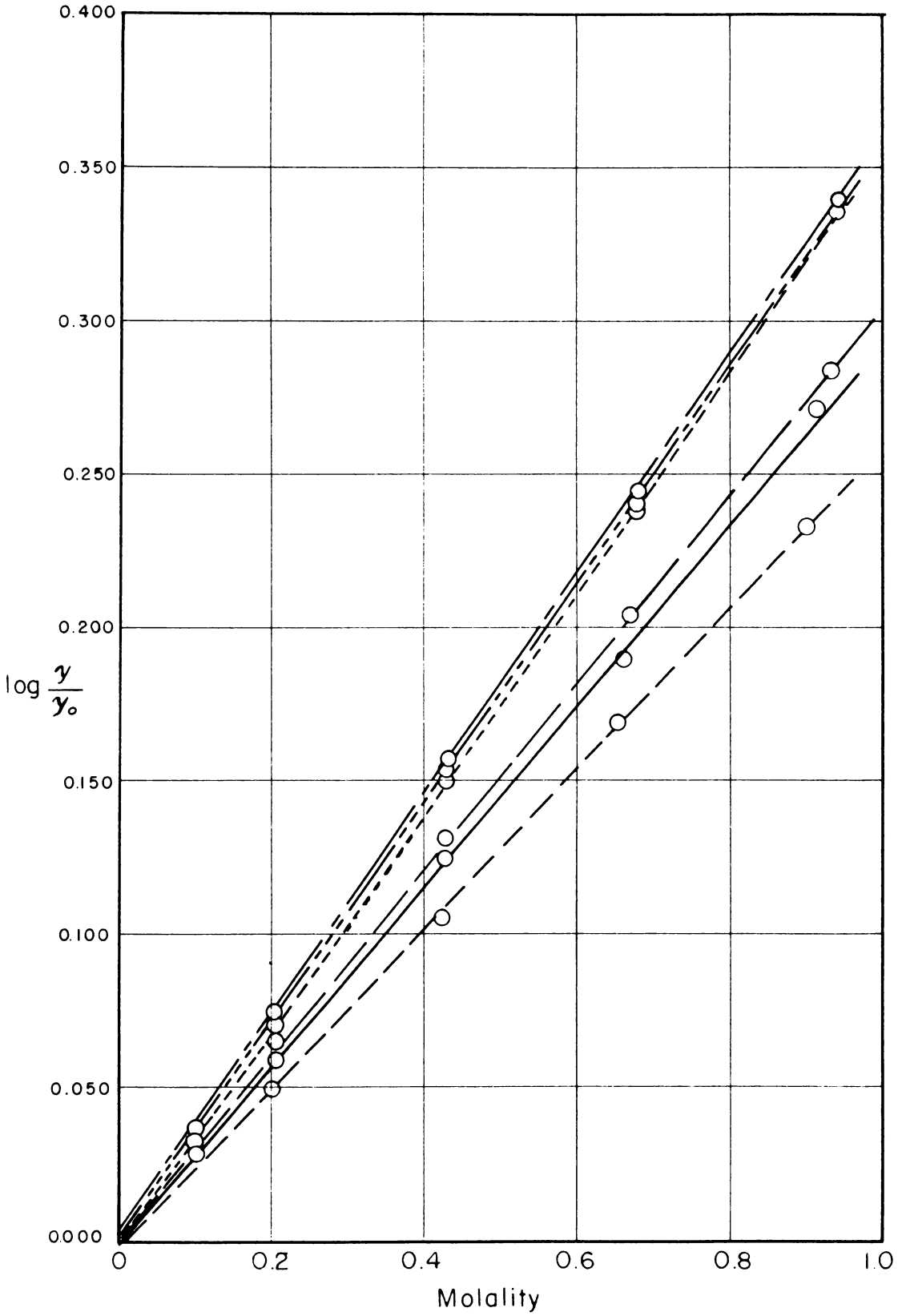

2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Relative viscosity $\frac{\eta}{\eta_0}$
0
0.2
0.4
0.6
0.8
1.0
Concentration - moles per liter

Fig. 7


0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
log $\frac{y}{y_o}$
0
0.2
0.4
0.6
0.8
1.0
Molality

Conductivity Measurements

The electrical conductivity of solutions of the barbiturates were measured by the Kohlrausch method. The source of alternating current was a 1000 cycle audio-oscillator. The slide wire set-up was of Leeds and Northrup make, wound over a bakelite cylinder with a contactor mounted on the inner surface of a hood, also of bakelite. A standard non-inductively wound resistance box, calibrated by the National Bureau of Standards, formed one arm of the Wheatstone bridge arrangement. A variable condenser connected parallel to the resistance served to minimize capacitance effects. Henry type cells with platinized electrodes were employed to measure the conductivity of the solutions. A pair of ear phones were used as the detector to determine the balance point indicated by a sharp minimum of sound.

The cell constants were determined by the use of Bakers CP Analyzed potassium chloride heated for one hour to about 400° C and cooled in a dessicator. The solutions of potassium chloride were made up by weight to conform to the Parker's standards (65) and their specific conductivities at the temperature coefficients given by Parker and Parker (66).

The conductivity water used in preparing all the solutions had the following mean specific conductances at the temperatures indicated.

25° C	1.22 10^{-6}	Mhos. $Cm.^2$
30° C	1.33 10^{-6}	" "
42.7° C	1.89 10^{-6}	" "

The solutions for the measurement of conductivity were made up by volume to known molar concentration at 25° C. Their concentrations at higher temperatures have been obtained by the relation, C x d_t/d_{25}, where C is the molar concentration at 25° C; d_t and d_{25}, their densities at t and 25° C. respectively. Since the solutions whose conductances were measured at the higher temperatures were dilute, the ratio d_t/d_{25} was taken to be approximately equal to that of water for these calculations.

The conductivity data for solutions of the sodium salts at various concentrations are given in Tables IX and IXa. The studies at 25° C. covered the concentration range 0.005 to 0.8 molar. Those at 30° C. and 42.7° C. were used only to estimate the equivalent conductivity at infinite dilution of the salts at these temperatures and were therefore confined to a few values in the region of low concentration, it being assumed that their variation with concentration at higher concentrations would follow a pattern similar to that at 25° C.

Plots of the equivalent conductance values against the square root of normality of the solutions yielded curves with nearly steady slopes at higher concentrations and with increasing steepness at high dilution. Extrapolation of the latter portion to zero concentration gave the limiting equi-

valent conductances of the salts tabulated on page 60.

Subtraction of the equivalent conductance of sodium ion at each temperature gave the limiting conductances of the anions which are included in the same table. The values of the equivalent conductance of sodium ion at each temperature employed were obtained directly or were calculated with the aid of the references indicated and are shown below.

Limiting equivalent conductance of sodium ion at

25° C.	50.1 mho cm^2	(67)
30° C.	56.2	
42.7° C.	71.5	

In order to obtain the limiting equivalent conductance of the hydrogen barbiturates the hydrogen ion conductances were added to the anionic conductance at the respective temperature. The hydrogen ion conductances were calculated for the higher temperatures by the use of the temperature coefficients given by Owen and Sweeton (68).

