The Heats of Combustion of Glycerol 1-Phosphate, Glycerol 2-Phosphate and Glucose 1-Phosphate

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Most of the accurate published data on heats of combustion relate to compounds containing only carbon, hydrogen and oxygen; little work has been done on biologically important compounds containing in addition nitrogen or phosphorus. It follows from the law of constant heat summation that when sufficient data on heats of combustion and other thermochemical data have been accumulated advances will be possible in working out the energy relationships in metabolism; further it will be possible to calculate many heats of formation and reaction which are experimentally unobtainable.

The alkali-metal salts of glycerol 1-phosphate, glycerol 2-phosphate and glucose 1-phosphate were chosen for investigation in the first instance, because of their importance in metabolism and their availability as highly purified compounds.

Many difficulties had to be overcome in this work before accurate results could be obtained. The compounds showed a reluctance to burn, which in 2-aminoethanol 1-phosphate could not be overcome; they also have low heats of combustion. The indeterminate nature of the phosphate residues after combustion leads to difficulties in correcting for the heat of solution of the products; because of this the only correction applied for changes in the internal energy of the contents of the bomb was for the heat of formation and solution in water of nitric acid derived from the oxidation of atmospheric nitrogen originally present in the bombs. It was assumed that all the carbon burnt was oxidized to carbon dioxide. In all cases a close similarity in experimental conditions was maintained between calibration experiments and those in which heats of combustion were determined. Because of these difficulties and the limitations of the apparatus available, it was impossible to obtain the degree of accuracy in this work that has been reported for some other compounds (Coops, Mulder, Dienske & Smittenberg, 1947; Pilcher & Sutton, 1955).

EXPERIMENTAL

Apparatus

A modified commercial bomb-calorimeter outfit (Baird & Tatlock Ltd., Cheadle Heath, Essex, Type LFA 6330) was used.

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Stirrer. This was driven by a constant-speed electric motor at 1475 ± 5 rev./min. instead of the mechanically governed 800 rev./min. motor supplied. The propeller tube was replaced with a similar one made of Perspex, but was approx. 10 cm. longer than the original. To prevent evaporation of water and heat loss the propeller shaft was connected to the motor shaft by a Perspex rod, 10 cm. long, which was a close fit in the propeller tube. This arrangement also greatly reduced the transfer of heat from the motor to the calorimeter.

Outer jacket. The outer jacket supplied was not used, and instead a polished nickel-plated cylindrical copper can, 37.5 cm. deep and 18 cm. in internal diameter, suspended in a constant-temperature paraffin bath was used. The temperature of the paraffin was measured with a platinum resistance thermometer; it was $25 \pm 0.01^\circ$ during the whole course of the experiments.

Thermometer. This was a solid-stem Beckmann instrument with a 5° scale graduated at intervals of 0.01° , readings being estimated to 0.001° with a telescope. The thermometer was vibrated along its axis by the rubber-covered clapper of an electric bell. The thermometer was calibrated over the required range by comparison with a platinum resistance thermometer, the R_{100}/R_0 ratio of which had been determined by the National Physical Laboratory. The zero setting of the thermometer, $0^\circ = 22.16^\circ$, was not changed during this work. For the combustion experiments the thermometer was immersed to the same depth as during calibration; this eliminated the need for emergent-stem corrections, as the room temperature was sufficiently close to that of the calorimeter, and sufficiently constant ($\pm 2^\circ$) to make such corrections negligible.

Fuse. A platinum wire (0.08 mm. in diam.) fuse was attached to the two electrodes in the bomb and a cotton thread (7.62 cm. long) tied to the platinum fuse; the ends of the cotton were arranged in good contact with the material in the platinum crucible.

Materials

The samples of the phosphate-ester salts were the same as those used for the determination of the acid dissociation constants: disodium glycerol 2-phosphate pentahydrate (Ashby, Crook & Datta, 1954), dipotassium glucose 1-phosphate dihydrate (Ashby, Clarke, Crook & Datta, 1955) and disodium glycerol 1-phosphate (S. P. Datta & A. K. Grzybowski, in preparation). The benzoic acid used to determine the water equivalent of the calorimeter was the AnalaR material (British Drug Houses Ltd., London) recrystallized once from water and dried *in vacuo* over P_2O_5 . The crystals were then finely powdered and dried again as before for at least a week; the melting point was 124° (corrected).

