308. *A Micro-flotation Method for the Precise Comparison of Liquid Densities and its Application to a Preliminary Investigation of the Distribution of Heavy Water in Certain Salt Hydrates and to Other Matters.*

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The flotation method of comparing liquid densities previously described has been refined by the use of smaller silica floats and concomitant changes in the technique of purifying samples and observing the flotation temperature so that comparisons with an error not exceeding $\pm$ 0·2γ^d are made with 2—5 c.c. of water obtained from an original sample of about 10 c.c. Temperature control of the thermostat has been improved by using a relatively small mercury regulator controlling the heater current by means of a valve-operated relay circuit. A rapid method for density comparisons within $\pm$ 2·0γ^d is also described.

These methods have been applied to additional survey experiments, especially on waters from cancer tissues, in the study of certain salt hydrates, and in examining the interchange between heavy water and certain amines and cobaltammines. Copper sulphate, hydrated with partly heavy water, on dehydration preferentially retains light water, and the experiments indicate that in this salt hydrated partly with heavy and partly with light water considerable interchange occurs in the solid state at temperatures below 100°.

The hydrochlorides of mono-, di-, and tri-methylamine, ethylamine, aniline, and pyridine, dissolved in heavy water, exchange the whole of the hydrogen linked directly to nitrogen within the duration of an experiment, say, 45 minutes. In agreement with Erlenmeyer and Gartner, various cobaltammines are found to exchange their ammine hydrogen completely with heavy water, but a study of hexamminocobaltic chloride has shown that this exchange proceeds at a measurable rate and may require several hours for completion.

A possible explanation is thus afforded for conflicting results recorded by Bankowski.

The flotation method of comparing densities described in a previous communication (J., 1934, 1207), although satisfactory in precision and trustworthiness,* was laborious and required a sample of at least a litre. We therefore attempted to refine the method so that, without loss of accuracy, a much smaller silica float and a correspondingly smaller sample could be used. The present communication records the technique whereby density comparisons with an error not exceeding $\pm$ 0·2γ^d have been made with about 2—5 c.c. of water, using an original sample of about 10 c.c. only.

Though the use of smaller samples facilitates the process of purification very materially, it involves certain special precautions which are described in the experimental part, notably a closer control of the temperature of the thermostat, a correction for the pressure at which the measurement is made (since the micro-floats appear to be practically incompressible), and extreme care to avoid mechanical damage to the float. It is a notable advantage of the micro-method that since the losses during purification are quite negligible, and there is no rejection of head and tail fractions, there is no risk of changing the isotope ratio by fractional distillation. Further, as the later stages of purification are effected in a vacuum, the specimen is secure from atmospheric contamination and is thoroughly outgassed. On the other hand, the demands made upon the purification process are very exacting, because a quantity of impurity insufficient to produce any appreciable change in the density of a macro-sample may possibly change the density of a micro-sample by 10γ^d.

To illustrate both the sensitivity of the method and the care it demands, the following experience may be cited. During a series of measurements the float was accidently shaken in its tube, and thereafter it was found that the flotation temperature of the specimen had

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* It is satisfactory to observe that certain doubts expressed on this point (Rakshit, *J. Physical Chem.*, 1935, **39**, 303) have been shown experimentally to be groundless (Dole and Weiner, *Science*, 1935, **81**, 45).

changed by 0·145°. On examining the float under the microscope it was found that a tiny flake of silica had been chipped off the tip. This, of course, involved a restandardisation of the float.

This technique has been applied in the extension of the survey previously reported (J., **1934**, 1207), and especially to certain specimens of water having a possible relationship to the incidence of cancer in man and animals. Some of the earlier results thus obtained suggested that the isotopic ratio in the blood might have some diagnostic value, and although this idea is not supported by the later results, the variations observed may well prove ultimately to have some meaning and it seems, therefore, desirable to record them. In certain cases, such as exchange reactions using water containing 0·5—5% D_2O, where sufficiently precise density comparisons are given by determinations of flotation temperature within ± 0·01°, the process of measurement can be greatly simplified and accelerated. In this way the dehydration of certain salt hydrates, especially copper sulphate pentahydrate, has been examined. Though the results recorded in the experimental part can only be regarded as preliminary, they afford clear indications that on dehydrating copper sulphate light water is preferentially retained in the monohydrate (or heavy water preferentially evolved), and that a considerable interchange occurs between the " monohydrate " water and the rest of the water of hydration in the solid state at temperatures no higher than 100°.