Limiting equivalent conductance of hydrogen ion at

25° C.	349.8 mhos. $cm.^2$
30° C.	373.9
42.7° C.	431.4

TABLE IX Conductance

Concentration eq/lit.	Barbital	Phenobarbital	Probarbital	Amobarbital	Pentobarbital	Secobarbital
	Specific and equivalent conductances at 25° C.					
0.8	0.02988 37.4	0.02542 31.8	0.02847 35.6	0.02541 31.8	0.02552 31.9	0.02573 32.2
0.6	0.02532 42.2	0.02219 37.0	0.02421 40.4	0.02240 37.3	0.02197 36.6	0.02226 37.1
0.4	0.01917 47.9	0.01702 42.6	0.01864 46.6	0.01737 43.4	0.01723 43.1	0.01744 43.6
0.2	0.01103 55.2	0.009972 49.9	0.01092 54.6	0.01018 50.9	0.01002 50.1	0.01023 51.2
0.1	0.006016 60.2	0.005551 55.5	0.005989 59.9	0.005684 56.8	0.05582 55.8	0.005719 57.2
0.05	0.003233 64.7	0.002930 58.6	0.003142 62.8	0.003010 60.2	0.002962 59.2	0.003012 60.2
0.02	0.001368 68.4	0.001237 61.9	0.001356 67.8	0.001269 63.5	0.001259 63.0	0.001290 64.5
0.01	0.0007033 70.3	0.0006314 63.1	0.0006942 69.4	0.0006558 65.6	0.0006466 64.7	0.0006601 66.0
0.005	0.0003595 71.9	0.0003253 65.1	0.0003527 70.5	0.0003317 66.3	0.0003338 66.8	0.0003338 66.8

TABLE IXa

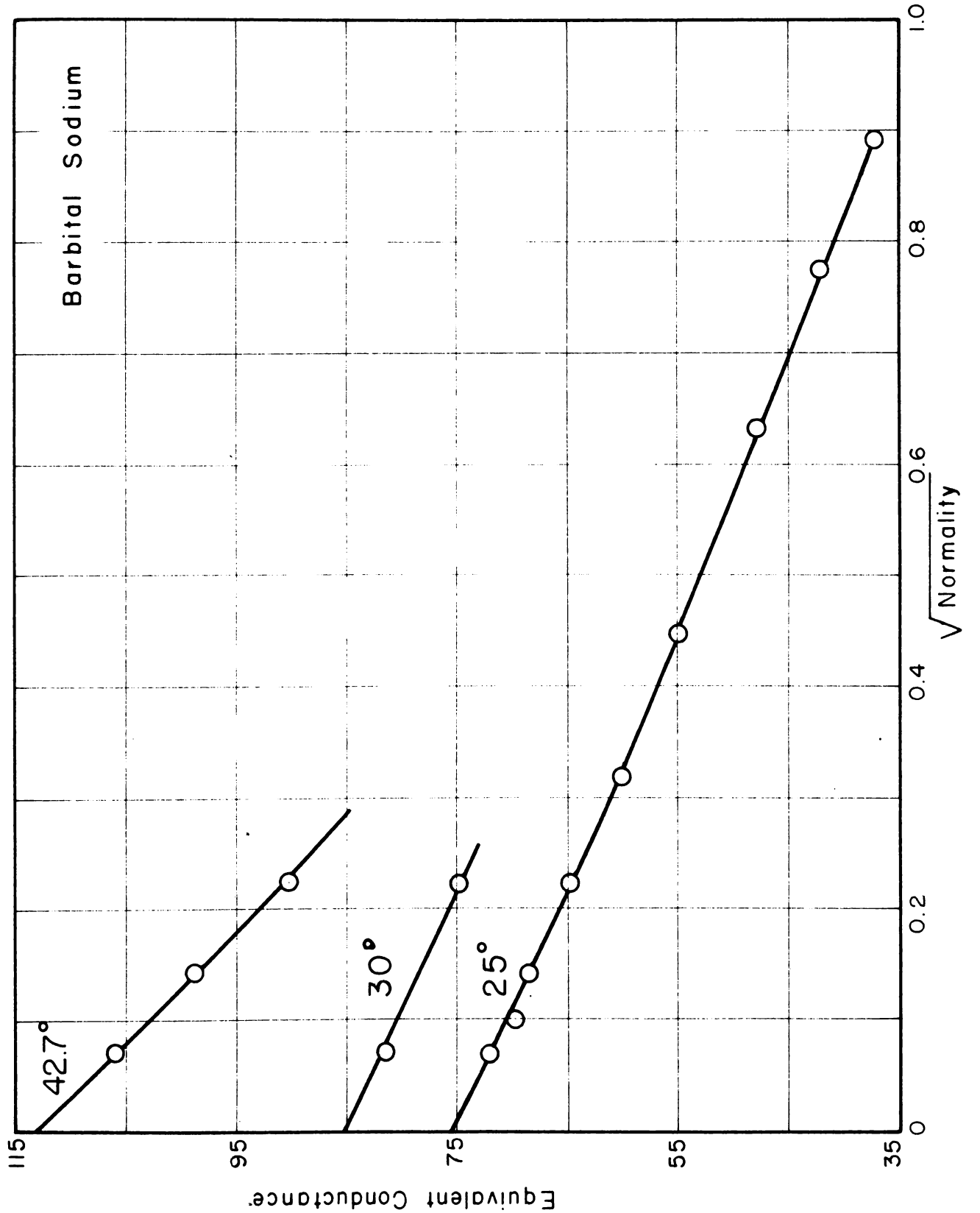
Equivalent conductance of aqueous sodium barbiturate solutions at 30° C.

	Molarity		Molarity		Molarity	
Barbital Sodium	0.0499	74.70			0.00499	81.73
Probarbital Na			0.01997	78.27	0.00499	80.84
Phenobarbital Na			0.01997	70.25	0.00499	73.14
Amobarbital Na			0.02497	69.14	0.00499	73.27
Pentobarbital Na	0.0499	69.38	0.01997	72.78		
Secobarbital Na	0.0499	68.21	0.01997	71.02	0.00499	74.66

Equivalent conductance at 42.7° C.

Barbital Sodium	0.0497	92.32	0.01988	98.75	0.00497	106.15
Probarbital Na .	0.0497	91.16	0.01988	97.86	0.00497	105.8
Phenobarbital Na	0.0497	84.56	0.01988	90.02	0.00497	96.63
Amobarbital Na			0.02485	86.79	0.00497	97.76
Pentobarbital Na	0.0497	84.25	0.01988	92.80	0.00497	98.71
Secobarbital Na	0.0497	85.66	0.01988	92.17	0.00497	99.33

Fig. 8


Barbital Sodium
42.7°
30°
25°
Equivalent Conductance
115
95
75
55
35
0
0.2
0.4
0.6
0.8
1.0
√Normality

