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THE COMPOSITION AND DISTRIBUTION OF THE FATTY SUBSTANCES OF THE HUMAN SUBJECT.

BY E. P. CATHCART AND D. P. CUTHBERTSON.

(*Institute of Physiology and Department of Biochemistry, Royal Infirmary, University of Glasgow.*)

EVEN a very cursory study of the literature concerning the metabolism of the various proximate principles makes it plain that the available information as regards the utilization of protein and carbohydrate, although far from complete, is abundant and varied, in comparison with our knowledge of the metabolism of fat.

The evidence that fat does serve as a source of energy is clear and unequivocal; and that in starvation, when the drain of energy is long continued, it is the depot fat that is involved is also beyond question. But in experiments of short duration we cannot say that this is so. Modern investigations of the energy exchange in muscle work would suggest that fat functions as a source of energy even in the presence of carbohydrate. Thus Krogh and Lindhard [1920] state: "The proportion of carbohydrate to fat catabolized is a function of the relative available quantities of the two substances, and substantially the same during rest and during muscular work." They also state that "neither fats alone nor carbohydrates alone are suitable for the supply of the energy requirements of the body, but that the catabolic disintegration of either of these substances requires the presence and the simultaneous catabolization of the other." We think it may now be taken as certain that in all tissue metabolism, as evidenced at any rate by the respiratory quotient, there is a varying quota of protein, carbohydrate and fat involved in the tissue oxidation processes. For the evaluation therefore of the energy exchanges in the body, which depends on the nature of the material oxidized, it is essential that some knowledge of the nature of the various possible available fats be gained. From whence is derived the quota of fat oxidized in tissue combustion? Is it in the form of neutral fat derived from the various depots; is it in the form of fatty acids such as are found in combination in average neutral fat; is it in the form of highly desaturated fatty acids as postulated by Leathes and his co-workers, or is it in some more complex "lipoid" form? The work of Zuntz [1911], Benedict and Cathcart [1913], Krogh and Lindhard [1920], and Furusawa [1925] would seem to point to the possibility that fat may be used as a source of energy for the muscles, yet the work of Leathes [1906] and Winfield [1915] suggests that neutral fat present even in muscle, the most active metabolic tissue, cannot, at any rate directly, be regarded as the source from which the muscle derives its energy.

Practically all the analyses of human fat which are available in the literature have been made on adipose tissue. Generally no attempt has been made to ensure the absence of lipides, cholesterol and allied substances from the material analysed, nor have corrections been made for the presence of such compounds in the final calculation. Hence most of the scanty data available are not beyond criticism.

One of the earliest observers, Chevreul [1823] reported fat as being composed of 77·85 p.c. carbon, and 11·42 p.c. hydrogen. These figures are the recalculated values of Schulze and Reinecke [1867] who corrected Chevreul's figures for the new atomic weight of carbon. Schulze and Reinecke [1867] in a more extended investigation on the composition of animal fat included in their observations two analyses of human fat. Fat from the kidney yielded carbon 76·44 p.c., hydrogen 11·94 p.c.; and fat from the panniculus adiposus 76·80 p.c. carbon and 11·94 p.c. hydrogen. The period of heating employed by these authors for the purification of the fat from the tissue involved several days' evaporation on a steam bath. More recent work has shown such a method of purification to be inadmissible. Benedict and Osterberg [1901] have made more exact analyses using methods of extraction which give an unchanged fat, but which are not applicable to the separation of muscle and liver fat. The tissues analysed were the panniculus adiposus abdominalis and perinephric adipose tissue. The sources of these materials were from poorly nourished or emaciated old persons, the youngest aged 51, the remaining ranging from 70 to 92 years. These workers noticed that generally the solid triglycerides, stearin and palmitin, settled leaving the liquid olein on top, an observation which the present authors have confirmed. Benedict and Osterberg state "that in light of the striking agreement between the samples as a whole the average 76·08 p.c. for carbon and 11·78 p.c. for hydrogen cannot be far from correct, and probably would be little, if any, affected by a multiplication of samples." In addition, Benedict and Osterberg determined the calorie value of 1 g. of each of their fats analysed. The average value obtained was 9·523 large calories per gram at constant volume, or 9·538 per gram using Stohmann's correction [1890] for reduction to constant pressure. These analyses were the first to be performed on human fat with the bomb calorimeter. The very earliest calorific determinations of human fat had been made by Stohmann [1884] using a Lewis Thomson calorimeter. Fat from the panniculus adiposus gave an average value of 9·379 large calories per gram, and that from perinephric fat 9·427 large calories per gram. Later Stohmann [1890], using the Berthelot bomb calorimeter, found that his earlier values were too low. His corrected values for human fat were 9·514 and 9·562 respectively. Zuntz and Loewy [1924] have given the following figures for the composition of human fat: 76·10 p.c. carbon, 11·80 p.c. hydrogen, 12·10 p.c. oxygen; respiratory quotient 0·7133 with a calorie value of 9·54 for 1 g. The calorie value of 1 litre of oxygen is given as 4·795. On recalculating this latter value by means of the recently determined weight of a litre of oxygen at 0° C. and 760 mm. [Baxter and Starkweather, 1924], viz. 1·4289 g., the figure obtained is 4·792.