Experimental procedure

The materials were made into pellets in a screw press and weighed in a platinum crucible. The crucible was placed in the bomb, the fuse arranged and the bomb filled with

oxygen to 30 atm. pressure. After the water in the calorimeter had been weighed, the bomb was lowered into position and the lid, stirrer, thermometer and leads were assembled as rapidly as possible to reduce the loss of water by evaporation. To allow the system to come to a steady state the stirrer was started and allowed to run for 10 min. before any temperature readings were taken.

Readings of the Beckmann thermometer were taken every 15 sec. for an initial period of 8 min., the bomb was then fired and readings were taken for a 'main' period of 10 min., during which time the heat developed by the combustion was distributed through the calorimeter; readings were continued for a final period of 8 min. In all cases the temperature of the calorimeter when the bomb was fired was $24.2 \pm 0.2^\circ$. After temperature readings had been completed the bomb was left at room temperature for 30 min. to allow the HNO_3 mist to settle. The contained gases were then allowed to leak out slowly, the crucible was removed and the contents of the bomb were washed into a beaker; the HNO_3 formed was determined by titration with standard CO_2 -free aqueous NaOH , with methyl orange as indicator.

After drying at 120° the weight of the carbon and phosphate residues in the crucible was obtained. The contents of the crucible were then washed into a weighed sintered-glass crucible with 2M-HCl and the insoluble carbon was washed on the sinter with more acid and then water. After drying, the weight of the carbon residue was obtained. The weight of any carbon adhering to the platinum crucible was determined from the difference in the weights of the dried and the glowed crucible.

Combustion of benzoic acid. When benzoic acid was melted in the crucible, to ensure perfect dryness, the mass disintegrated on combustion, leaving extensive carbon residues. If, however, the finely powdered material was compressed into pellets the combustion was much more complete. The latter method was therefore adopted in the determination of the water equivalent of the calorimeter. Similar difficulties were encountered by Pilcher & Sutton (1955) in the combustion of fused benzoic acid.

Combustion of disodium glycerol 2-phosphate. The pentahydrate was dried at 120° , at which temperature it lost all its water of crystallization. The anhydrous material was compressed into pellets; these were returned to the oven overnight before weighing. The errors in the weight of the compound due to hydration were thus reduced to a minimum.

Combustion of disodium glycerol 1-phosphate. Attempts to dry the hexahydrate in the same way as the pentahydrate were unsuccessful. The salt dissolved in its own water of crystallization and set to a glass; further, the last traces of water could not be removed. The hexahydrate was therefore weighed directly into the crucible and subsequently dehydrated by heating slowly up to 130° . The cotton thread was placed in contact with the crystals before drying, and the melt thus set round the cotton. Only in this way was a satisfactory firing contact obtained. The crucible was transferred rapidly from the oven to the bomb, which contained no water.

The hydrated forms of disodium glycerol 1- and 2-phosphate could not be burnt satisfactorily in the bomb.

Combustion of dipotassium glycerol 1-phosphate. It was impossible to remove the water of crystallization from the dihydrate without decomposing the salt. Pellets were

therefore made from the dihydrate and were successfully burnt with very little carbon residue.

Attempted combustion of 2-aminoethanol 1-phosphate. Although this compound was anhydrous, combustion in the bomb, even with increased oxygen pressure, was always incomplete. This result was not altogether surprising, considering the low proportion of oxidizable atoms in the molecule.

Heat of ignition. The heat developed by the combustion of the cotton thread was estimated by weighing a known length of thread after drying at 120° ; the heat of combustion of cotton was assumed to be the same as that of pure cellulose. The heat developed by the electric current in the platinum fuse wire amounted to less than 1 cal. and was ignored.

The following densities were used in reducing weights to vacuum: benzoic acid, 1.3; disodium glycerol 1-phosphate hexahydrate, 1.6; disodium glycerol 2-phosphate (anhydrous), 1.4; dipotassium glucose 1-phosphate dihydrate, 1.7.