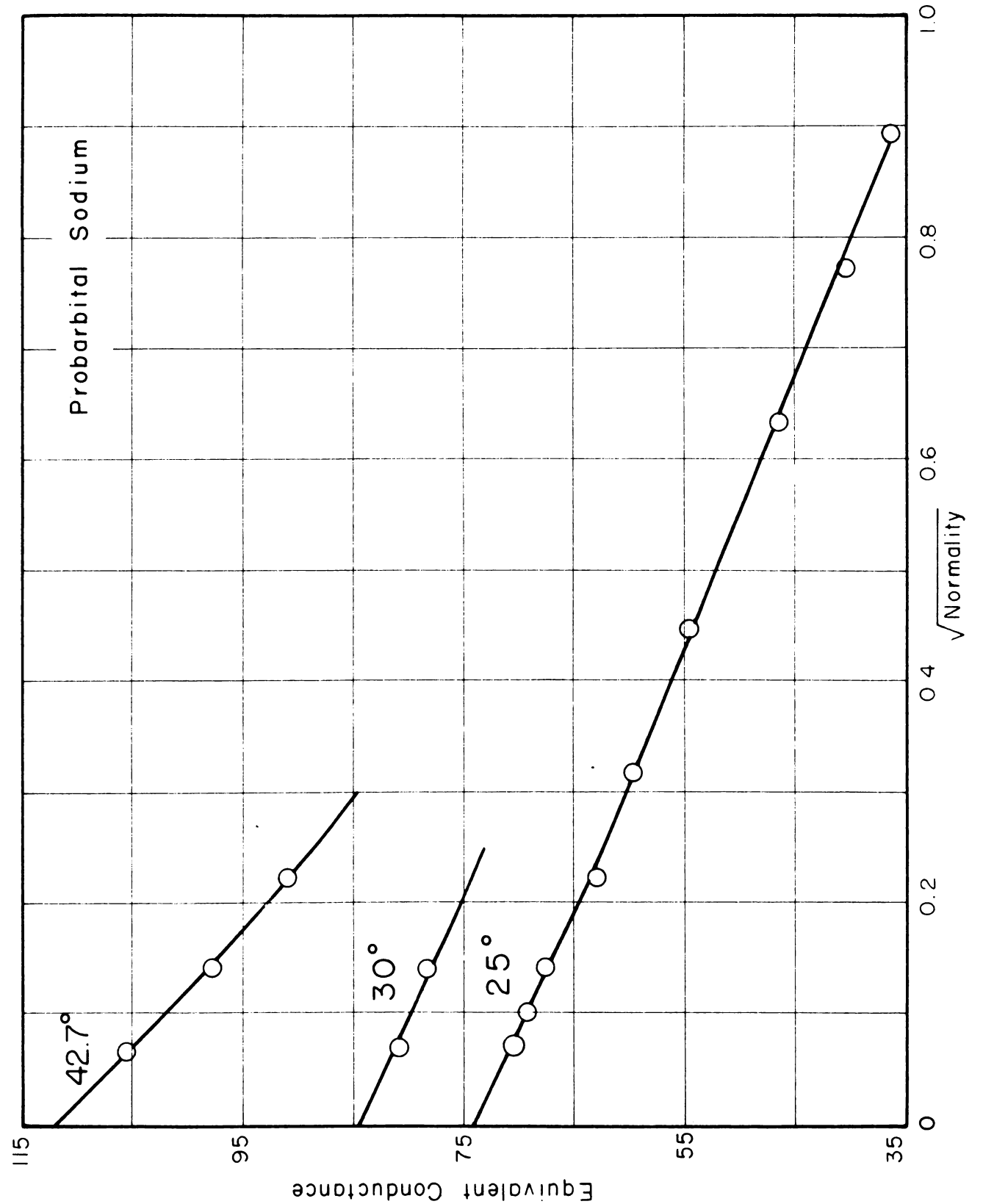

Probarbital Sodium
42.7°
30°
25°
Equivalent Conductance
115
95
75
55
35
0
0.2
0.4
0.6
0.8
1.0
√Normality

Fig. 10

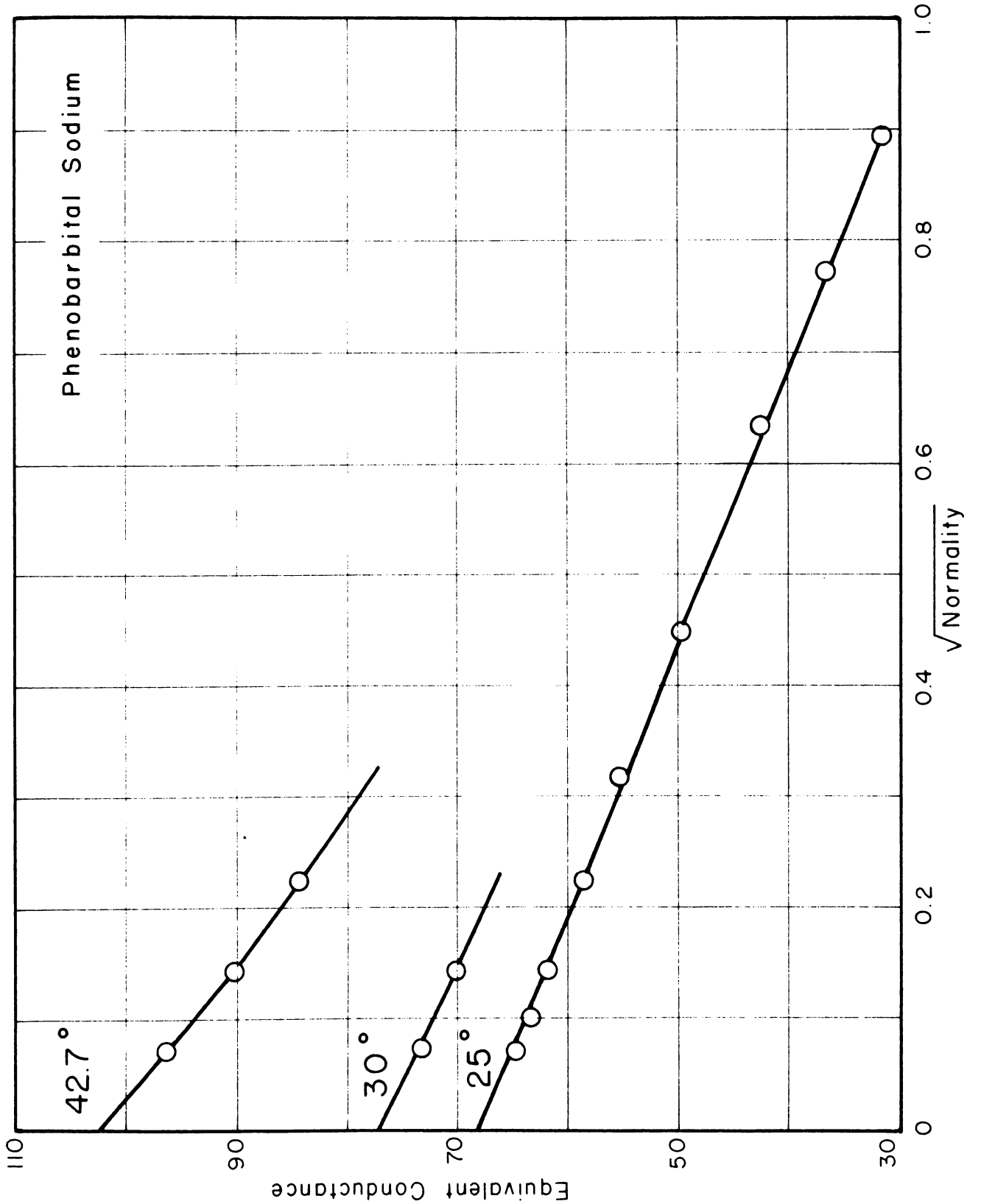

Phenobarbital Sodium
42.7°
30°
25°
Equivalent Conductance
110
90
70
50
30
0
0.2
0.4
0.6
0.8
1.0
√Normality