The general theoretical considerations here involved have been clearly set out by Ingold and his collaborators (J., **1934**, 1593) and need not be recapitulated. They inferred from their experiments that any structure involved in the linking of water of hydration to ions in solution has a loose and evanescent character. The interchange we have now observed in the solid state would appear to indicate that this is also true for the water of hydration of ions in the crystal lattice of copper sulphate, but, on the other hand, the preferential retention of light water on dehydration seems to imply a " chemical " rather than a " physical " linkage of water. Clearly, further experimental investigation of this matter is desirable, and this we hope to undertake in the near future.

The same technique of density comparison has been applied to a study of the interchange between water and certain cobaltammines. Erlenmeyer and Gartner (*Helv. Chim. Acta*, 1934, **17**, 1008) have stated that interchange in such cases is complete, but Bankowski (*Monatsh.*, 1935, **65**, 266; cf. Erlenmeyer and Lobeck, *Helv. Chim. Acta*, 1935, **18**, 1213) has reported that it is only partial. Our experiments reconcile these conflicting statements by showing that, although the interchange does indeed involve all the ammine hydrogen in such salts, yet it proceeds at a relatively slow rate and was presumably not completed in Bankowski's experiments. A kinetic study of this reaction is now being undertaken.

EXPERIMENTAL.

Purification of Specimens.—The method of purification, although similar in principle to that used previously for large samples, differs therefrom in detail and may be regarded as falling into three main stages : (1) Repeated dry oxidation with air over copper oxide at 800°; (2) distillation with and from sodium peroxide and permanganate; (3) simple distillation in a vacuum. For stage (1) the apparatus shown in Fig. 1 is employed; *A* and *B* are two similar vessels of Pyrex glass, carrying side tubes as shown and fitted with standard interchangeable ground joints. By means of the adaptors *C* and *D*, they are connected through the silica tube *E*, packed with oxidised cylinders of copper gauze isolated from the walls by a wrapping of asbestos paper, and heated by a multiple Bunsen burner. The specimen of water is distilled very slowly from *A*, through *E* into *B*, which is cooled to − 80° with solid carbon dioxide and alcohol, and during the whole distillation a slow current of air, dried over calcium chloride, is drawn through the system by means of an aspirator connected to the side limb of *B*. When the whole of the specimen has thus been distilled over, the apparatus is dismantled, the silica furnace *E* is heated with the blow-pipe throughout its length and especially at the inlet end (*A*) to destroy traces of organic deposit, and then the parts are re-assembled with *A* and *B* interchanged so that the distillation may be repeated.

How effective this procedure is may be inferred from the fact that, with blood as the starting material, only three or four distillations were required to produce a perfectly colourless and odourless specimen of water.

Stage (2) is important, for it removes nitric and nitrous acid, sulphuric and sulphurous acid, and carbon dioxide, all or any of which may be formed as oxidation products during stage (1). In the vessel *F* (Fig. 2), having a sealed-in reflux condenser, the specimen is boiled with sodium peroxide (about 0·01 g.) for about 20 mins. and with added potassium permanganate (about 0·01 g.) for a further 10 mins. Larger samples are poured into *F* : smaller samples, with which loss must be avoided, are transferred to *F* by means of the apparatus shown in Fig. 2, *F* in this case replacing *H*, so that the specimen may be distilled into it directly from *B* (Fig. 1) which replaces *G*.

In every case this same apparatus is used both to transfer the sample from *F* and to effect the final distillations and the transference to the flotation tube. The specimen being at *G*, the traps *J* and *K* are cooled with solid carbon dioxide, the apparatus is thoroughly evacuated, and the cock *L* closed. Then *H* is cooled to − 80° while *J* is allowed to warm up, and *G* is warmed to about 30° so that the specimen and any condensate in *J* distil wholly into *H*. Provided the specimen has been thoroughly out-gassed, distillation proceeds quite smoothly, but in case any bumping should occur, a trap *M*, packed with glass-wool, is interposed between *G* and *H*. This distillation is then repeated, if necessary, and finally the sample is distilled into the flotation vessel *N* (Fig. 3), which for this purpose is fitted in place of *H*. After the first density determination has been made, the specimen is poured into a clean distilling vessel (*G*) and redistilled into the flotation vessel for a second determination. At the end of a series of determinations the specimen is poured away, and the flotation vessel is pumped dry without washing. To make the apparatus vacuum-tight, the ground joints are lubricated with a very thin film of Apiezon Grease L, which is carefully restricted to the outer part of the joint. Whenever it is observed that the grease has crept, as it must in time, towards the inner parts of the joints, the apparatus is cleaned by digestion with sulphuric–chromic acid mixture, well washed first with tap water and then with good distilled water, and finally steamed out for ½ hour. Immediately after steaming, the joints are capped with tin-foil to keep out dust during cooling, and then the apparatus is set up as before and warmed under vacuum till dry. The joints dry first, and are then greased.