As regards the composition of human fat, it is difficult to give exact values as various factors play important determinate rôles such as the nature of the diet, the age of the subject, the exact position from which the sample for analysis has been taken.

Heintz [1852] concluded that the basis of human fat was a mixture of triolein, tripalmitin and tristearin. Jaeckle [1897] gave the composition of fat as stearic acid 4·9 to 6·3 p.c., palmitic acid 16·9 to 21·1 p.c., oleic acid 65·6 to 86·7 p.c., unsaponifiable matter 0·33 p.c., and lecithine 0·084 p.c. More recently Erben [1900] found in human fat 1·18 p.c. free fatty acids, 0·56 p.c. lecithine, and 1·17 p.c. cholesterol. Heiduschka and Handritschk [1928] found no other fatty acids than oleic, palmitic and stearic in a series of analyses of liver, heart, mesenteric and adipose fat. Such observations would suggest that these impurities are not present in sufficient amount to alter appreciably analyses such as those of Benedict and Osterberg [1901] and of Zuntz and Loewy [1924] on adipose fat. This may be true as regards adipose tissue proper, but these substances must be reckoned with in the purification of fat from such sources as liver and muscle.

Many factors have recently been elucidated which play a rôle in the determination of the character of the fat of adipose tissue. The nature of the food consumed may bring about wide variations in its composition. Ellis and Hankins [1925], and Anderson and Mendel [1928] have shown that diets rich in protein or carbohydrate produce "harder" fats than a diet containing higher amounts of fat. More striking still is the effect of the type of fat in the food, *e.g.* the effect of coco-nut oil on the Polynesians and fish fat on the Eskimos [Rosenfeld, 1902; Knöpfelmacher, 1898].

Currie [1922] has quoted some hitherto unpublished results of Duncan on the variation of the iodine value of normal human fat with the age of the subject, a finding which had earlier been noted in "beef" animals by Moulton and Trowbridge [1909]. Jaeckle [1902] found that the iodine value of the three-day-old child's fat was 47, of the two to three-weeks-old child 58, and of the adult 62–73. Later Duncan [1922] noted that under 11 years the iodine value was 44, from 16 to 19 years 60·9, from 20 to 25 years 61·0, 62·1 from 26 to 38 years, and 63·98 from 52 to 65 years of age. Duncan in forty-three cancer patients found an average of 72·6, the ages varying from 39 to 68 years.

The present series of analyses was performed with a view to determining if the fat obtained from muscle and liver differed appreciably from the fat of adipose tissue in its elementary composition and caloric value. The question of the rôle of the co-existent lipides and their composition is delayed for a subsequent communication.

The material was obtained from fourteen subjects of ages ranging from 18 to 57, six of whom died from accidental injuries (B., E., K., L., M. and N.). The necropsy was in most cases within a few hours of death, the bodies being kept in a refrigerator till required for examination.

METHOD OF EXTRACTION OF FAT FROM TISSUES.

The tissue was finely minced and extracted three times with absolute alcohol in the cold. This was followed by three extractions with ether in the cold. The alcoholic extracts were combined, filtered, and the filtrate evaporated to dryness. The ethereal extracts were similarly treated. These and all subsequent evaporations were carried out under reduced pressure at a temperature not exceeding 45° C. To avoid bumping during the distillation of the various solvents a gentle stream of nitrogen bubbles was drawn through the solution being distilled. This latter precaution was to minimize any tendency for oxidation of the unsaturated fatty acids.

