AN APPLICATION OF THERMODYNAMICS TO PHYSIOLOGY

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ON THE FREE ENERGY OF GLUCOSE AND OF TRIPALMITIN

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The theoretical maximum amount of work derivable from a chemical reaction is a quantity which we may designate as the reversible work, and which is equal to the decrease in the free energy of the system plus the change in the pressure–volume product. In the form of an equation this is: $W_R = -\Delta F + \Delta(PV)$.

Since the magnitude of the change in the pressure–volume product is rarely significant compared to the free energy change, for practical purposes we may consider the theoretical maximum amount of work derivable from the chemical reactions discussed here as a quantity equal to the decrease in

free energy. It is possible now-a-days to compute the free energy change for most reactions involving only inorganic reactants with a high degree of precision. On the other hand, until quite recently the standard free energy data for organic compounds were too scanty to be useful in considerations of the energy changes in physiological reactions. Instead, tacitly it has been assumed that the change in the heat content, e.g., the heat of combustion, was equal to the theoretical maximum available work. This assumption is made, for instance, in the studies of the relative efficiencies of carbohydrate and of fat as fuels for muscular work; in the principle of replaceability of "isodynamic" quantities of different foodstuffs.

Recently Parks and his collaborators[1] have determined the entropies of a number of organic compounds among which are such elementary metabolites as glucose, palmitic acid and glycerol. From the values of the standard molal entropies and the available data on the heats of combustion the standard free energies of these compounds were computed.

This work renders it possible to compute now with some precision the energy changes incurred in the oxidation of glucose and of tripalmitin. The ratios for each of these metabolites of the reversible work as defined above to the total energy change, i.e., to the heat of combustion plus the change in the pressure-volume product, can be considered as measures of their theoretical "efficiencies" as sources or generators of energy other than heat, i.e., as fuels for work, whether mechanical, chemical, or electrical.

The conventional term "efficiency" is not used with exactly the same meaning always. In the physiological literature it is employed in the sense of the ratio of work performed by the animal to the heats of combustion of the metabolites consumed in the performance of this work. In accordance with this usage we have considered the theoretical maximum "efficiency" of a physiological fuel as the ratio of the reversible work to the total energy change. When "efficiency" is defined in this way the ratio may in some cases, as Burk has shown,[2] be greater than 1. Here, obviously the balance sheet is incomplete: some reactions have been omitted, or the process is an endothermic one when it proceeds under reversible conditions, drawing heat from the surroundings. "Efficiency" might also be defined in the sense of the ratio of the work performed to the calculated maximum work obtainable from the reactions involved. For clarity the term "efficiency" will be defined here explicitly in each case. The available data are sufficient also for the calculation of the free energy change involved in the conversion of tripalmitin to glucose, or vice versa.

The usefulness of these calculations is limited before all else, of course, to those systems in which the second law of thermodynamics is obeyed. In spite of the hesitancy prevalent,[3,4] there does not appear to be any strong *a priori* reason for rejecting the conclusions of thermodynamic calculations when the available data are as adequate as they are now, in such macro-

scopic physiological chemical changes as the oxidation of glucose or of tripalmitin to carbon dioxide and water. The accuracy of the data depends now only on the accuracy of the values for the heats of combustion, and to a lesser degree on the values employed for the concentrations of the reactants in these physiological reactions.

The results of the calculations given in detail below are that the reversible work, which is practically the same as the free energy change, and which is equivalent to the theoretical maximum obtainable work in the physiological combustion of glucose, is 101 per cent of the heat of combustion; and of tripalmitin 98 per cent of the heat of combustion. This difference between glucose and a saturated fat represents, therefore, their relative theoretical "efficiencies" as fuels. In the case of the oxidation of carbohydrate the pressure-volume product change is practically zero, as the respiratory quotient for the reaction is 1. In the case of the oxidation of tripalmitin the change in the pressure-volume product is equivalent approximately to 13,000 calories. Even this quantity is negligible in comparison with the values for the heat of combustion and the free energy change which are both over 7,000,000 calories per gram mol. The computations show also that the production of fat from glucose is a process in which there is a large gain in free energy. From the assumption made here, that the second law of thermodynamics is obeyed in the body, it follows that this process is not a spontaneous one: i.e., when it does occur it is effected at the expense of the energy derived from some other reaction.