Fig. 1.

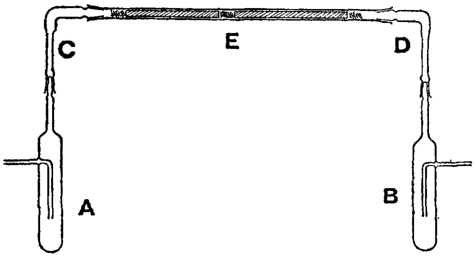
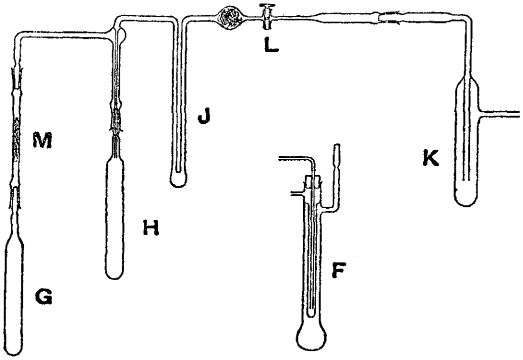


Fig. 2.



The importance of the trap *J* is evident from the fact that if it be omitted a small head fraction is lost, and specimens containing 0·5% D_2O show after each distillation a rise of flotation temperature (increase of density) of about 0·003°. With specimens much poorer in D_2O, for example the standard tap water, no perceptible change in density results from the rejection of this small head fraction.

The flotation vessel is cleaned only when it becomes contaminated with grease. Then it is pumped dry, washed successively with chloroform, alcohol, and water, soaked overnight in chromic–nitric acid mixture, washed thoroughly with distilled water, and lastly washed with three changes of conductivity water and dried by evacuation. The specimen to be measured is then distilled into the vessel as a final wash and poured back into another vessel for redistillation into the flotation tube for measurement.

The Measurements.—In general, the method of measurement resembles that used with larger floats and need not be described again here. The present floats are about 40 mm. long × 1 mm. diam., and the flotation tube is about 5 mm. internal diameter × 25 cm. long overall, containing a column of water about 7—10 cm. long, representing about 1·5—2 c.c. For small differences of temperature, the relationship between velocity and Δt for these micro-floats is not materially different from that observed with macro-floats.

In addition to the Calderara thermometers used in the earlier work, two standard Beckmann thermometers were used for the micro-determinations, chiefly because it proved desirable and possible to control the temperature of the bath more precisely. The intervals on the Beckmann thermometers were checked against each other and against the Calderara thermometers : no appreciable difference was observed. Fluctuations of 0·001° in the temperature of the thermostat, which were adequately smoothed out in transmission to a sample of 150 c.c., caused marked variations in the temperature of the micro-specimens of 5—10 c.c. Therefore a more sensitive thermo-regulator was employed, having 16 vertical toluene bulbs, each 20 cm. × 1·2 cm., well distributed throughout the bath. An inner glass vessel of water, 40 cm. × 6 cm., was also used to contain the flotation tube, this inner bath being agitated by a slow stream of air previously raised to the bath temperature. It was found that if the stream of air bubbles exceeded 3—4 per second, an upward drift of temperature occurred, of the order of 0·001° in 10 minutes. Since this drift varied in magnitude with the air stream, it was doubtless due to the conversion of work into heat. A cooling coil was incorporated in the main thermostat bath, as it was found that this facilitated measurement when the temperature of bath was within 2—3° of its surroundings.

FIG. 3.