The residues from these two evaporations were combined and extracted with ether in the cold. This ethereal extract was filtered and concentrated to a small volume. To this solution a small quantity of NaCl and five times its volume of acetone were added and the mixture allowed to stand over night. In the morning the precipitated lipides were filtered off and the filtrate evaporated to dryness. The residue from this evaporation was dissolved in a small quantity of ether and again five times its volume of acetone added to remove any remaining lipide. This process was repeated a third time. The filtrate from this last acetone precipitation was evaporated to dryness and extracted with petroleum ether. The petroleum ether extract was filtered and evaporated to dryness. The fat obtained was dried and stored in a vacuum desiccator over $CaCl_2$ and P_2O_5 and was kept in the dark.

It was found possible to precipitate out all the cholesterol of the subcutaneous fatty extracts with digitonin, the digitonin, after filtering off the cholesterol complex, being precipitated by the addition of excess petroleum. This process had to be repeated. In the muscle and liver extracts, however, owing to the presence of cholesteryl esters, all the cholesterol could not be precipitated out. Recourse was then made in all subsequent preparations to the quantitative determination of the total cholesterol by the modified Liebermann reaction. In calculating the final composition of the fats after analysis, correction was made for the cholesterol using the following figures: for the iodine value 135 (determined by Holde using Wijs' solution, which gives approximately twice the theoretical value), and for the calorie value of 1 g. 10·289 [Bills, Cox and Steel, 1929]. The figures for the amounts of cholesterol present are not appended, as they only signify the amount present at the particular stage of purification of the neutral fat.

The elementary analyses were performed in triplicate, so also the caloric values. A Berthelot bomb calorimeter was used. It was checked against pure benzoic and salicylic acids. No heat was used in the process of sampling, only thorough mixing. Nitrogen and phosphorus were tested for, and where present in appreciable amount the values were recorded and due allowance made in the final calculations. The nitrogen was determined by combustion with H_2SO_4 and subsequent nesslerization. Hydrogen peroxide (perhydrol) could not be used owing to the presence in it of acetamide. The phosphorus was determined, subsequent to the oxidation of the fat by HNO_3 and H_2SO_4, by precipitation as ammonium phosphomolybdate and weighing directly. The calculated values for oxygen are included, also the respective respiratory quotients on the assumption that these fats are oxidized in the body to CO_2 and H_2O.

Iodine values were determined using Wijs' solution. The entire estimation was performed in a stoppered 500 c.c. bottle. This avoided the unnecessary transference of reagents, etc. Blank determinations were made with each fresh batch of fats, and from day to day during these operations.

In determining the calorie equivalent of a litre of oxygen used in the burning of these fats the volume of 1 g. oxygen at 0° C. and 760 mm. was taken as 0·6998 litre [Baxter and Starkweather, 1924].

Attempts were made to determine the melting points of several of these fats, and their fatty acids. No definite data were gained owing to the fact that these fats consist of triglycerides of various melting points, and therefore the final point of disappearance is often considerably higher than the temperature at which the main bulk fuses. The same criticism applies in the reverse sense to the determination of the solidifying point.

Discussion of analytical data.

The fats of the panniculus adiposus abdominalis were pale yellow in colour, the depth of colour varying somewhat with the distance from the skin surface. Whitish flakes of the more saturated triglycerides floated throughout the fat, tending later to sediment. These fats showed much less liability to change their character with keeping, than did the liver and muscle fats which tended to harden after storage for several weeks in a desiccator. A muscle fat which had hardened into a wax-like material gave an elementary analysis of 68·42 p.c. carbon, 10·54 p.c. hydrogen, and 21·04 p.c. oxygen, the respiratory quotient being 0·742, the fuel value 7·71 large calories, and the iodine value 60·2.

TABLE I.