This is shown in the feeding experiments of Bleibtreu,[5] Rapport, Weiss and Csonka,[6] and Wierzuchowski and Ling.[7] Bleibtreu pointed out that the high respiratory quotients which are obtained when carbohydrate is converted to fat are most frequently accompanied, not by a decreased, but by an increased consumption of oxygen. In the experiments of Wierzuchowski and Ling in which a respiratory quotient as high as 1.55 was obtained, the oxygen consumption was nearly trebled, while the CO_2 production was quadrupled. It was calculated that, over a period of 24 hours, fat equivalent in calories to 184 per cent of the basal was manufactured from carbohydrate and stored by the animal, while the increase in metabolism over the same period was 60 per cent of the basal. Their most striking experiment was "When starch and glucose were given together on the morning of a day following a large ingestion of starch, the metabolism rose 100 per cent above the basal, and the production of fat from carbohydrate amounted to a storage of caloric energy in the form of fat equal to 260 per cent of the basal metabolism." Similarly Rapport, Weiss and Csonka observed in the hog that, when a quantity of fat whose caloric equivalent was equal to 84 per cent of the basal, was produced from carbohydrate, there was an increase in metabolism over the basal of 22 per cent. The evidence is not conclusive because it is not possible from this data to sepa-

rate the specific dynamic action due to fat production, from that due simply to the ingestion of large amounts of carbohydrate, i.e., to the effect which Lusk has designated as the specific dynamic action due to plethora. The latter effect, however, subsides within 4 or 5 hours after the ingestion of the carbohydrate and has never been found to provoke an increase in metabolism of the order of magnitude of 100 per cent of the basal. In one experiment Wierzuchowski and Ling observed, 20 hours after the ingestion of 700 gm. of corn starch, a respiratory quotient of 1.4 and an increase in the metabolism of 45 per cent over the basal. At this time the fat production from carbohydrate was 4.1 grams per hour. The power of the dog to transform carbohydrate to fat is apparently much less than that of the hog, and the increase in metabolism following the administration of large amounts of carbohydrate apparently is less, and is over sooner than in the case of the hog. Here also the evidence is not conclusive because there are no experiments on record in which as large amounts of sugar were given to dogs as were provided in the diets of the hogs in the experiments of Wierzuchowski and Ling. The experiments of Boyd, Hines and Leese, quoted by Lusk[8] suggest, however, that the specific dynamic action of carbohydrate in dogs would not under any circumstances approach the high figures observed in hogs. In these experiments glucose was administered continuously intravenously to a dog in such amounts that the blood sugar rose from 0.1 per cent to 0.7 per cent. Under these conditions "the maximal increase in metabolism ever recorded in a dog after glucose administration was obtained." During the injection the increase over the basal was 48 per cent. One hour after the cessation of injection the blood sugar had returned to a normal level, and the metabolic rate was now only 16 per cent above the basal. Three hours after the injection the increase over the basal was only 5 per cent. The mere deposition of carbohydrate in the tissues, therefore, cannot, it seems, account for the very high values for the specific dynamic action of carbohydrate observed in the hog many hours after ingestion of the carbohydrate.

The difference between these two animals appears to be that in the hog much more carbohydrate is converted to fat. The source of the energy for this conversion under the conditions of these experiments is derived largely from the oxidation of carbohydrate. The very large values for the specific dynamic action indicate that only a fraction of the energy released by this oxidation is actually used in the conversion; the remainder escapes unused as heat.

In the equation of Bleibtreu which was employed by Rapport, Weiss and Csonka, and by Wierzuchowski and Ling for the comparison of the values obtained from indirect with those from direct calorimetry, the specific dynamic effect of the process of fat-production from carbohydrate is tacitly implied. Bleibtreu considered that in the formation of 100 grams

of pork fat with an elementary composition of C, 76.54%, H, 11.94%, and O, 11.52%, 191.35 grams of glucose would be required in order to provide the carbon. The hydrogen of the glucose, not used to form fat, combines with some of the unused oxygen to form water; and the remaining oxygen is consumed in the combustion of more glucose, over and above the basal requirement. If the process of fat formation did not exert a specific dynamic action, this unused oxygen presumably would be available for purposes of the basal metabolism, so that, under these conditions, the consumption of inspired oxygen would be reduced. But, as Bleibtreu pointed out, the oxygen consumption is increased, in spite of the release of a large quantity of oxygen from the carbohydrate. The observations on plants of Terroine and Bonnet and their collaborators[20,21,22] show similarly that the formation of fat from carbohydrate involves an expenditure of energy which manifests itself in an addition to the basal metabolism. Their results are discussed in detail below.