RESULTS AND CALCULATIONS

The temperature/time observations were reduced by the method of Coops, Ness, Kentie & Dieneske (1947) and all calculations were made in calories, allowance being made for the specific heat of water (0.998) at the mean temperature of the experiments (25°). The final results are also given in kJ/mole. The following heats of combustion (Coops, Ness, Kentie & Dieneske, 1947) were used in the calculations: benzoic acid, 6315 cal.g.⁻¹; cellulose, 3890 cal.g.⁻¹; carbon, 8080 cal.g.⁻¹. The heat of formation and solution in water of HNO_3 in the bomb was taken to be 14.0 kcal.mole⁻¹. The heat, Q_1 , developed by the combustion of the cotton thread was taken as 18 cal. in all experiments.

The results of the experiments to determine the water equivalent of the apparatus are given in Table 1 and those of the combustion experiments in Table 2. In these tables are shown the weight of material taken, m , the weight of water added to the calorimeter, W'' , the temperature rise corrected for heat exchange with the surroundings and heat of stirring, ΔT_c , the heat loss due to the residual carbon, q_c , and the heat of formation and solution in water of HNO_3 , q_{HNO_3} . The last column in Table 1 shows the calculated water equivalent of the calorimeter, W' , including 1 ml. of water added to the bomb. The mean value of W' and the standard error of the mean are also given. The total water equivalent, $W (= W' + W'')$, is of the order of 3000 cal.deg.⁻¹. The last two columns of Table 2 give the calculated heats of combustion of the various substances studied, in cal.g.⁻¹ (Q_c) and kJ.mole⁻¹ (Q_m). The mean values and the standard errors of the means of Q_m are also given. The molecular weights of the substances as weighed, M , and the value of the water equivalent, W' , used in

Table 1. *Determination of the water equivalent of the calorimeter by the combustion of benzoic acid*

m represents the weight of benzoic acid used, W'' the weight of water added to the calorimeter, ΔT_C the temperature rise corrected for heat exchange and heat of stirring, q_C the heat loss due to carbon residue, q_{HNO_3} the heat gain due to the formation and solution in water of nitric acid, and W' the calculated water equivalent of the calorimeter. In all experiments the heat of ignition, Q_I , was taken as 18 cal. and the heat of combustion of benzoic acid as 6315 cal.g.⁻¹.

Expt.	m (g.)	W'' (g.)	ΔT_C	q_C (cal.)	q_{HNO_3} (cal.)	W' (cal.deg. ⁻¹)
A 1	0.94301	2505.5	1.984°	0.1	8.1	514
A 2	0.95219	2563.0	1.967	0.5	8.6	512.5
A 3	0.94710	2539.6	1.9745	0.2	8.5	508
A 4	0.95396	2501.7	2.014	0.0	8.7	508
A 5	0.94111	2491.6	1.991	0.0	8.3	511
A 6	0.93222	2533.7	1.948	3.4	8.2	505
A 7	0.96735	2561.3	2.001	6.3	8.2	507
A 8	0.48382	2567.6	1.002	0.3	4.5	509
A 9	0.51507	2613.6	1.0505	0.0	4.6	509
A 10	0.94950	2385.8	2.086	0.3	8.3	506
					Mean	509.0
					S.E.M.	± 0.9

Table 2. *Determination of the heats of combustion of dipotassium glucose 1-phosphate, disodium glycerol 1-phosphate and disodium glycerol 2-phosphate*

m represents the weight of substance used; W'' , ΔT_C , q_C and q_{HNO_3} are as defined in Table 1; Q_C represents the heat of combustion/g. of substance, Q_M the heat of combustion/mole of substance and M the molecular weight of the substance as weighed. In Expts. B1-6 and D1-6 the water equivalent of the apparatus, W' , was 509 cal.deg.⁻¹; in experiments C1-6 and D7-8 W' was 508 cal.deg.⁻¹. In all experiments the heat of ignition, Q_I , was taken as 18 cal.