Source of fat	Case	Age	C	H	O	N	P	Iodine value	R.Q.	Calorie value of 1 g.	Calorie value of 1 litre of oyxgen
Panniculus adiposus abdom.	A.	57	76·70	11·70	11·59	vft	vft	68	0·714	9·523	4·750
	B.	27	75·29	11·70	13·01	vft	vft	69	0·7135	—	—
	C.	30	77·01	12·03	10·92	0·04	vft	70	0·706	—	—
	D.	41	75·64	11·86	12·43	0·07	vft	—	0·7095	—	—
	K.	18	76·17	11·80	11·94	0·02	0·067	66·5	0·711	9·490	4·747
		Average	76·16	11·82	11·98	0·03	0·01	68·4	0·711	9·506	4·749
		Standard deviation	...	...	...	...	...	...	0·0029		
		Average value reduced to constant pressure				...	...	...	...	9·521	4·755
Panniculus adiposus abdom. of two very fat women	I.	59	75·48	11·67	12·80	0·05	vft	74	0·714	9·511	4·820
	J.*	52	75·02	11·98	13·00	vft	vft	70	0·708	9·390	4·762
			75·11	11·82	13·07	vft	vft	68	0·711		
		Average	75·28	11·79	12·41	0·02	vft	71·5	0·712	9·450	4·791
		Average value reduced to constant pressure				...	...	...	...	9·465	4·798
Liver	A.	57	73·63	11·46	14·91	Trace	Trace	—	0·719	—	—
	B.	27	71·01	11·26	17·73	Trace	Trace	—	0·723	—	—
	D.	41	71·50	11·24	17·26	Trace	Trace	—	0·724	—	—
	G.	24	75·32	11·41	13·27	Trace	Trace	—	0·720	—	—
	K.	18	75·16	11·76	11·76	0·47	0·86	134	0·709	9·378	4·742
	M.	38	74·57	11·42	12·97	0·70	0·34	127	0·718	8·880	4·581
	N.	56	75·33	11·39	12·59	0·47	0·22	121	0·720	9·059	4·638
		Average	73·79	11·42	14·36	0·23	0·20	127	0·719	9·105	4·654
		Standard deviation	...	...	...	...	...	...	0·0045		
		Average value reduced to constant pressure				...	...	...	...	9·120	4·663
Liver of two very fat women	I.	59	75·23	11·93	11·91	0·32	0·61	69	0·706	9·5205	4·787
	J.	52	74·55	11·28	13·32	0·58	0·27	77	0·721	—	—
		Average	74·89	11·605	12·615	0·45	0·44	73	0·713		
		Average value reduced to constant pressure				...	...	...	...	9·536	4·795

Skeletal muscle	A.	57	71·62	11·59	16·79	Trace	Trace	—	0·715	—	—
	B.	27	73·89	11·48	14·63	Trace	Trace	66	0·721	—	—
	E.	56	73·13	11·19	14·42	0·63	0·63	72	0·722	—	—
	F.	28	72·35	11·33	14·93	0·71	0·68	69	0·718	—	—
	K.	18	70·81	11·05	16·66	0·525	0·955	72	0·724	8·940	4·898
	L.	50	74·78	11·72	12·69	0·50	0·31	76	0·711	9·440	4·810
	M.	38	74·83	11·80	13·12	0·25	Trace	73	0·711	9·318	4·744
	N.	56	73·92	11·64	13·49	0·66	0·29	91	0·713	—	—
		Average	73·17	11·47	14·59	0·41	0·36	74	0·717	9·233	4·817
		Standard deviation	...	...	...	...	...	...	0·0048		
		Average value reduced to constant pressure				...	...	...	...	9·248	4·824

vft = very faint trace. * Outer and inner tangential layers.

TABLE II.

Source of fat	Case	Heat of formation of products of combustion	Heat of combustion of 1 g. of fat at constant pressure	Heat of formation of 1 g. of fat
Panniculus adiposus abdom.	A.	10·196	9·538	0·658
	I.	10·088	9·526	0·562
	J.	10·131	9·405	0·726
	K.	10·188	9·505	0·683
			Average	0·657
Skeletal muscle	K.	9·499	8·955	0·544
	L.	10·048	9·455	0·593
	M.	10·079	9·333	0·746
			Average	0·628
Liver	I.	10·156	9·5355	0·6205
	K.	10·092	9·393	0·699
	M.	9·929	8·895	1·034
	N.	9·980	9·074	0·906
			Average	0·814
			Total average	0·707

The fats of liver and muscle were fairly similar in appearance. They were of a golden brown colour, the liver fat being slightly the softer. There was also a greater degree of solidification of the saturated fats. Exposure to the air even for a short space of time, as, for example, during the process of weighing, resulted in an increased fluidity of all these fats, and heating in a hot air oven for half an hour at 95° C. caused considerable darkening.

The theoretical respiratory quotient for tristearin is 0·699, for tripalmitin 0·703, for triolein 0·7125, the percentage of oxygen in the molecule of these fats being 10·78, 11·91 and 10·86 respectively. It is apparent that the highest calculated respiratory quotient is not a property of the fat with the highest percentage of oxygen in its molecule, but that the respiratory quotient of the saturated fats decreases with increasing complexity, and further, that the respiratory quotients of the unsaturated fats are higher than the corresponding members of the saturated series. It will be noticed that in the present series of fat analyses the iodine value of the adipose tissue fats is generally a little lower than that of muscle fat and very much lower than that of normal liver. It is interesting to note the relatively great difference between the iodine values of the normal liver fats and the values of the liver fats of very stout women. The normal liver fats gave values ranging from 121 to 134, whilst those showing a visible fatty change gave values ranging from 69 to 85. The elementary analyses of the fats of the panniculus adiposus abdominalis of the present series agree very closely with the average values, 76·08 p.c. carbon, 11·78 p.c. hydrogen, and 12·14 p.c. oxygen, determined by Benedict and Osterberg [1901], and the almost identical values reported by Zuntz and Loewy [1924]. The respiratory quotients for average fats as calculated by these workers are 0·712 and 0·7133 respectively. As previously mentioned the majority of Benedict's and Osterberg's subjects were elderly and in many cases emaciated subjects.