It seems improbable that the production of fat from carbohydrate is carried on with the same expenditure of energy in all animals. The specific dynamic action of this process in any animal can be considered as the reciprocal of the ability of that animal to transform carbohydrate into fat. The final equation of Bleibtreu is

$$270.06 \text{ gm. glucose} = 100 \text{ gm. fat} + 115.45 \text{ gm. } CO_2 + 54.6 \text{ gm. } H_2O$$

In animals such as the hog or goose, that transform carbohydrate into fat very readily, the amount of glucose used and CO_2 and water formed to produce 100 grams of fat will be less than in a dog. The procedure of Lusk and his pupils, of first deducting the specific dynamic action, as indicated by the increased oxygen consumption, and then estimating the calories equivalent to the CO_2 production over and above that required for an R. Q. of 1, tends to obscure the differences between animals, and by an apparent more or less satisfactory agreement between direct and indirect calorimetry endows the empirical equation of Bleibtreu with an unwarranted generality.

The converse reaction, the hypothetical conversion of tripalmitin to glucose, is accompanied by a work content decrease which amounts to 21 per cent of the theoretical maximum amount of work available, and to 20 per cent of the total energy change in the combustion of tripalmitin. If fat is converted into carbohydrate before its energy can be used in muscular work, the energy equivalent to the decrease in work content must be wholly dissipated as heat instead of used for work. Under these conditions fat must necessarily be less efficient as a source of energy for work than carbohydrate.

There are described in the literature a number of attempts to determine experimentally the relative efficiencies of carbohydrate and of fat as fuels for

muscular work in the animal body. The careful study by Krogh and Lindhard of about ten years ago[9] led to a general acceptance of a figure for the efficiency of fat as a fuel for muscular work 11 per cent less than that of carbohydrate. Unfortunately the reliability of this estimate has been impaired by the subsequent work of Hill, Long and Lupton[10] on the oxygen debt, and by the studies of Rapport and Ralli,[11] and particularly of Best, Furusawa and Ridout[12] on the respiratory quotient of the excess metabolism during the performance of and recovery from muscular work. The serious deficiency in the data of Krogh and Lindhard is their omission of the metabolism during recovery. Their estimate of 11 per cent difference in efficiency between the two fuels is based upon observations on the "excess" metabolism only during the performance of the work.

The later observations of Rapport and Ralli on the "excess" metabolism of mild exercise by dogs are in accordance with those of Krogh and Lindhard in so far as they found that according to whether the diet is preponderantly carbohydrate or fat, respectively more carbohydrate or fat is burned. It is possible, therefore, that in the experiments of Krogh and Lindhard, the respiratory quotient during recovery was the same as that during the exercise. If this was the case, and if the recovery period is of the same length on the two diets, then the same relative differences that were observed on the carbohydrate and fat diets might have been found for the total excess metabolism.

Leathes and Raper[13] calculated that a quantity of heat equivalent to 20 per cent of the heat of combustion of fat is evolved in the conversion of fat to carbohydrate. They consider that this figure is at variance with the figure of 11 per cent lower efficiency of fat obtained by Krogh and Lindhard, if it is considered that fat is converted to carbohydrate before it is burned. This difference between 20 per cent and 11 per cent constitutes a discrepancy only if it is assumed that before fat can provide energy for any kind of work in any tissue of the body it must first be converted to carbohydrate. If the energy available for work released by the combustion of fat can be utilized by the viscera, without its previous conversion to carbohydrate, then even if such a conversion is obligatory in skeletal muscle, it would still be possible to reconcile the difference between the calculated 20 per cent and the observed 11 per cent lower efficiency of fat, by postulating a selective utilization of fat by the viscera and of carbohydrates by the muscles when muscular work is carried out on a high fat diet; or by some such mechanism as that suggested by Rapport and Ralli, that the energy of combustion of fuels other than a fraction of the free lactic acid may serve in the re-synthesis of glycogen in the recovery phase of muscular contraction.