Expt.	m (g.)	W'' (g.)	ΔT_C	q_{HNO_3} (cal.)	q_C (cal.)	Q_C (cal.g. ⁻¹)	Q_M (kJ.mole ⁻¹)
Dipotassium glucose 1-phosphate (M , 372.35)							
B 1	2.36923	2530.2	1.3755°	1.8	43.0	1770	2758
B 2	1.42462	2521.2	0.839	0.8	7.8	1774	2764
B 3	1.47541	2555.7	0.859	0.9	7.5	1773	2762
B 4	1.96273	2574.7	1.133	1.2	9.2	1772	2761
B 5	1.74341	2543.4	1.018	1.0	6.2	1772	2761
B 6	1.63471	2590.5	0.9425	1.0	0.0	1773	2762
						Mean	2761.3
						S.E.M. ±	0.8
Disodium glycerol 1-phosphate (M , 324.15)							
C 1	1.7773	2527	0.715	0.6	1.0	1209	1640
C 2	2.1048	2524	0.850	0.8	0.4	1214	1646
C 3	2.0940	2446	0.8695	0.8	0.1	1216	1649
C 4	2.0027	2578	0.799	0.6	1.4	1221	1656
C 5	2.0420	2559	0.8135	0.7	0.3	1210	1641
C 6	2.0141	2473	0.830	0.6	1.1	1218	1652
						Mean	1647
						S.E.M. ±	3
Disodium glycerol 2-phosphate (M , 216.055)							
D 1	1.2937	2551	0.778	1.2	0.0	1823	1648
D 2	1.3594	2546	0.816	1.3	0.3	1817	1643
D 3	1.4426	2568	0.8645	1.6	0.0	1827	1652
D 4	1.4369	2550	0.869	1.6	1.8	1834	1658
D 5	1.4940	2518	0.9135	1.2	2.3	1837	1661
D 6	1.2915	2541	0.775	1.3	2.3	1814	1640
D 7	1.2975	2489	0.800	1.3	2.1	1832	1656
D 8	1.1759	2532	0.7085	0.8	2.9	1815	1641
						Mean	1650
						S.E.M. ±	3

Table 3. *Heats of combustion of some phosphate esters*

The errors given are the standard errors of the means.

	Reference	Q_M (kJ.mole ⁻¹)
Calcium glycerol 1-phosphate	Collatz (1936)	1644 ± 8
Disodium glycerol 1-phosphate	This paper	1647 ± 3
Disodium glycerol 2-phosphate	This paper	1650 ± 3
Dipotassium glucose 1-phosphate	This paper	2761 ± 1
Calcium hexose diphosphate	Collatz (1936)	2807 ± 7

the calculations are shown. In the experiments with disodium glycerol 1-phosphate and in experiments no. D7 and D8 with disodium glycerol 2-phosphate no water was added to the bomb; hence $W' = 508$ cal.deg.⁻¹. A summary of the heats of combustion of phosphate esters obtained in this work and by Collatz (1936) is given in Table 3.

DISCUSSION

The results given in Tables 1 and 2 show that a reproducibility of $\pm 0.2\%$ has been obtained both in the determination of the water equivalent and in the heats of combustion. This is probably the limit of accuracy obtainable with the apparatus. During the work great attention was paid to the details of the experimental technique. The relative positions of the various parts of the apparatus were always the same, approximately the same amount of water was added to the calorimeter in each experiment and an attempt was made to obtain the same temperature rise. In these experiments a difference in the temperature rise of 1° did not appear to affect the results.

The temperature of the calorimeter water at the moment of ignition was $24.2 \pm 0.2^\circ$ and the outer jacket was kept at $25.00 \pm 0.01^\circ$; these conditions, together with a temperature rise of $1-2^\circ$, reduce the magnitude of the heat-exchange correction and hence the effect of errors in its calculation. The calculation is based on the validity of Newton's law of cooling and assumes that convection is negligible. Coops & Ness (1947*a*), from their own experiments and the results of other workers, concluded that convection is negligible in most calorimeters if the following relation holds:

$$(\text{Air gap, cm.})^3 \times (T_v - T_w) \leq 5.9,$$

where T_v and T_w are the temperatures of the calorimeter and outer jacket. In our experiments

$(T_v - T_w)$ did not significantly exceed 1° and the air gap was 1.9 cm. Thus the heat exchange due to convection was small.

The complete distribution throughout the calorimeter of the heat from the combustion is assumed for the calculation of the results. Coops & Ness (1947*b*) have demonstrated that, assuming a total temperature rise of 2° , 99.99% of the available heat from the combustion is accounted for if the duration of the 'main' period of temperature-time readings is 10 times that required for the temperature to rise from its initial value to two-thirds of its maximum value. In our experiments a 'main' period of 10 min. satisfied this condition.