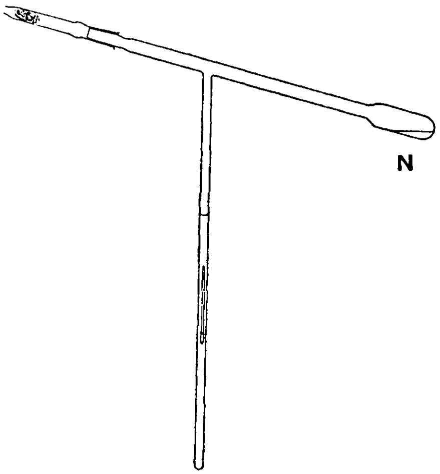
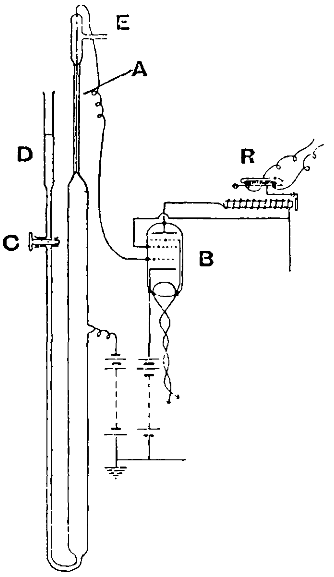


FIG. 4.



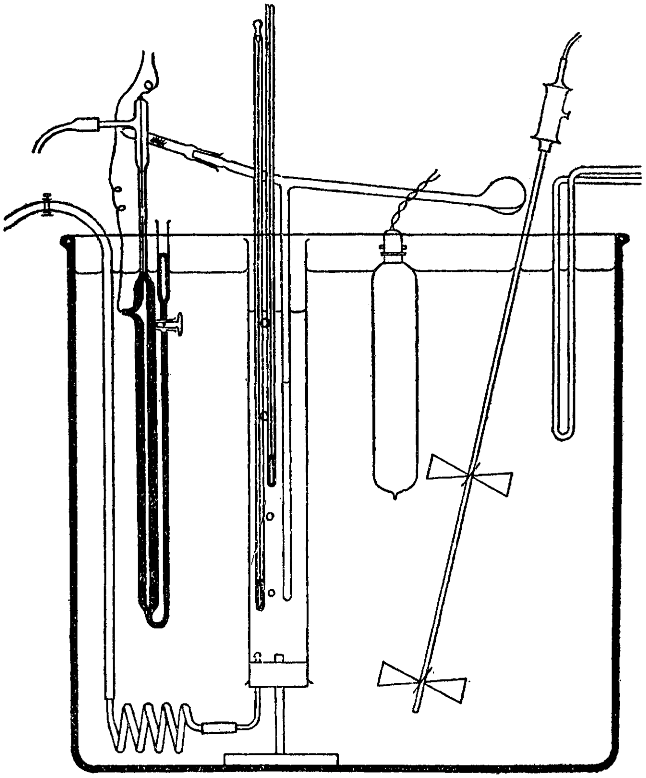
When the heater current was adjusted by the series resistance so that the " on " and " off " periods were about equal, no difficulty was experienced in keeping the temperature of the inner bath constant within ± 0·001° for several hours.

As any vibration of the flotation vessel caused inconsistency in the flotation temperature observed, and produced a movement of the float towards the wall of the tube, the motor and stirrer were carried by a support independent of the bench carrying the thermostat.

Subsequently, it was found advantageous to substitute for the very large toluene–mercury regulator (described above) the much more efficient mercury regulator shown diagrammatically in Fig. 4. This is of Pyrex glass, and the controlling contact is made at *A* between a very fine tungsten wire, sealed through the top, and the column of mercury in a capillary tube about 0·3 mm. in diameter. This contact is inserted in the control grid circuit of an indirectly heated pentode valve *B* of the type commonly employed in the output stage of a broadcast receiver. The potentials applied to the anode, the auxiliary grid, and the cathode are such that when the control grid circuit is closed there is a negligible current in the anode circuit. On opening the control grid circuit by breaking contact at *A*, a current of about 40 milliamps. flows in the anode circuit and serves to actuate a moderately sensitive mercury-switch relay *R* controlling the heater current. At first some irregularity in action was experienced : this was traced to the effect of leakage along the glass surface from mercury to tungsten and was immediately eliminated by covering the mercury with heavy liquid paraffin. Under these conditions the operation of the regulator is highly reliable and so sensitive that with a mercury-filled bulb of only about 60 c.c. capacity the bath temperature could be held constant within the readable limits (± 0·001°) for many hours at a stretch. Indeed, the sensitiveness is such that variations of atmospheric pressure, by varying the size of the regulator bulb, have an appreciable effect on the temperature at which the regulator operates. For this reason the top of the regulator must be closed to maintain the pressure constant. Regulation of the operating temperature is simple, as a coarse adjustment is afforded by the cock *C* in conjunction with the reservoir *D*, while a fine adjustment is secured by expansion or contraction of the bulb itself, by controlling through *E* the air pressure above the mercury.