But until the observations of Best, Furusawa and Ridout on the excess metabolism during muscular exercise have been explained, any conclusions

regarding the nature of the fuel used in muscular exercise can only be tentative so long as they are based, as they are above, upon inferences drawn from respiratory quotients. Best, Furusawa and Ridout found that "The respiratory quotient of the excess metabolism of muscular exercise is not invariably unity, but increases with the severity of the exercise from a value approximately the same as the basal quotient for very mild exercise, through unity for moderate exercise, to one considerably above unity for very severe exertion." These observations in so far as they pertain to excess metabolism during mild exercise confirm the similar earlier findings of Rapport and Ralli.

If eventually it is found that muscular work is performed on a fat diet with an efficiency considerably less (more than a few per cent less) than on a carbohydrate diet, this inefficiency cannot be ascribed to differences between the free energy and heat energy of these two metabolites; but rather to differences in their intermediary metabolism, whereby more energy escapes as heat in the case of fat than in the case of carbohydrate.

These conclusions regarding the losses of energy incurred both in the conversion of fat to carbohydrate and vice versa are supported by the observations of Terroine and Bonnet and their collaborators on germinating seeds and moulds. The problem is simplified with these forms on account of the absence of any specific dynamic action due to plethora.[20] In the mould Sterigmatocystis nigra Terroine and Bonnet[21] found that for every 100 calories of potential energy contained in the glucose used in the process of conversion to fat, 11 per cent was lost as heat when the fatty acid content of the mycelium rose from 3.1 per cent to 9.0 per cent; 13 per cent was lost when the fatty acid content was 10.5 per cent; and 17 per cent was lost when the fatty acid content was 12.3 per cent. If in the feeding experiments on the hog one ascribes the whole of the specific dynamic action of the carbohydrate to the work of converting carbohydrate to fat, the ratio of energy lost to the total employed is 18 per cent in the experiment of Rapport, Weiss and Csonka, and 23 per cent in the experiment of Wierzuchowski and Ling. The latter estimates are certainly too high, as only a fraction of the total specific dynamic action of carbohydrate in these feeding experiments could have arisen from the production of fat. There must have been another fraction due simply to the plethora effect. The "specific dynamic action" of formation of fat from carbohydrate will therefore be apparently the same in plants as in the hog.

The problem of the energy losses incurred in the utilization of fat in animals also is elucidated by the observations made on plants, where the production of carbohydrate from fat is unequivocal and easily demonstrated. In germinating grains Terroine, Trautmann and Bonnet[22] observed that when fat is converted into cellulose, 23 per cent of the energy of the fat used is lost. Similarly in the mould Sterigmatocystis nigra the

amount of energy lost in the conversion to carbohydrate varied, depending upon the fat used between 18.8 per cent and 25.8 per cent. These values are in accord with that calculated, and substantiate the criticism, based upon calculated energy changes, of the view that fat must first be converted to carbohydrate before it can be utilized in muscular work.

Calculations: The symbols employed are those of Lewis and Randall.[14] Throughout, the activity of the reactants will be considered as equal to their molar concentrations. This is permissible because even the allowances for the free energy of solution and dilution, and for temperature, are very small compared with the free energy changes when the reactions are considered as occurring with the metabolites and end products in their standard states. In the re-calculation of the standard free energies at 37°C. instead of at 25°C., the values of the heats of formation and of combustion will be taken to be the same at the two temperatures. The free energy values employed in the calculations which follow are taken from the table of revised values of Parks, Kelley and Huffman.

Glucose:

ΔF_{298} (solid) $= -219{,}000$ calories.