Fig. 11

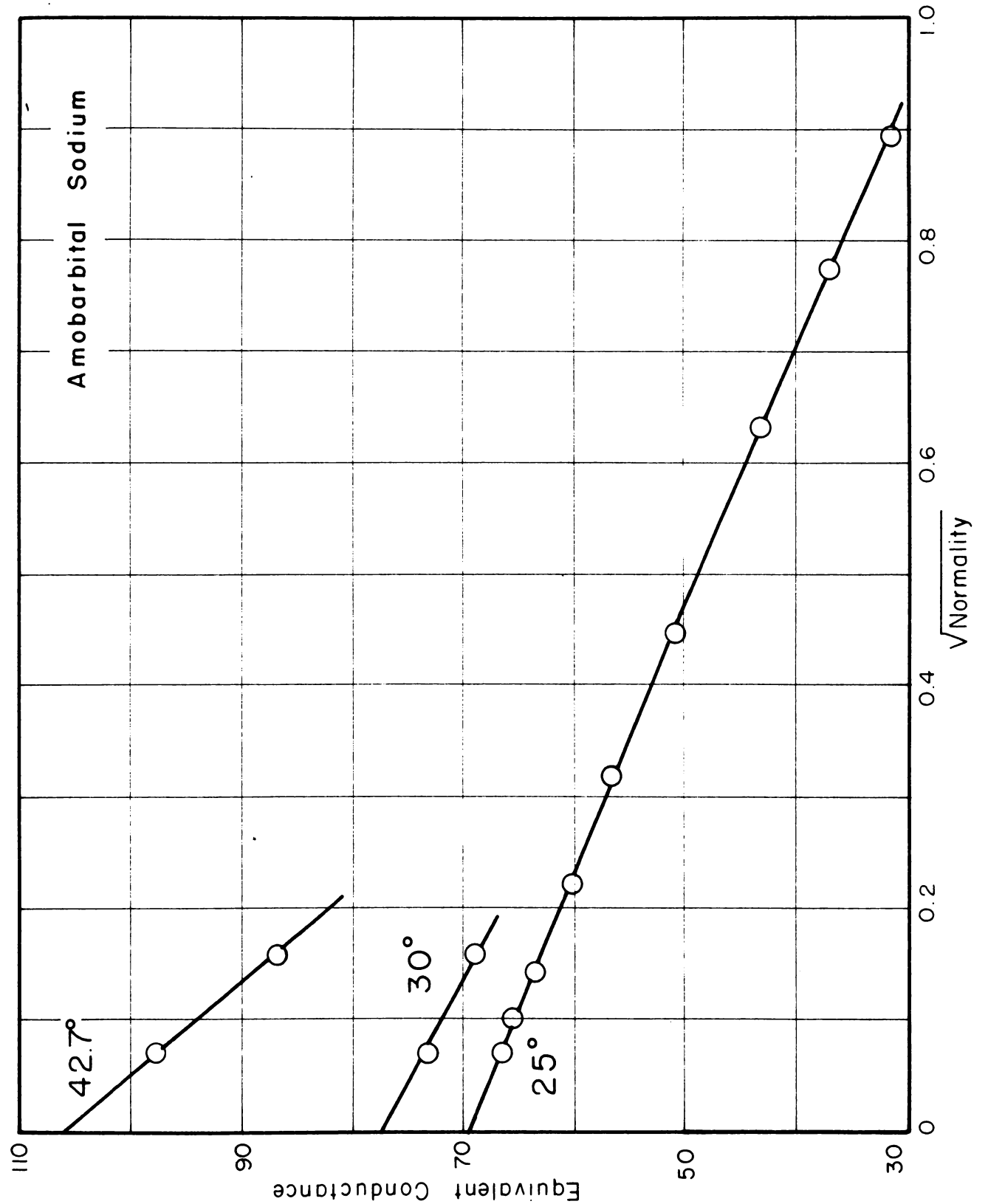

Amobarbital Sodium
42.7°
30°
25°
Equivalent Conductance
110
90
70
50
30
0
0.2
0.4
0.6
0.8
1.0
√Normality

Fig. 12

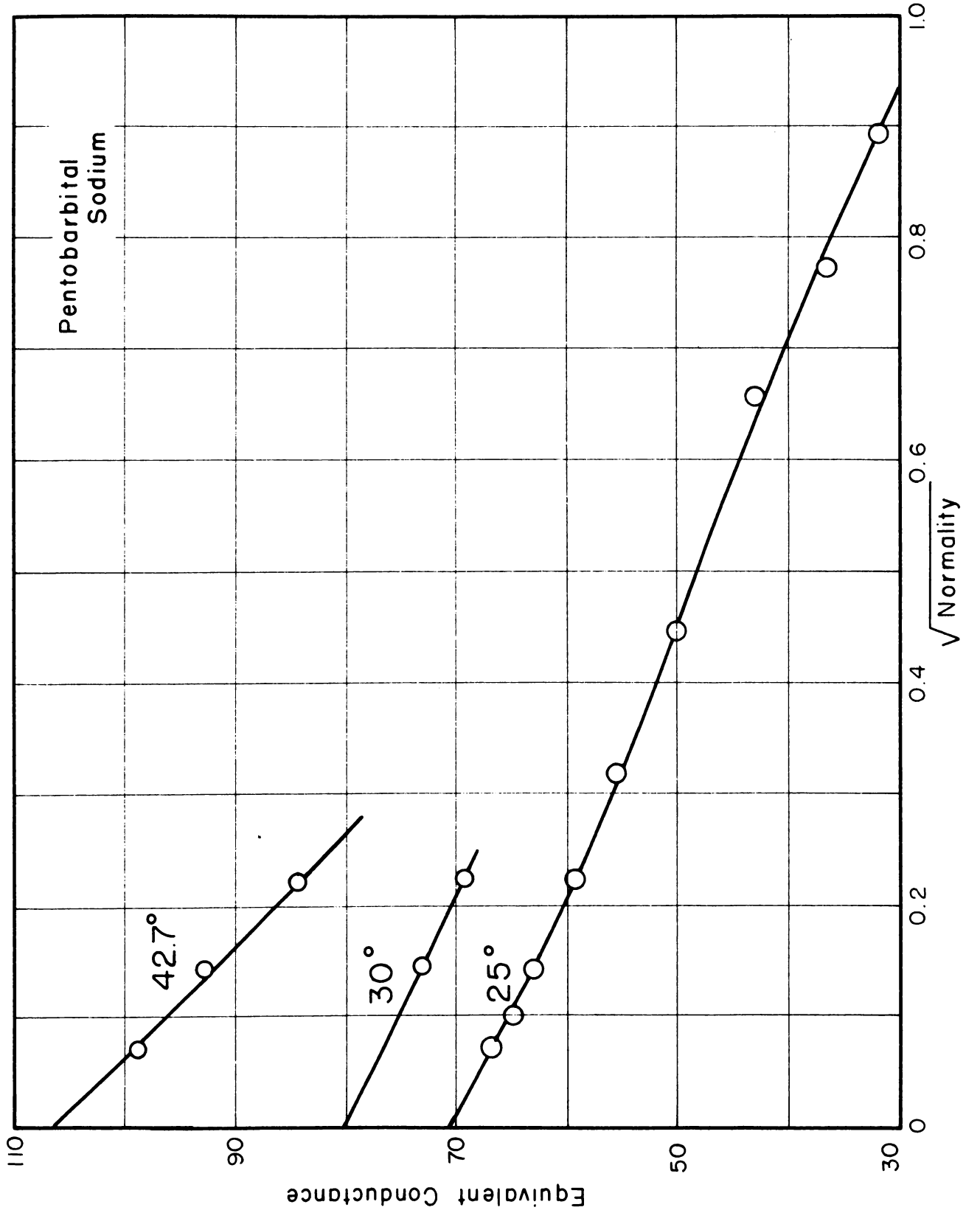

Pentobarbital Sodium
42.7°
30°
25°
Equivalent Conductance
110
90
70
50
30
0
0.2
0.4
0.6
0.8
1.0
√Normality