Evidently, variations in the valve circuit are possible, and several other arrangements of this type have been described (see, *e.g.*, Beaver and Beaver, *J. Ind. Eng. Chem.*, 1923, **15**, 359; Hutchinson's Testing Apparatus Ltd., *J. Sci. Instr.*, 1934, **11**, 227; Folley and Temple, *ibid.*, 1935, **12**, 392; Temple, *ibid.*, 1936, **13**, 414). It seems worth while, however, to emphasise the practical value of this device, and to point out the importance for its success of using tungsten contacts sealed through Pyrex, of immersing the contacts in oil, and of controlling the pressure in the regulator.

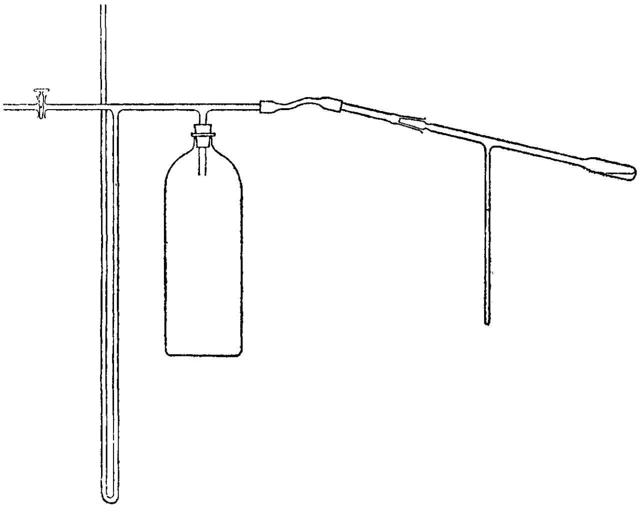
FIG. 5.



The general arrangement of the thermostat is shown in Fig. 5.

Effect of Pressure Change on the Flotation Temperature.—In the early stages of the present investigation the flotation tube was protected by means of a closed cap, and the flotation temperatures observed were very erratic. A suspicion that these variations were due to pressure changes (which must, of course, accompany temperature changes in a closed tube) was confirmed by direct experiment with the apparatus shown in Fig. 6, whereby any desired pressure difference could be applied to the atmosphere in the flotation tube. The results for floats No. 1 and No. 2 showed that there is a linear relationship between pressure and flotation temperature, a change of 0·001° in the latter resulting from a pressure change of 0·52 cm. Hg for No. 1 and 0·54 cm. Hg for No. 2. It appears therefore that these small floats are practically incompressible—a striking difference from the macro-floats, which showed no pressure effect over a range of ± 30 cm. Hg and so, by mere chance, were almost exactly as compressible as water.

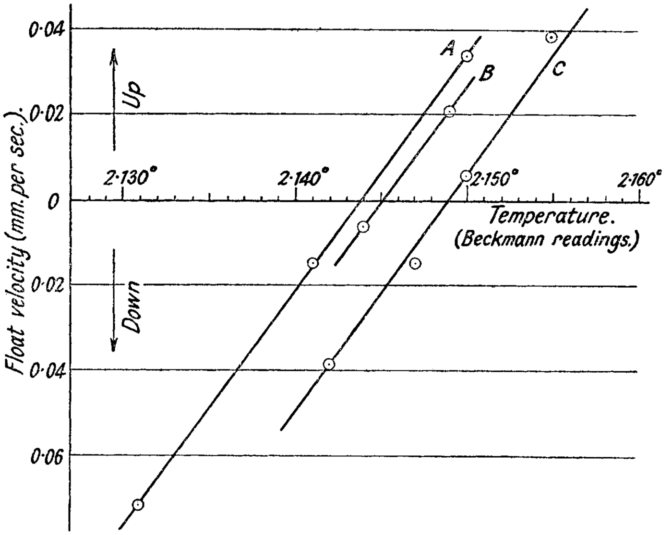
FIG. 6.



The general consistency of the results and the apparent validity of the correction for pressure differences are illustrated by the data for a single sample of water given in Fig. 7.