From the second-law free energy equation $\frac{-\Delta F_2}{T_2} - \frac{-\Delta F_1}{T_1} = \int_{T_1}^{T_2} \frac{\Delta H}{T_2} dT$; and $\Delta H_{298} = -303{,}000$ calories, $\Delta F_{310} = -215{,}600$ calories. Interpolating from the data in the International Critical Tables, the solubility of glucose at 37°C. is 146 gm. $C_6H_{12}O_6.H_2O$ in 100 gm. water. The mol fraction of the saturated solution at 37°C. is $\frac{\frac{132.7}{180}}{\frac{132.7}{180} + \frac{113.3}{18}} = .11$. Considering the concentration of glucose at the site of oxidation to be nearly the same as it is in the blood, normally approximately 0.1 gm. in 100 cc., the mol fraction is $\frac{\frac{0.1}{180}}{\frac{90}{18}} = .00011$, and the free energy of the dilution per mol is therefore $-\Delta F = RT \ln \frac{.11}{.00011} = 4250$ cal. $\therefore \Delta F$ glucose (mol fraction $= .00011) = -219{,}900$ calories.

The concentrations of oxygen, carbon dioxide and water in the blood and tissues are not those of their standard states, and accordingly slight corrections are introduced in the free energy computations for these deviations, in addition to the modification of the standard free energy values for the change in temperature from 25°C. to 37°C.

Oxygen: Krogh[15] gives the following values for the difference in oxygen tension between the blood in the capillaries and the muscles under the following physiological conditions: rest, 6.5 − 3.5 per cent of an atmosphere; massage, 2.5 per cent; work, 0.4 per cent; maximum circulation, 0.25 per cent. Considering, therefore, the oxygen tension in the muscles during work to be 100 mm. mercury, the free energy decrease per mol of oxygen due to "dilution" is $-\Delta F = RT\ln\frac{760}{100} = 1250$ calories.

H_2O:

$$\Delta F_{298} = -56560.$$
$$\Delta H = -68270.$$
$$\therefore \Delta F_{310} = -56100.$$

In plasma the mol fraction of water is .995.[16] The correction for dilution is therefore negligible.

CO_2:

$$\Delta F_{298}\ CO_2\ (g) = -94{,}260 \text{ calories.}$$
$$\Delta H = -94{,}300 \text{ calories.}$$
$$\therefore \Delta F_{310}\ CO_2\ (g) = -94{,}260 \text{ calories.}$$

Considering the pressure of CO_2 in the tissues to correspond to 40 mm. of mercury, the correction for the free energy decrease due to the dilution of the CO_2 is

$$-\Delta F = RT\ln\frac{760}{40} \text{ calories} = 1800 \text{ calories.}$$

$$\Delta F_{310}\ CO_2\ (g)\ (40 \text{ mm. Hg}) = -96{,}100 \text{ calories.}$$

Oxidation of Glucose: $C_6H_{12}O_6$ (mol fraction .00011) + $6O_2$ (g) (100 mm. Hg) = $6CO_2$ (g) (40 mm. Hg) + $6H_2O$ (l); $-\Delta F_{310} = (-219{,}900) + (-6 \times 1250) - (-6 \times 96{,}100) - (-6 \times 56{,}100) = 685{,}800$ calories. The heat of combustion of glucose in solution is 676,080 calories.[17]

$$\therefore \frac{\text{Free Energy of Combustion}}{\text{Heat of Combustion}} = \frac{685{,}800}{676{,}080} = 1.01.$$

The change in the pressure–volume product is negligible here.

The fact that the free energy of combustion is greater than the heat of combustion means only that if it were possible to convert into work all the free energy released by the oxidation of glucose, approximately 9000 calories would be absorbed per gram mol of glucose oxidized. Actually in the body, of course, only a small fraction, between 15 and 30 per cent of the free energy, is utilized, the remainder being dissipated as heat.

The experimentally determined values for the standard molal free energies of palmitic acid and of glycerol permit a fairly good approximate computation of the free energy of tripalmitin. In the hydrolysis and formation of a great variety of esters Berthelot and Saint-Gilles[18] found that the equilibrium constant for the reaction, acid + alcohol = ester + water, was always in the neighborhood of 4; and that the value of the equilibrium constant seemed to be independent of temperature in the range from 100°C. to 220°C. One may assume from these observations that for this type of reaction ΔF and ΔH are equal, or nearly so. The value of ΔF at 25°C. then becomes about −800 calories per linkage mol of ester. The value for the heats of combustion in the International Critical Tables, however, yield values for the heats of formation of esters with the higher acids as high as 15,000 calories. No heat data are available in regard to glycerol esters. Since it is insignificant in the final result here whichever value is chosen for ΔH we have preferred the alternative of assuming that the heat of ester formation in the case of tripalmitin is approximately 1000 calories per linkage. The free energy of formation of tripalmitin from glycerol and palmitic acid is accordingly taken as +2400 calories.