Fig. 13

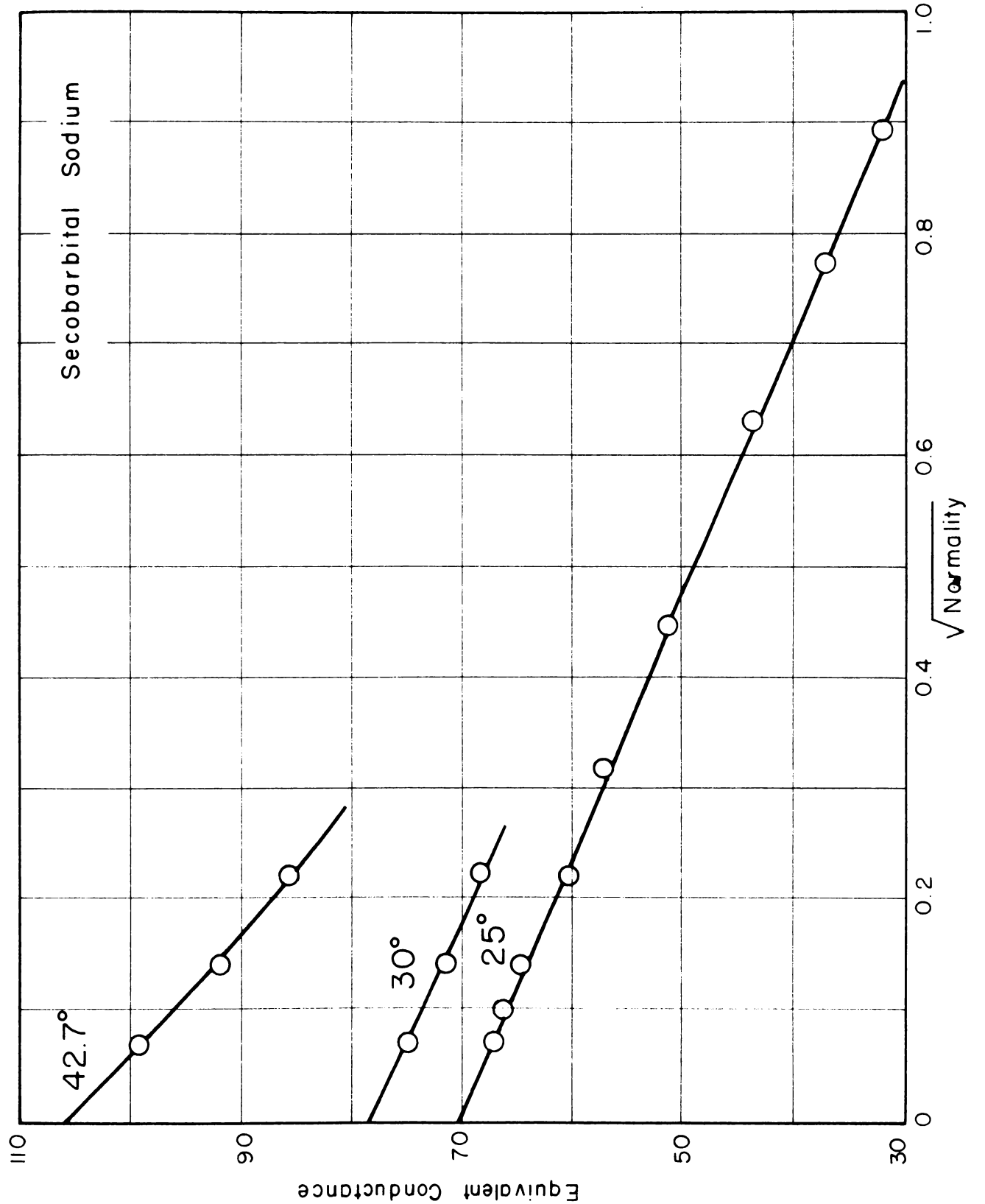

Secobarbital Sodium
42.7°
30°
25°
Equivalent Conductance
110
90
70
50
30
0
0.2
0.4
0.6
0.8
1.0
√Normality

TABLE X

Limiting equivalent conductances

	λ_o of sodium salt			λ^- Anionic conductance			λ_o of the acid		
	25° C.	*30° C.	*42.7° C.	25° C.	30° C.	42.7° C.	25° C.	30° C.	42.7° C.
Barbital	75.5	85.3	113.1	25.4	29.1	41.6	375.2	403.0	473.0
Probarbital	74.0	84.5	112.2	23.9	28.3	40.7	373.7	402.2	472.1
Phenobarbital	68.2	76.8	102.5	18.1	20.6	31.0	367.9	394.5	462.4
Amobarbital	69.3	77.2	106.0	19.2	21.0	34.5	369.0	394.9	465.9
Pentobarbital	70.4	79.8	107.0	20.3	23.6	35.5	370.1	397.5	466.9
Secobarbital	70.2	78.5	106.2	20.1	22.3	34.7	369.9	396.2	466.1

* The values at 30° and 42.7° C. are precise to ±0.3 units

Diffusion

The law of diffusion of electrolytes and the relation between the diffusion coefficient and ionic mobilities due to Nernst (69) have received considerable attention in recent times (70) experimentally as well as theoretically. Reference may be made in this connection to the works of Onsager and Fuoss (71) and later workers in this field (72).

The diffusion coefficient for an infinitely dilute aqueous solution of a 1:1 electrolyte is given by

$$D = 17.863 \ 10^{-10}. \ T. \ \frac{\lambda^+ \lambda^-}{\lambda^+ + \lambda^-}$$

By the use of this equation, the diffusion coefficients of the sodium salts of the barbiturates at infinite dilution have been calculated and listed in Table XI.

TABLE XI

Diffusion coefficients in cm^2 per sec.

$x10^5$

	25° C.	30° C.	42.7° C.
Barbital Sodium	0.90	1.04	1.48
Probarbital Sodium	0.86	1.02	1.46
Phenobarbital Sodium	0.68	0.81	1.22
Amobarbital Sodium	0.74	0.83	1.31
Pentobarbital Sodium	0.77	0.90	1.34
Secobarbital Sodium	0.76	0.86	1.32

Ionization constants of the hydrogen barbiturates

According to Arrhenius dissociation theory the degree of ionization of a weak electrolyte is given by the conductance ratio λ/λ_o, if the mobilities of the ions are assumed not to change with concentration.

The hydrogen barbiturates under study were prepared as described on page 16. Their low solubilities in water, which are in the range 0.5 to 2 grams per liter (7) except for barbital, restricted the measurements to very dilute solutions, all solutions being well below saturation. As the substances dissolved in water slowly, dissolution was effected at a temperature of 80-100° C., and the solution cooled before making it up to the desired volume in each case.

The specific conductances of the hydrogen barbiturates were measured at known concentrations. Subtraction of the specific conductance of water, which was of comparable magnitude and therefore necessitated constant check, gave the specific conductance of the solute.

The degree of ionization at the specified concentrations were calculated by the use of λ_o values of the hydrogen barbiturates given on page 61.

The ionization equilibrium of the acids HB, where B represents the barbiturate radical, has according to the law of mass action

$$\frac{(H^+)(B^-)}{(HB)} = \frac{c^2x^2}{c(1-x)} = K_a,$$ the ionization constant

where c is the concentration and x the degree of ionization. The activity coefficients have not been considered.

The ionization constants of the hydrogen barbiturates, so derived, have been tabulated on page 65, as also their $pK_a = (-\log_{10} K_a)$ values.