In some of the later interchange experiments, where fairly heavy water containing 1% or more of D_2O was used, it became convenient to employ a less precise and more speedy method of comparing flotation temperatures. The bath in this case is a 2-litre cylindrical silvered Dewar vessel, with clear strips for observation. It is heated by a steam jet, cooled by circulation of water in a cooling tube, and stirred as required by a stream of air. The temperature of the bath can thus be very quickly adjusted and can be held constant within ± 0·01° for several minutes, a time sufficient for observation of the micro-float when flotation temperatures within ± 0·01° ≡ ± $2{\cdot}0\gamma^d$ are accurate enough for the purpose in hand.

FIG. 7.



Typical flotation curves (sample from $NiSO_4,7H_2O$).
Curve A. Barometer 736 *mm.*
,, *B.* ,, 743 ,,
,, *C.* ,, 740 ,,

Corrected values of flotation temperature (768 *mm.*).
From curve A. 2·138°
,, ,, *B.* 2·140° } *mean* 2·41°.
,, ,, *C.* 2·144°

Results.

The purposes to which this micro-method has been applied are diverse, but may be grouped under two heads: (*a*) an extension of the survey already reported; and (*b*) a preliminary enquiry into the mode of interchange between heavy water and certain salt hydrates, amines, and ammines.

Additional Survey Experiments.—Since these are in principle quite similar to our earlier survey, they are adequately presented by Table I. We were enabled to examine the specimens of

TABLE I.

Specimen.	T_{H_2O}.	T_S.	$10^3(T_S - T_{H_2O})$.	$\Delta\gamma^d$.
Starches.				
Potato starch	*3·623°	3·618° 3·620	+ 4°	+0·8°
Rice starch	3·623	3·607 3·605	+17	+3·4
Wheat starch	3·623	3·616 3·614 3·618	+ 9	+1·8
Alga.				
Spirogyra (by combustion)	3·623	3·623 3·624	0	0
Micas.				
Muscovite (from Tanganyika Territory, E. Africa)	3·623	3·618 3·619	+ 4	+0·8
Muscovite (from Nellore district, Madras, India)	3·623	3·605 3·607	+17	+3·4
Specimens from the Cancer Hospital.				
Normal rat blood, average from 6 rats	3·626 3·626	3·622 3·625 3·623+ 3·623−	+ 3	+0·6
Blood from cancerous rats: average from 6 rats (J.R.S. tumours)	3·624 3·626	3·653 3·650+	−27	−5·4
Water from rat-cancer tissue: average from 6 rats (J.R.S. tumours)	3·640 3·638 3·638 3·643	3·660+ 3·659	−19	−3·8
Normal human blood: man aged 61	3·623	3·623 3·625	0	0
Blood from cancer patient: carcinoma of colon	3·623	3·623 3·623 3·626	0	0
Water from cancer tissue: carcinoma of breast; woman aged 61	3·623	3·620 3·622 3·621 3·618 3·621 3·620	+ 3	+0·6
Normal mouse blood from 25 mice	3·631 3·629 3·629	3·622 3·620+ 3·624−	+ 8	+1·6
Blood of 12 mice with malignant sarcoma	3·630	3·628− 3·628+	+ 2	+0·4
Blood of 12 mice with Crocker sarcoma	3·630	3·622 3·622	+ 8	+1·6
Necrotic tissue, mouse; Crocker sarcoma	3·630	3·630 3·627 3·626	+ 2	+0·4
Breast carcinoma	21·301	21·317 21·318	+17	+3·4
Carcinoma of rectum: blood supplied by Mr. Johnstone	21·301	21·304 21·303	+ 3	+0·6
Carcinoma of breast: primaries. Woman aged 61	21·301	21·302 21·302	+ 2	+0·4
Carcinoma of breast: secondaries. Woman aged 61	21·301	21·306 21·306	+ 5	+1·0
Rat blood; J.R.S. tumour	21·301	21·303+ 21·304	+ 3	+0·6
Cancer tissue: rats; J.R.S. tumours	21·301	21·302 21·301	0	0
Normal mouse blood	21·301	21·310 21·309	+ 8	+1·6
Water from combustion of cholesterol	21·301	21·293 21·295	− 7	−1·4

* All readings of the order of 3° are readings on the arbitrary scale of a Beckmann thermometer.

blood, tissue, etc., dealt with in the lower part of Table I, through the kind collaboration of Professor Kennaway and Dr. Boyland, of the Cancer Hospital, who gave us crude distillates from the various sources. Though no meaning can be attached to the few results here recorded, it seems noteworthy that substantial variations in the isotopic composition of the water are observable in cases of cancer, and may perhaps, by more extended study, later acquire significance.