The analyses of the subcutaneous fats of two of the three very fat women indicated little divergence from that of normal adipose tissue fat. The carbon was slightly lower, the oxygen somewhat higher, the calculated respiratory quotient identical. The calorie value was practically the same as the normal average in one case and somewhat lower in the other. Elementary analyses of the outer and inner tangential layers of case J. revealed no real difference.

The liver fats were found to be more variable in composition, the carbon values being distinctly lower and the oxygen values distinctly higher than the subcutaneous fats. The respiratory quotient was also very variable, the average 0·719 being definitely higher than that of adipose tissue fat. The calorie value also varied considerably, the average value 9·11 being much below that of depot fat. In some respects the liver fats of the stout women were more closely allied to the adipose tissue fats, particularly in Case I. The calorie value 9·52 in this case was also high.

As was rather to be expected the series of muscle fats presented a variable group. Although every effort was made to get rid of every visible trace, the variable factor would appear to be the fat present in the areolar connective tissue surrounding the bundles. Muscles vary greatly in regard to the amount of this material present. While it cannot be termed the true muscle fat it is impossible to free the muscle surface entirely of its presence. Muscle fat as determined by its elementary analysis is very like liver fat. Again the average respiratory quotient is high, 0·717, and the calorie value low, 9·23.

Both liver and muscle fats are much more readily oxidized than are the fats of adipose tissue, and although all precautions were taken to minimize the risk of oxidation, such a possibility cannot be completely ruled out of account.

It is possible also that, as the liver almost certainly receives the relatively saturated fats to work over into the desaturated forms, death may find the laboratory of the liver at various metabolic stages.

It is of interest that the analyses of the liver of Case G., who died in diabetic coma, did not reveal any obvious deviation from the values found in the other cases.

A practical point emerges from the consideration of the foregoing data in connection with the determination of the energy expenditure. If it be assumed, and we believe correctly, that the fat utilized, at least for immediate combustion, is not that of the depots in the subcutaneous tissue but the more highly oxygenated fats and probably more labile fats found in the muscle and liver, and if our analyses of these fats can be accepted as reasonably accurate, then the data, as given either in the Zuntz table or as modified by Lusk, must be somewhat changed. If then a composite value from the liver and muscle analyses be employed and the table recalculated the following result (Table III) is obtained.

It will be observed that there is naturally little change as we approach a respiratory quotient of unity when it is generally assumed that no fat is undergoing combustion, but that with the lower respiratory quotients,

TABLE III. Significance of the non-protein respiratory quotient.

R.Q.	Percentage total O_2 consumed by		Percentage total heat from		Calories per litre O_2 Values of	
	Carbohydrate	Fat	Carbohydrate	Fat	Lusk	Zuntz
Lusk's data from Zuntz and Schumburg (condensed):						
0·707	0·0	100·0	0·0	100·0	4·686	—
0·75	14·7	85·3	15·6	84·4	4·739	4·829
0·80	31·7	68·3	33·4	66·6	4·801	4·875
0·85	48·8	51·2	50·7	49·3	4·862	4·921
0·90	65·9	34·1	67·5	32·5	4·924	4·967
0·95	82·9	17·1	84·0	16·0	4·985	5·012
1·00	100·0	0·0	100·0	0·0	5·047	5·058
New data from present analyses:						
0·718	0·0	100·0	0·0	100·0	4·735	
0·75	11·4	88·7	12·0	88·0	4·770	
0·80	20·1	70·9	30·4	69·6	4·827	
0·85	46·8	53·2	48·4	51·6	4·881	
0·90	65·5	35·5	66·0	34·0	4·936	
0·95	82·3	17·7	83·2	16·8	4·992	
1·00	100·0	0·0	100·0	0·0	5·047	

the quotients which are more commonly met with in the determination of the metabolic rate, the divergence becomes quite definite.