The reaction may be represented as follows: 1 glycerol + 3 palmitic acid ⟶ tripalmitin + 3 water; $-\Delta F = +2400$ calories.

Parks, Kelley and Huffman give −115,700, and −94,000 as the free energy values at 25°C. of glycerol and palmitic acid respectively. The free energy of water at 25°C. is −56,560. The value for the free energy of palmitic acid is based upon a value for the heat of combustion of 2,379,000 calories. The value given in the International Critical Tables is slightly higher, 2,380,000 calories. With this value, the free energy of palmitic acid is −93,000 calories. From these figures an approximate value for the free energy of tripalmitin is −227,000 calories.

Assuming that the heat of ester formation of tripalmitin is +3000 calories, ΔH, the heat of formation of tripalmitin, is simply the sum of the heats of formation of 1 gram mol of glycerol and three of palmitic acid plus 3000 calories, minus the heat of formation of three gram mols of water, and is equal to −610,500 calories. The free energy, ΔF, at 37°C. then is −211,000 calories.

Because fat in the tissues probably exists as a saturated solution in equilibrium with the solid phase there is no free energy of solution to be taken into account for tripalmitin.

Oxidation of Tripalmitin: $2C_{51}H_{98}O_6$ (solid) + $145O_2$(100 mm. Hg) ⟶ $102CO_2$ (40 mm. Hg) + $98H_2O$; $-\Delta F = (2 \times -211{,}000) + (145 \times -1250) - (102 \times -96{,}100) - (98 \times -56{,}100) = 14{,}696{,}750$ calories; = 7,348,000 calories per gram mol of tripalmitin. The diminution in the pressure–volume product is equivalent to 13,000 cal. per gram mol of tripalmitin oxidized.

There are no data available on the direct measurement of the heat of combustion of tripalmitin; but little error will be involved in assuming it to be equal to the sum of the heats of combustion of 1 mol of glycerol and 3 mols of palmitic acid. The value then is 7,537,000 calories, minus 3000 calories, the heat of esterification.

$$\therefore \frac{\text{Free energy of combustion}}{\text{Heat of combustion}} = \frac{7{,}348{,}000}{7{,}534{,}000} = .98; \quad \frac{\text{Reversible work}}{\text{Total energy change}} = \frac{7{,}335{,}000}{7{,}521{,}000} = .98.$$

The theoretical maximum work obtainable from the oxidation of tripalmitin is therefore relative to their respective heats of combustion, only 3 per cent less than that of glucose.

The free energy changes in the conversion of tripalmitin to glucose under physiological conditions may be considered in three stages: (1) the hydrolysis of tripalmitin, (2) the conversion of glycerol to glucose, (3) the conversion of tripalmitin to glucose.

The hydrolysis may be taken to incur a free energy change of -2400 calories.

Conversion of Glycerol to Glucose under Physiological Conditions:

ΔF_{298} glycerol $= -115{,}700$ calories.
ΔH_{298} glycerol $= -159{,}300$ calories.
$\therefore \Delta F_{310}$ glycerol $= -113{,}700$ calories.

Glycerol exists in the blood in a minute concentration. In order to assign some figure for the free energy of dilution we shall assume that in the liver, where the conversion may be said to occur, its concentration is of the order of magnitude of a mol fraction of 10^{-4}, so that the free energy of dilution is approximately 5000 calories.

$\therefore -\Delta F_{310}$ (mol fraction 10^{-4}) $= -118{,}700$ calories.

$\therefore 2C_3H_8O_3$ (mol fraction 10^{-4}) $+ O_2$ (100 mm. Hg) $\longrightarrow C_6H_{12}O_6$ (mol fraction .00011) $+ 2H_2O$; $-\Delta F = 103{,}400$ calories $= 51{,}700$ calories per mol of glycerol.