Investigations by Dole (*J. Amer. Chem. Soc.*, 1935, **57**, 2731; *J. Chem. Physics*, 1936, **4**, 268) and others (*e.g.*, Hall and Jones, *J. Amer. Chem. Soc.*, 1936, **58**, 1915), published since these measurements were made, show that the variation of density found may, where small in magnitude, be due in part to variations in the ratio of the oxygen isotopes : the larger variations, however, must be due to changes in the hydrogen isotopic ratio.

The Dehydration of Copper Sulphate Pentahydrate.—A quantity of crystallised copper sulphate was dehydrated completely by heating to about 300° in a vacuum until no further condensation of water could be observed in a trap cooled to − 80° interposed between the salt and the pump. The anhydrous salt was then recrystallised from heavy water containing about 0·5% D_2O, and the crystals after drainage at the pump and thorough drying between filter-papers, were dehydrated in two stages, so that they first lost 4 mols. of water and later were completely dehydrated. The water evolved at each stage was condensed, and the densities of these two specimens of water were subsequently determined in the micro-flotation apparatus.

Batch I of copper sulphate was first heated in a retort immersed in an oil-bath at 110° until distillation of water ceased. The end-point of this operation was quite sharp and corresponded with the elimination of $4H_2O$. The retort was then connected with an evacuated receiver and heated with a free flame until the remaining molecule of water had distilled over.

Batch II of copper sulphate was dehydrated in a similar fashion, except that the first 4 mols. of water, as well as the remaining molecule, were evaporated off in a vacuum and condensed in a receiver cooled to − 80°. Under these conditions about 95% or more of the fifth molecule can be removed without appreciable decomposition of the salt.

Batch III was dehydrated in the same fashion as Batch I, except that during the first stage the oil-bath was maintained at 120—130°. The results given in Table II were obtained on examining the distillates, the temperature readings being those observed on the arbitrary scale of the Beckmann thermometer, which is, of course, inverted.

TABLE II.

Batch.	Fraction of crystal water.	Flotation temperature.		Diff. in flotation temp.
		Readings.	Mean.	
I	1st—4th	2·911°		
		2·914	2·913°	
	5th	3·016		
		3·016	3·016	−0·103°
II	1st—4th	2·452	2·452	
	5th	2·568	2·568	−0·116
III	1st—4th	1·920		
		1·924	1·922	
	5th	1·942		
		1·944	1·943	−0·021

From these data it appears, rather unexpectedly, that in hydrated copper sulphate containing some heavy water the first four molecules eliminated in dehydration are *heavier* than the fifth. All three experiments agree on this point, so there is reason to believe that light water is preferentially held by copper sulphate. A possible alternative view, that heavy water is preferentially eliminated, seems inherently improbable. Nevertheless, the unexplained variations in the data suggest that the phenomenon is complex and, therefore, that no quantitative significance can yet be attached to these results. The difference between the first four molecules and the fifth is greatest in the case where dehydration was effected wholly under vacuum and where, consequently, there was least opportunity for interchange either in the solid or in the vapour phase, and the difference is least in the case where the removal of the first 4 mols. at 120—130° under atmospheric pressure gave conditions which are obviously more favourable for either type of interchange. This also appears to be significant.

In a second experiment anhydrous copper sulphate was first rather less than monohydrated with ½% heavy water and then treated with rather less than 4 mols. of normal water, the water being introduced slowly as vapour from a cool vessel into the dry solid maintained at about 30° so that no condensation could possibly occur. On dehydrating this salt in the fashion already described, it was again found that the water first evolved was markedly heavy.

In yet another experiment, a quantity of copper sulphate, carefully dehydrated completely in a vacuum, was first partly hydrated with 1% heavy water, slightly less than the quantity required to form the monohydrate being used. This product was then suspended in alcohol, and treated with normal water in quantity almost sufficient to convert the salt into pentahydrate. The salt was then dehydrated first at 100° and later at 300°, the fractions of water being collected separately and with all precautions to avoid their contamination with each other. On examining these the following results were obtained :

Sample.	Flotation temp.	$T_{H_2O} - T_S$.
Standard water	21·10°	—
Stock heavy water	26·74	5·64°
1st—4th H_2O from salt	21·79	0·69
5th H_2O from salt	22·36	1·25

Although a good deal more work is required to elucidate this problem, it seems clear that, as between the first molecule and the remaining molecules of hydrate water, a substantial degree of interchange occurs in the solid state.