The free energy of ionization for the process

$$HB_{(aq.)} = H^{+} + B^{-}$$

is given by the relation $\Delta F^{o} = -RT \ln K_a = 2.3026\ RT\ pK_a$ (73). The values for the free energy of ionization of the hydrogen barbiturates have been recorded in the same table.

TABLE XII

Ionization of Hydrogen Barbiturates

Temperature 25° C

	eq/lit.	sp.cond.	eq.cond.	x	K_a x 10^8	pK_a	ΔF° in Kilocals.
Barbital	0.0100	0.00000381	0.381	0.001015	1.03	7.99	10.89
Probarbital	0.0040	0.00000223	0.558	0.001494	0.89	8.05	10.98
Phenobarbital	0.0030	0.00000312	1.04	0.00283	2.66	7.58	10.33
Amobarbital	0.0016	0.00000123	0.769	0.00208	0.70	8.16	11.13
Pentobarbital	0.0040	0.00000202	0.505	0.00136	0.74	8.13	11.08
Secobarbital	0.0020	0.00000117	0.585	0.00158	0.50	8.30	11.32

Temperature 30° C

Barbital	0.00998	0.00000408	0.409	0.001014	1.03	7.99	11.07
Probarbital	0.00399	0.00000243	0.609	0.001514	0.91	8.04	11.14
Phenobarbital	0.00299	0.00000350	1.17	0.00296	2.63	7.58	10.51
Amobarbital	0.001597	0.00000138	0.864	0.00219	0.76	8.12	11.25
Secobarbital	0.001597	0.00000126	0.789	0.00199	0.63	8.20	11.37

Discussion

Structural features affecting physical state and the hypnotic activity of barbiturates.

The compounds which have been the subject of this study, belong to a closely related class characterized by an identical trioxypyrimidine portion, polar in character, at one end of the molecule, while at the other positions are differing substituents in the 5,5 position. It may be noticed (page 14) that barbital and probarbital differ only by a $-CH_2$ group in the substituents and pentobarbital and amobarbital are isomeric with difference in the position of branching in the substituent chain. Phenobarbital has an aromatic substituent while the other members of the series contain only aliphatic chain substituents. Secobarbital contains an ethylenic bond in its chain. It has been observed that a minimum of four carbon atoms is necessary among the substituents in the 5,5 position for the members of this class to exhibit hypnotic activity, the activity increasing up to eight carbon atoms. A further increase in the number of carbon atoms (beyond eight) produces a decrease in activity (74). Introduction of polar groups such as hydroxyl, amino or carbonyl, in the substituents generally deprive the barbiturates of their hypnotic activity. The presence of a lipotropic portion within certain size limits and a hydrotropic portion constituted by the pyrimidine ring thus seem to be common features of the members of this series which possess hypnotic activity.

With no suggestion of any direct relation, it may be stated that the same "amphipathic" nature (75) viz. a non-polar hydrocarbon portion and a comparatively small polar group, distinguishes the class of compounds called colloidal electrolytes. It has been observed that among the salts of fatty acids, at least seven carbon atoms are necessary in the hydrocarbon chain for colloidal properties to be exhibited in solution (76). Such solutions are stable and fulfill all the strict thermodynamic requirements as do solutions of ordinary electrolytes. They are, however, especially at higher concentrations, heterogeneous in character, from the point of view of the phase rule (77). Structurally some of the sodium barbiturates studied here come close to satisfying the conditions above stated with the exception of the chain length. In such cases it may be open to question, to what extent the configuration of the pyrimidine moiety makes additional contribution, if any, to the paraffinic character of the substituents in the 5,5 position.

Hydrolysis of sodium barbiturates in solution

The sodium salts of barbiturates are hydrolyzed in aqueous solution, the barbituric acids being weakly acidic. The pH of the solutions lie in the vicinity of 8-9. The possibility of the acids formed by hydrolysis saturating the solution and causing their own deposition has been considered but it has been calculated that the free acids produced would be considerably below the concentration required for saturation.

Physical properties in solution

Only the more salient features arising out of the study of some of the physical properties of sodium barbiturates in solution will be reviewed here.

The low density of aqueous solutions of sodium barbiturates even at high concentration is evident. This low-density corresponds to a high molar volume in comparison with salts like sodium halides in solution. A regular increase in apparent molar volume of the substances at any given concentration is noted with increase in the number of carbon atoms and this conforms to our knowledge of molecular volume as an additive property.

Departures are noted in the forms of apparent molar volume vs. composition curves and the general forms possessed by ordinary electrolytes, marked rises of apparent molar volumes at concentrations occuring at concentrations close to 0.5 normal in the case of amobarbital, pentobarbital and secobarbital sodium. In this respect their behavior is similar to that of potassium n-octoate, one of the short chain soaps studied by Davies and Bury who have recorded a quite marked increase in partial molal volume of this substance over the concentration region 0.5 normal which has been attributed to aggregation of the molecules (78). Alexander and Johnson have advanced a suggestion in this connection that the lack of affinity between hydrocarbon chain and water leads to a curling

up of the molecules to reduce the hydrocarbon / water interface to a minimum, subjecting them to great compressive force. On aggregation, the surface energy is lessened and the pressure is released causing an increase in partial molal volume (79).

It has been pointed out in the experimental section that amobarbital, pentobarbital and secobarbital sodium tend to deviate from linearity of refractive index vs. molarity plots at medium concentrations. This deviation need not necessarily imply any change of state of the solute nor may the latter influence the apparent molar refraction to any appreciable extent (see p. 32).

McBain and collaborators describe three types of surface tension vs. concentration curves in water solution (80). Two of these typify the concentration dependence of surface tension of sodium barbiturates. One of these types of behavior indicates general positive adsorption of the solute in agreement with Gibbs adsorption isotherm wherein surface tension is lowered gradually by increasing concentration of the solute. Barbital, probarbital and phenobarbital apparently follow this pattern. The other type, exemplified by the data of this study in amobarbital, pentobarbital and secobarbital sodium, belong to the pattern of soap and detergent solutions. These substances are characterized by a great lowering of surface tension in dilute solution, the surface tension remaining constant thereafter or passing through a minimum. McBain

sought to explain the rise of surface tension after passage through a minimum as due to decrease in the effect of a submerged electrical double layer, while Miles and Shedlovsky attribute such minima to the presence of foreign organic impurities (81). Irrespective of any minimum, it is to be noticed that the surface tension lowering by amobarbital, pentobarbital and secobarbital are even greater than that due to a complete coating of insoluble oleic acid on a water surface (surface tension 40 dynes/cm.). The surface behavior of sodium barbiturates in this respect may be contrasted with an increase of surface tension caused by increasing concentration of a solution of sodium chloride.

Studies in the distribution of various hydrogen barbiturates between water and charcoal surface by Tabern and Shelberg have shown the percentage adsorption of the substances on charcoal follows the order of increasing hypnotic activity of the barbiturates. The results of adsorption studies on sodium barbiturates given on page 42 reveal that the salts follow the same order qualitatively.

The rate of increase of viscosity with increase of concentration of sodium barbiturates is higher than that of ordinary electrolytes and less than that of soap solutions of comparable molecular weight. Among the salts studied here, there is a more rapid increase in the case of amobarbital, secobarbital and pentobarbital analogous to the pattern of colloidal electrolytes (82).

The empirical formula of Arrhenius

$$\eta' = A^{c}$$

is a relation which has been found to represent the behavior both ordinary and colloidal electrolytes (82), the difference being mainly in the exponential factor which represents the slope in log η' vs. $\underline{c}$ graph and this value is relatively greater for colloidal electrolytes. In view of the complexities of concentrations it is perhaps accidental that an equation of this kind should fit the results for a variety of solutes; and the sodium barbiturates are no exception.