SUMMARY.

1. Elementary analyses of fat from the panniculus adiposus abdominalis of normal subjects gave an average value of 76·16 p.c. carbon, 11·82 p.c. hydrogen, and 11·98 p.c. oxygen. The average respiratory quotient was 0·711; the average calorie value 9·505 cal. per g. at constant volume, and the equivalent of 1 litre of oxygen at constant volume was 4·75 cal. The average iodine value was 68·4.

Similar analyses of fat from two very stout women gave 75·28 p.c. carbon, 11·79 p.c. hydrogen, and 12·41 p.c. oxygen; 0·712 for the respiratory quotient; 9·45 for the calorie value and 4·79 for the calorie equivalent of a litre of oxygen at constant volume. The average iodine value was 71.

2. Elementary analyses of liver fat practically free of lipoid gave 73·79 p.c. carbon, 11·42 p.c. hydrogen, and 14·36 p.c. oxygen. The average respiratory quotient was 0·719, the average calorie value 9·11 and the calorie equivalent of a litre of oxygen at constant volume 4·65. The average iodine value was 127.

The average iodine value of the fat from two abnormally fat women's livers which demonstrated visible fatty change was 73.

3. From voluntary muscle fat practically freed of lipoid gave on

elementary analyses 73·17 p.c. carbon, 11·47 p.c. hydrogen, and 14·59 p.c. oxygen. The average respiratory quotient was 0·717, the calorie value of 1 g. 9·23 cal. at constant volume and the equivalent of 1 litre of oxygen at constant volume 4·82 cal. The average iodine value was 74.

4. A modification is suggested on the basis of the new analyses of the values given in the Zuntz-Schumburg table, and of necessity of the Lusk modification of this table, of the calorie values of 1 litre of oxygen at different respiratory quotients.

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APPENDIX A.

Calculation of heat of formation of human fat.

The thermal value due to the formation of a compound from its elements C_a, H_{2b} and O_c is equal to the thermal effect on direct formation of the products of combustion, aCO_2 and bH_2O. Using the symbols of Thomsen [1908] the equation of the combustion may be written:

$$C_aH_{2b}O_c + (2a + b - c)\,O = aCO_2 + bH_2O.$$

To express the heat of combustion the abbreviated formula $\int(C_aH_{2b}O_c)$ may be used. Thus the equation form of the above law may be written

$$(C_a, H_{2b}, O_c) + \int C_aH_{2b}O_c = a\,(C, O_2) + b\,(H_2, O).$$

In the calculations of the heats of formation we have utilized for the heat of formation of CO_2 from amorphous carbon, the value 96,960 cal. per g. atom [Favre and Silbermann, 1852] and for the heat of formation of liquid water the value 68,360 cal. per g. mol. [Thomsen, 1908]. The respective figures per gram of C and H are therefore 8·080 and 34·180 kilocalories. Values for the heats of formation so calculated are at constant pressure.

Favre, P. A. and Silbermann, J. T. (1852). *Ann. Chim. phys.* **34**, 357.
Thomsen, J. (1908). *Thermochemistry*, trans. Burke. Longmans, London.

APPENDIX B.

The correction of the value of the heat of combustion of fat as determined at constant volume to that at constant pressure, can be conveniently calculated by determining what energy the apparatus would gain by gas entering the bomb to make up the deficient volume.

If we assume a fat of constant composition C = 76 p.c., H = 12 p.c., and O = 12 p.c., then each gram of fat requires 2·864 g. oxygen. Each unit volume of oxygen is replaced by 0·708 of a unit volume of CO_2, leaving a volume equal to that filled by $0·292 \times 2·864$ at the original pressure. This volume is filled by internal expansion of the contents of the calorimeter. The temperature changes are negligible.

Work done by gas entering at constant pressure:

$$\Sigma\pi = \Sigma\pi \times \text{vol. of } 0·292 \times 2·864 \text{ g. oxygen at } 0^\circ \text{ C.} \quad (\pi = 1 \text{ atmosphere.})$$

Now 1 g. mol. of oxygen at pressure π has volume 22·4 litres, and πv for 1 g. mol. of oxygen at 0° C. = 2 cal. per degree = 2 × 273 cal.

$$\Sigma\pi \times \text{vol. of } 0·298 \times 2·864 = \frac{2 \times 273 \times 0·298 \times 2·864}{32} = 14·47 \text{ cal.}$$

If temperature 16°,

$$\text{work done} = \frac{289 \times 14·47}{273} = 15·32 \text{ cal.}$$