The pressure–volume product change is approximately 600 calories, and is negligible.

Conversion of Palmitic Acid to Glucose under Physiological Conditions:

ΔF_{298} palmitic acid $= -93{,}000$ calories.
ΔH_{298} palmitic acid $= -221{,}000$ calories.
$\therefore \Delta F_{310}$ palmitic acid $= -88{,}000$ calories.

Therefore, under physiological conditions, for the reaction:

$3C_{16}H_{32}O_2 + 21O_2$ (100 mm. Hg) $= 8C_6H_{12}O_6$ (mol fraction .00011);

$-\Delta F$ = 1,469,000 calories; pressure–volume product change, $\Delta(PV)$ = $-13{,}000$ calories. The total change in work content in the conversion under physiological conditions of tripalmitin to the maximum possible amount of glucose is 1,505,000 calories. Therefore for tripalmitin,

$$\frac{\Delta W_R \text{ conversion}}{W_R \text{ oxidation}} = \frac{1{,}505{,}000}{7{,}335{,}000} = .21;$$

and

$$\frac{\Delta W_R \text{ conversion}}{\text{Total energy change in oxidation}} = \frac{1{,}505{,}000}{7{,}521{,}000} = .20$$

The conversion of tripalmitin to glucose involves the liberation of a large fraction of the energy of the fat. The reverse reaction, the formation of one gram mol of tripalmitin from $8^1/_2$ gram mols of glucose, would incur a gain in the quantity of that energy which we have designated as reversible work, equivalent to the total energy of oxidation of approximately 2 gram mols of glucose. The reaction almost certainly does not take place in a reversible manner, and a better guess, it seems, would be that at least 4 gram mols of glucose would be required. The unused energy would escape as heat and in direct calorimetry would represent the specific dynamic action of this process.

A concise statement of the free energy relationship between carbohydrate and fat is obtained from the comparison of the free energy content per gram mol of carbon in glucose and in tripalmitin. Per gram mol of carbon the free energy content of glucose is $-36{,}500$ calories; and of tripalmitin -2060 calories. It is clear that any process by which carbohydrate is converted into fat in such a manner that all of the carbon of the one compound is re-constituted into the other involves a large gain in free energy which must be supplied by other processes. If the mechanism by which this energy is supplied is not a perfectly reversible one, a larger amount of energy will be produced than is used in the conversion of carbohydrate into fat; and the excess, lost as heat, will appear as specific dynamic action. Similarly the converse production of carbohydrate from fat is accompanied by a large decrease, i.e., liberation of free energy, which will appear, wholly or in part, as heat or work.

In plants, as the observations discussed above show, this released energy is not used for any other purpose, and escapes, presumably, as heat.

It follows from the small difference in the ratios of the reversible work obtainable to the total released in the combustion of glucose and of tripalmitin, that the explanation for any large differences observed experimentally in their respective efficiencies as fuels must be sought in their intermediary metabolism. If fat must be converted to glucose before it can serve as a fuel for muscular work, the large free energy change involved

in the conversion of fat into glucose renders it probable on theoretical grounds (i.e., the process probably is not perfectly reversible) that fat will be less efficient as a fuel. On the other hand, if the oxidation of fat *per se* can serve, as suggested by Rapport and Ralli, in the re-synthesis of lactic acid to glycogen, then there is no *a priori* reason for considering that fat will be less efficient than lactic acid. The plausibility of the suggestion of Rapport and Ralli is enhanced by the observations of Himwich, Koskoff and Nahum[14] that the liver possesses a great avidity for the lactic acid liberated in the blood-stream during exercise; and by the observation of Rapport and Ralli on dogs, confirmed by Best, Furusawa and Ridout on men, that the excess metabolism of mild exercise is that of the basal metabolism at that time.