An examination of the equivalent conductances at various concentrations of the sodium barbiturates show their magnitudes to be of the same order as sodium salts of organic acids of comparable molecular weights. Their variation with increase of concentration follows an approximate linearity with the square root of concentration, the slopes being smaller than that required by the Onsager equation. Undoubtedly, hydrolysis of the salts is bound to contribute to the total conductance and cause a small error inherent in all conductance work with salts of weak acids. It is estimated that the conductance values may not be in error on this account by over 1%. Moreover, it is noted that the anionic conductance of barbital at 25° C obtained by extrapolation (viz. 25.4) is actually less than the value 27.7 derived from an estimated semiempirical value for the limiting conductance of barbital given in the International Critical Tables (83).

Colloidal electrolytes are known to reveal the transition from simple ions to micelles by characteristic breaks in conductivity curves. These breaks are sharp for long chain compounds (of the order of 12 carbon atoms and above) and become less pronounced with decreasing number of carbon atoms in the chain. In the case of sodium barbiturates nowhere is a sharp break noticeable although secobarbital, pentobarbital and amobarbital seem to indicate a gradual inflexion at higher concentrations. It is possible, as suggested by the consideration of their structural features, that the compounds lie in the border region wherein the break in conductance may not be expected to be conspicuous.

Although the general physical properties discussed earlier seem to point to the colloidal behavior of pentobarbital, amobarbital and seconal sodium in particular, more fundamental and detailed investigation of the thermodynamic properties such as the lowering of vapor pressure, depression of freezing point or the magnitude of osmotic pressure - colligative properties which indicate the number of particles, ionic, molecular or colloidal - is considered necessary to trace the progress of ionic or molecular association in solution to give rise to a colloidal system.

Extrapolation of equivalent conductance vs. $\sqrt{\text{Normality}}$ curves of the sodium barbiturates to infinite dilution has provided values of the limiting conductances of the salts, their anions and of the corresponding hydrogen barbiturates.

Information on most of these are not on record. Taft and Patton have given the value for the limiting conductance of barbital anion at 30° C as 26.3, a value lower than the value 29.1 obtained in the present work on the same compound.(83 a)

The ionic conductances have been helpful in determining the values of the diffusion coefficients of sodium barbiturates. The diffusivities show no correspondence to the hypnotic activity of barbiturates. It may be expected - and this has been shown by conductivity measurements* - that in the medium of gastric juice, which has a mean pH of 1.6, the orally administered sodium barbiturates would be present almost completely as undissociated acid and therefore ionic diffusion may have no part in diffusion through stomach walls. Its role in transference of the drug from blood plasma to the sites of action also seems to be minor in the light of the principles discussed in an earlier section.

Ionization constants of hydrogen barbiturates: Relation to their rapidity of action.

The ionization constants of hydrogen barbiturates which have been determined with an estimated precision of 2% reveal the following features. The value which lies close to 1×10^{-8}

*For example, a medium was made up of hydrochloric acid and sodium chloride, 0.025 molar with respect to each, to approximate the principal electrolyte content of gastric juice. This solution had a pH 1.67. The addition of an exact amount of sodium barbiturate to this solution also 0.01 n with respect to the barbiturate (a concentration value resulting from a normal dose of barbiturate) gave a solution whose conductance was almost identical with that of a mixture 0.015 moles of HCl and 0.035 moles of NaCl per liter.

for barbital is more than doubled by the substitution of a phenyl group in place of an ethyl as is shown in the case of phenobarbital. The lengthening of the substituent in the 5 position causes a consistent fall in the value of ionization constant. The 5,5 substituents thus seem to have a direct effect on the ionization process.

Several workers have sought to determine the pK value of barbital. Wood in 1906 measured the conductance of acid barbital and reported a value 7.43 for its pK (84). The limiting conductance value employed appears to have been the one quoted in International Critical Tables (see p. 71). The following pK values have been obtained by later workers, all by E. M. F. measurements: 7.4 by Kolthoff (85), 7.86 by Michaelis (86), 7.89 by Britton and Robinson (87), 7.93 by Bush (47), 7.91 by Krahl (88) and 7.980 by Manov, Scheutte and Kirk (48). The value of 7.99 obtained in this work is considered in good agreement with the last of these. (Reported values have been corrected to 25° C)

Bush has obtained pK values for phenobarbital, pentobarbital and amobarbital by pH measurements in acid-salt mixtures. The values are shown below in comparison with the results of this work.

	By conductance	by pH (Bush)
Pentobarbital	8.13	8.04
Phenobarbital	7.58	7.34
Amobarbital	8.16	7.89

The values obtained by conductance are uniformly higher in the three cases.

The general physico-chemical features which have plausible validity in the hypnotic action of the barbiturates have been covered in the introduction to this work. Although the operative factors determining their activity are undoubtedly interrelated, it appears, as a result of this investigation, that the ionization constant of acid barbiturates, which determines the proportion in which the lipid soluble free acid barbiturates are present in plasma, is a dominant factor and determines the rapidity of onset of hypnotic action of the barbiturates. An examination of the pK values of the acid barbiturates show their increase in the following order: Phenobarbital < Barbital < Probarbital < Pentobarbital < Amobarbital < Secobarbital. This order corresponds to increasing abundance of undissociated acid barbiturates at the same concentration in any medium, other conditions being equal. This result may be compared with the order of rapidity of their onset of action presented in p. 10. to which it remarkably corresponds.

Similar conclusions have been reached by Klimesch under other premises by his studies on a number of barbiturates (46). The barbiturates investigated by Bush also yield results which would support this contention save for the lower pK value of 7.89 recorded for amobarbital (47). This also corresponds to the observation of Kindler mentioned on page 8.

The present work provisionally offers a better under-

standing of the probable cause of the differences in the time of onset of hypnotic action of the barbiturates. It becomes meaningful when we recall that a greater concentration of the lipid soluble undissociated acid barbiturate in plasma means faster transport across the nerve cell membranes and a more rapid build up of the minimum concentration, requisite for hypnosis, at the site of action of the barbiturates. Only an extension of a similar approach to a far greater number of barbiturates - or for that matter to drug action in general - may justify any claim for real validity or otherwise of the assumptions involved.

Bibliography

1. Beutner, "Physical Chemistry of Living Tissues and Life Processes" Williams and Wilkins Co., Baltimore, (1933)

2. Albert, A., Pharm. J., 145, 84-92-100, (1940)

3. Thomas C. Butler, Pharmacological Reviews, 2, 121-160, (1950)

4. Meyer, Arch. expt. Path. Pharmakol., 42, 109 (1899); 46, 338 (1901)

5. Overton, Arch. ges. Physiol. 92 , 115, (1902)

6. Tiffeneau, Soc. Biol., 84, 540, (1921)

7. Tabern, D. D. and E. Shelberg, J. Am. Chem. Soc., 55, 328, (1933)

8. Brown, D., Soc. Biol. 100, 703, (1929)

9. Meyer and Hemmi, Biochem. Z., 277, 59, (1935)

10. Dawson, H., and Danielli, J. F., "The Permeability of natural membranes" Cambridge University Press, (1943)

    Höbner, Physical Chemistry of cells and Tissues" The Blakiston Company, Philadelphia (1945)