The values of the heats of combustion given in Table 2 are not accurate enough to warrant the calculation of the energy change for the combustion reaction at 1 atm. pressure and for the substances involved in their standard states; nevertheless, the values of Q_M given may be compared with other data quoted for 25° .

Apart from shortcomings in the apparatus some lack of reproducibility and ambiguity in the results may arise from other causes which are discussed below.

State of hydration of the compound. About 20 min. elapsed between weighing the anhydrous disodium glycerol 2-phosphate and its ignition. During this time some hydration occurred as the substance was in proximity to the water in the bomb, though separated from it by oxygen at 30 atm. To determine the amount of water taken up under these conditions weighed pellets of anhydrous disodium glycerol 2-phosphate were placed in the bomb as for combustion and left at room temperature for 1 hr. The gases were then released slowly and the pellets reweighed. The increase in weight was found to be 0.692 and 0.762, mean 0.727%, which corresponds to less than 0.1 mole of water/mole of salt. Thus in 20 min. the amount of hydration must have been very small and could not have had a significant effect on the heats of combustion observed. This is supported by the results of Expts. D7 and D8, where there was no water in the bomb and in which the values of Q_M obtained are within the range of the other values.

The amount of water retained by the disodium glycerol 1-phosphate, after drying, was very small and cannot have had much effect on Q_M . This substance was always burnt in the bomb in the absence of water. Thus the two glycerol esters were burnt virtually in the anhydrous condition, in contrast to the dihydrate of dipotassium glucose 1-phosphate, which could not be dehydrated and was therefore burnt as the stable dihydrate.

Completeness of oxidation. It was assumed that all the carbon burnt was completely oxidized to carbon dioxide and the contents of the bomb were

not tested for carbon monoxide. Pilcher & Sutton (1955), using a bomb of similar size (300 ml. internal volume) and the same oxygen pressure, found significant amounts of carbon monoxide only when 0.3 g. or less of benzoic acid was burnt, the oxidation of the carbon usually being complete with larger charges. In the determination of the water equivalent the weight of the benzoic acid charge was between 0.48 and 0.97 g. and no correlation was found between W' and m (Table 1). Thus it is unlikely that significant amounts of carbon monoxide were formed.

In the combustions of the disodium glycerol phosphates the minimum heat quantity measured was about 2150 cal. (Expt. C1, Table 2), compared with approximately 3000 cal. (Expt. A8, Table 1) in the determination of the water equivalent. Since W was virtually constant the temperatures reached during the combustions of the disodium glycerol phosphates were probably considerably lower than during the combustion of benzoic acid, and small amounts of carbon monoxide may have been formed.

This possible error, together with the smaller proportion of oxidizable atoms in the disodium glycerol phosphates, may account in part for the greater variance in Q_M for these substances than for dipotassium glucose 1-phosphate, though even for the latter substance the heat quantities were not as large as for benzoic acid.

Nature of the phosphate residue. The residues in the platinum crucibles after combustions in the bomb were dried at 120° and weighed and the weights compared with the expected weights of the corresponding pyrophosphates. The weights of the residues remaining after the combustion of dipotassium glucose 1-phosphate and disodium glycerol 1-phosphate were high (after allowing for the carbon and fused platinum wire present), possibly indicating the presence of orthophosphate. In one experiment the residue from a combustion of dipotassium glucose 1-phosphate was ignited to constant weight over a Meker burner and was found then to agree with the expected weight of potassium pyrophosphate. The weights of the residues from the combustion of disodium glycerol 2-phosphate were about 0.1 % lower than expected. This may have been due to small amounts of the phosphate residue being blown out of the crucible as only with this compound was a fused mass of material burnt. The washings from the bomb were tested for ortho- and pyro-phosphate, and only in some instances were traces of these salts found. No direct chemical analyses of the residues from the combustion experiments were undertaken.

To test whether significant amounts of orthophosphate were present in the residues from combustions, in the bomb, similar quantities of the

three salts studied were subsequently burnt in the same bomb under the same conditions as during heat measurements. These experiments showed that only 0.1–0.2 % of the weight of the residues was orthophosphate as estimated by Allen's (1940) method. Thus it appears likely that during the heat measurements also the main part of the phosphate residue is as the alkali-metal pyrophosphate.