Interchange Experiments with Amine Hydrochlorides.—These were carried out by using 1% heavy water and density determinations to ± 0·01°. Quantities of the amine hydrochlorides were weighed out such that each contained the same weight of hydrogen directly attached to nitrogen. Each was then dissolved in 5·00 g. of the same sample of heavy water at a temperature of 40—45°, and after the whole had been kept at about this temperature for 45 minutes, the water was distilled off in a vacuum and purified in the usual manner. The results of the flotation temperature determinations are given in Table III.

TABLE III.

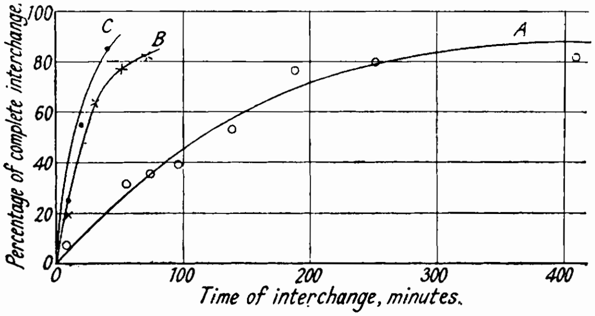
Solute.	Wt. of solute, g.	Wt. of solvent, g.	Flotation temp. of solvent. Initial.	Flotation temp. of solvent. Final.
NH_4Cl	1·00	5·00	26·62°	25·93°
NH_2Me,HCl	1·68	5·00	26·62	25·92
NHMe,HCl	3·05	5·00	26·62	25·92
NMe_3,HCl	7·14	5·00	26·62	25·85
NH_2Et,HCl	2·03	5·00	26·62	25·92
NH_2Ph,HCl	3·23	5·00	26·62	25·92
C_5H_5N,HCl	8·64	5·00	26·62	25·94
$CO(NH_2)_2$	1·12	5·00	26·62	25·92

If it be granted that ammonium chloride exchanges four hydrogen atoms with water, it appears that these substituted amines and urea similarly exchange the amino-hydrogen atoms and that of the hydrogen chloride. In the one case, that of trimethylamine hydrochloride, where the result does not accord quite closely with this conclusion, some difficulty was encountered in the process of purification, so that the difference here is probably not significant.

Interchange Experiments with Complex Salts.—Preliminary experiments were made with the following salts dissolved in partly heavy water : (*a*) 1 : 6-Dinitrotetramminocobaltic nitrate, (*b*) carbonatotetramminocobaltic nitrate, (*c*) hexamminocobaltic chloride, (*d*) trisethylenediaminocobaltic chloride. As it was found that in every case interchange occurred, quantitative experiments were begun with (*a*) and (*b*) by making a solution of the salt in partly heavy water, allowing it to stand for about 30 minutes at laboratory temperature and then distilling off the water in a vacuum for subsequent density comparisons. It was soon found that, while considerable interchange occurred, the quantitative results were not reproducible because the interchange, being much slower than was anticipated, was incomplete.

Attempts were then made to follow the progress of the reaction by withdrawing samples at definite times and distilling off the water from these as rapidly as possible in a vacuum. In this way curve *A*, Fig. 8, was obtained, but the method was unsatisfactory, as the samples froze during distillation. Better results (curve *B*) were obtained by putting a relatively large volume of mercury into the sample and shaking continuously during the distillation. An attempt to arrest the reaction by freezing the samples at − 80° and subsequently subliming off the water into a receiver cooled in liquid air failed entirely of its purpose, as, apparently, the exchange proceeds to completion overnight in the solid phase. Finally, a satisfactory method was found in arresting the exchange by precipitating the complex cobalt salt from the solution as the insoluble mercuric chloride compound. This was carried out by pipetting the sample from the reaction vessel at the desired time and running it immediately on to an excess of finely divided mercuric chloride. The supernatant liquid was then sucked off through a filter and subsequently distilled and purified in the usual way prior to the determination of its flotation temperature. Curve *C* shows that this method gives consistent and apparently trustworthy results. It is thus established that hexamminocobaltic chloride exchanges its hydrogen with water completely but at a measurable rate.

FIG. 8.



A more extended investigation of the rate of this reaction is being undertaken.

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