11. Butler, T. C., J. Pharmacol. Exper. Therap., 74, 118-128, (1942)

12. Bear, R. S., Palmer, K. J., and Schmitt, F. D., J. Cellular Comp. Physiol. 17, 355-367, (1941)

13. Dode, M., Gavaudan, P., and Pousal, H., Compt. rend., 223, 521-523, (1946)

14. Fergusson, J., Proc. Roy. Soc., London, B.127, 387-404, (1939)

15. Brink, F., and Posternack, J. M., J. Cellular Comp. Physiol., 32, 211-233, (1948)

16. Richet, C., Compt. Rend. Soc. Biol., 45, 775-776, (1893)

17. Overton, "Studien uber die narcose", (1901)

18. Traube, Pflügers Arch. ges. Physiol., 218, 749 (1928) 153, 276, (1913); 161, 530, (1915)

19. Antonio Giacalone, Gazz. chim. ital., 70, 656-660, (1940)

20. Rideal, E. K., and Schulman, J. H., Nature, 144, 100, (1939)

20a. Rideal, E. K., Chem and Ind. 58, 830, (1939)

21. Eyster, H. C., Science, 96, 140, (1942)

22. Burr, H. S., and Barton, D. S., Yale J. Biol. Med., 10, 271, (1938)

23. Ravitz, L. J., Arch. of Neurol. and Psychiat., 65, 413, (1951)

24. Barnes, T. C., and Beutner, R., Science, 110, 511 (1949)

26. Vermast, B. G., Biochem. Z., 125, 106, (1921)

27. Clowes, G. H., Keltch, A. K., and Krahl, M. E., J. Pharmacol. Exper. Therap., 68, 312, (1940)

28. Albert, A., Rubbo, Goldacre, Davey, and Stone, Brit. J. Exp. Pathol., 26, 160, (1945)

29. Simmons, E. W., Nature, 166, 343, (1950)

30. Clowes, G. H., Keltch, A. K., Proc. Soc. Exper. Biol. Med., 29, 312 (1951)

31. Breyer, Biochem. Z., 301, 65 (1939)

32. Kindler, B., Arch. Pharm., 276, 107 (1938) and ibid. 277, 14 (1939)

33. Hawk, Osser and Summerson, "Practical Physiological Chemistry"

34. Mathews, "Physiological Chemistry"

35. Weese, H., Arch. exper. Path. Pharmakol., 181, 46 (1936)

36. Albert, A., Pharm. J., 145, 84 (1940)

37. Keeser and Keeser, J. Pharm. Exper. Therap., 53, 137 (1935)

38. Maynert, E. W., and Van Dyke, H. B., Pharmacol. Revs. 1, 217 (1949)

39. Andrew, R. L., and Neubauer, L. G., Analyst, 72, 21 (1947)

Koppanyi and Dille, J. Pharmacol. Exper. Therap., 54, 84 (1935)

40. Tatum, A. L., Physiol. Rev., 19, 472, (1939)

Grollman and Slaughter, "Pharmacology and Therapeutics," Lea and Febiger (1947)

41. Fisher, R. S., Walker, J. T., and Plummer, C. W., Am. J. Clin. Pathol., 18, 462 (1948)

42. Quartet, J. M., Current Researches Anesthesia and Analgesia, 30, 151 (1952)

43. Grollman and Slaughter, "Pharmacology and Therapeutics" Lea & Febiger (1947)

44. Pratt, J. Pharmacol. Exper. Therap., 48, 285 (1933)

45. Murphy and Koppanyi, Proc. Soc. Exper. Biol. Med., 31, 376 (1933)

Murphy and Koppanyi, J. Pharmacol. Exper. Therap., 52, 70 (1930)

Koppanyi, Dille and Linegar, ibid., 58, 119 (1936)

45$_1$. Dorfman, Albert and Goldbaum, J. Pharmakol. Exper. Therap., 90, 330 (1947)

46. Klimesch, Arch. Exper. Path. Pharmakol., 172, 10 (1933)

Klimesch and Starkenstein, Ibid., 176, 494 (1934)

47. Busch, M. T., J. Pharmakol. Exper. Therap., 61, 134 (1937)

48. Manov, G. G., Schuette and Kirk, J. Research Nat. Bur. Standards, 48, 84 (1952)

49. Beilstein, U. S., Pharmacopoeia, New and Non-Official Remedies, National Formulary.

50. Parker and Parker, J. Phys. Chem., 29, 132 (1925)

51. Reilly and Rae, "Physico-chemical Methods" Van Nostrand Co.

52. Masson, Phil. Mag., 8, 218 (1929)

53. Redlich and Rosenfeld, Z. phys. chem., 155, 65 (1931)
A. Electrochem., 37, 705 (1931)

54. Partington,J.R., "An Advanced Treatise on Physical Chemistry", Longmans, Green and Co., 1951.

55. Weissberger, "Physical methods of organic chemistry", Interscience (1949)

56. Kohner, Z. physic. Chem., B1, 427 (1928)

57. Geffcken, W., Z. Electrochem., 37, 233 (1931)

58. Kruis, A., and Geffcken, W., Z. physik. Chem., B34, 51 and 70 (1936)

59. Ersenlohr, F., ibid., 75, 585 (1910), 79, 129 (1912)

60. Manov, Schuette and Kirk, J. Research Nat. Bur. Standards, 48, 84 (1952)

61. Nouy, J. Gen. Physiol., 1, 521 (1918); 5, 429 (1923)

62, Dorsey, "Properties of ordinary water-substance," Reinhold Publishing Corp., New York (1900)

63. Arrhenius, Z. phys. chem., 1, 285 (1887)

64. Onsager and Fuoss, J. Phys. Chem., 36, 2689 (1932)

65. International Critical Tables

66. Parker and Parker, J. Am. Chem. Soc., 46, 312 (1924)

67. Johnston, J., ibid., 31, 1010 (1909)

Gunning and Gordon, J. Chem. Phys., 10, 126 (1942)

68. Owen and Sweeton, ibid., 63, 284 (1941)

69. Nernst, W., Z. physik, chem., 2, 613 (1888)

70. Ann. Rev. Phys. Chem., 1, 65 (1950)

71. Onsager and Fuoss, J. Phys. Chem., 36, 2689 (1932)

72. Harned and Owen, "The Physical Chemistry of Electrolytic Solutions" Reinhold Publishing Corp., (1950)

73. Klotz, I.M., "Chemical Thermodynamics", New York, Prentice Hall,(1950)

74. Dox and Yoder, J.Am.Chem.Soc.,44, 1141 (1922)

75. Hartley, Ann. Repts. Progr. Chem.(Chem.Soc.London) (1948)

76. McBain, Dye and Johnston, J. Am. Chem. Soc.,61,3210(1939)

77. McBain, "Colloid Science",D.C.Heath and Co.,Boston (1950)

78. Davies and Bury, J.Chem.Soc., 2263 (1930)

79. Alexander and Johnson, "Colloid Science", Oxford University Press (1950)

80. McBain, Ford and Wilson, Kolloid Z., 78, 17(1937)

81. Miles and Shedlovsky, J. Phys. Chem., 48, 57 (1944)

82. McBain, "Colloid Science", Heath and Co., (1950)

83. International Critical Tables

83a. Taft and Patton, Trans.Kans. Acad. Sci., 53, 585 (1950)

84. Wood, J.K., J. Chem.Soc., 89, 1831 (1906)

85. Kolthoff, I.M., Pharm. Weekblad., 57, 514 (1920)

86. Michaelis, L., J.Biol. Chem., 87, 33 (1930)

87. Britton, H.T.S., and Robinson, R.A., J. Chem. Soc., 1456 (1931)

88. Krahl, M.E., J. Phys.Chem., 44, 449 (1940)