Thompson (1952) has determined the heat of formation of disodium methylphosphonate from combustion measurements. In that work the stoichiometry of the bomb reaction was established by the analysis of the residue, which was found to correspond to that of sodium pyrophosphate. The phosphorus content of the residue was found to be 23.08 % which, in fact, corresponds to 14.5 % of Na_2HPO_4 in $\text{Na}_4\text{P}_2\text{O}_7$.

Heats of formation. For the purpose of calculating the heats of formation of the substances studied here we assume that the following represent the bomb reactions, within the limits of error indicated above.

For both disodium glycerophosphates:

and for dipotassium glucose 1-phosphate dihydrate

The following values in kcal.mole⁻¹ of the heats of formation, ΔH_f° , at 25° were used: $\text{Na}_4\text{P}_2\text{O}_7$ (c) –760.8, CO_2 (g) –94.05 and H_2O (l) –68.32 (Rossini, Wagman, Evans, Levine & Jaffe, 1952). No data are available for the heat of formation of $\text{K}_4\text{P}_2\text{O}_7$ (c), though the value for KH_2PO_4 (in water) of –370 kcal.mol.⁻¹ is near that of NaH_2PO_4 (in water), –368 kcal.mol.⁻¹, so the assumption is made that the heat of formation of $\text{K}_4\text{P}_2\text{O}_7$ (c) is the same as for $\text{Na}_4\text{P}_2\text{O}_7$ (c).

Table 4. *Heats of formation*

	Reference	$-\Delta H_f^\circ$ (kJ. mole ⁻¹)
Disodium glycerol 1-phosphate (c)	This paper	2126
Disodium glycerol 2-phosphate (c)	This paper	2123
Dipotassium glucose 1-phosphate (2H ₂ O) (c)	This paper	2764
α-D-Glucose (anhydrous) (c)	Huffman & Fox (1938)	1274.6
Glycerol (l)	Parks, West, Naylor, Fujii & McClaine (1946)	668.6

The heats of formation calculated from the results of this work are given in Table 4, together with those for glucose and glycerol for comparison.

SUMMARY

1. The heats of combustion of dipotassium glucose 1-phosphate, disodium glycerol 1-phosphate and disodium glycerol 2-phosphate have been determined in a bomb calorimeter.

2. Possible errors in the determinations are discussed and the stoichiometry of the combustion reactions are established.

3. The heats of formation of the compounds examined have been calculated.

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Some Observations on the Measurement of 'Binding' of Cyanocobalamin by 'Intrinsic Factor' Preparations

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In certain body tissues and fluids, particularly gastric juice and serum, there are present substances having the property of combining with cyanocobalamin so that its activity for micro-organisms is altered and on dialysis it does not pass through a cellophan membrane. The ability of gastric juice to combine with added cyanocobalamin is closely associated with its 'intrinsic factor' activity, but there have been conflicting reports on the identity of 'binding' activity with intrinsic-factor activity. To some extent this confusion has come about because the different groups of workers have used different methods for measuring binding activities.

For example, Bird & Hoevet (1951) measured the binding activity of an intrinsic-factor concentrate by three methods and obtained three different results. The first method was that of Ternberg & Eakin (1949), in which graded doses of the concentrate were added aseptically to a series of autoclaved tubes, each containing assay medium plus 0.5 μ mg. of cyanocobalamin. The minimum amount of protein required to inhibit the growth of the test organism (*Lactobacillus leichmannii*) was taken as

the amount which bound 0.5 μ mg. of cyanocobalamin. In the second method, a constant amount of intrinsic-factor concentrate was added aseptically to increasing amounts of cyanocobalamin. The binding activity was calculated from the lowest level of cyanocobalamin at which growth occurred. This level was three times higher than that found by the first inhibition method. The apparent binding activity was increased a further tenfold if the method of measurement was based on the non-dialysability of the bound vitamin. Bird & Hoevet suggest that the last method is the more reliable and that in the growth-inhibition test *L. leichmannii* is able to utilize all but a small part of the bound vitamin.

Rosenthal & Sarett (1952) also used a dialysis method and *L. leichmannii* to measure the cyanocobalamin-binding activity of normal human serum. Their results were considerably higher than those obtained for normal serum by Pitney, Beard & Van Loon (1954), who measured the activity by the *Euglena gracilis* tube assay method of Ross (1952). This method depends on the assumption that bound cyanocobalamin is unavailable to *E. gracilis* unless