If fat is first converted to glucose, as Best, Furusawa and Ridout maintain, then the free energy of conversion must be considered as lost for purposes of muscular work. It is interesting to compute from the calculations carried out above the minimum theoretical difference in efficiency of fat and carbohydrate under these circumstances. If the efficiency of the process of muscular contraction is taken first at 30 per cent, then 30 per cent of the energy of glucose derived from tripalmitin would be used, which corresponds to 23.1 per cent of the heat of combustion of the tripalmitin. If the muscular work is carried out at a 10 per cent efficiency then 7.7 per cent of the heat of combustion of tripalmitin is used. In each case fat is 77 per cent as efficient a fuel as carbohydrate. The experimental investigations into the difference in efficiency of fat and carbohydrate have in no case yielded figures as high as this, showing, if subsequent investigations do not yield a very different result, that fat is burned during muscular work as such; and that the efficiency of the processes by which this energy is used is not much less than is the case with glucose.

Krogh and Lindhard criticize the estimations of the energy changes in the conversion of fat to carbohydrate as follows: "We are not convinced of the validity of any of these summary methods of calculating the waste of energy incidental to the conversion of fat into sugar or any other substance. As a reason for suspicion against summary methods of calculating the loss of energy in question we would suggest the making up of 1 molecule of fat (tripalmitin) into 12 molecules of sugar by the addition of 21 molecules of CO_2 and 23 of H_2O, which would result in a *gain* of energy amounting to about 18 per cent." It is interesting to compute the free energy change for this proposed hypothetical reaction.

$$C_{51}H_{98}O_6 + 21CO_2 + 23H_2O \longrightarrow 12C_6H_{12}O_6$$

From the data given above the free energy change, $-\Delta F$ is $-881{,}000$ calories. In other words, this reaction could not occur except at the cost of other reactions. As it is improbable that a reaction such as this would

proceed under ideal, reversible conditions, we may expect instead of a gain in energy of 18 per cent, a wastage as heat of approximately 1,000,000 calories for each molecule of tripalmitin converted to glucose.

The computations carried out above show that the cycle of storage of carbohydrate by conversion to fat and its later reconversion to carbohydrate preliminary to its ultimate oxidation, is one which is carried out at the cost of a considerable amount of energy to the organism. From studies of the degree of reversibility of reactions *in vivo* it may be possible to estimate how much of this expended energy, i.e., not contained in the newly formed molecules as chemical potential energy, is, from the nature of the reactions and substances involved, inevitably wasted as heat, and how much under suitable conditions can be converted into work.

Summary—1. The theoretical maximum amount of work obtainable from the oxidation under physiological conditions of glucose, and of tripalmitin has been computed from the standard free energies and heats of combustion of these compounds.

2. It has been shown that the theoretical difference in efficiency, i.e., the ratio of the theoretical maximum work obtainable to the total energy change, is little different for glucose and for tripalmitin.

3. The process of conversion of glucose into tripalmitin probably exerts a considerable "specific dynamic action" on metabolism.

4. From the fact that the conversion of tripalmitin to glucose involves a release of energy approximately equivalent to 20 per cent of the theoretical maximum work available from the oxidation of tripalmitin, and the observation that the difference in efficiency of fat as a fuel for muscular work, as compared with carbohydrate, is much less than 20 per cent, it follows either that fat is burned as such in the provision of energy for muscular work, or that the energy released in the hypothetical conversion of fat to cabohydrate is not dissipated as heat, but is used for work; and the efficiency of utilization of this energy is little different from that in the consumption of carbohydrate.

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THE WORK OF THE KIDNEY IN THE PRODUCTION OF URINE

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The work performed by the kidney in the production of urine appears to be more readily susceptible of analysis by means of the laws of thermodynamics than any other complete function of the animal body. The recognition of this possibility, of course, is not new. It has attracted a number of investigators whose essays have been collected and reviewed by Cushny.[1] The purpose and method were essentially the same in all of these studies: the computation of the theoretical minimum amount of work necessary to elaborate a solution such as urine from another such as blood. None of these computations was complete; and even in the last, made in 1914,[2] a number of processes were not taken into account, viz.: the suppression of ionization of the phosphates, and other weak acids by the greater acidity of the urine, and the production of ammonia from urea by the kidney.

In the account presented below of the minimum work necessary for the production of the urine, an attempt has been made to appraise the factors omitted in previous studies; and an alternative method has been employed in the analysis of the work involved in the transport of water. It is felt that a clearer understanding of the energetics of water transport in the body in general is obtained from the alternative treatment. The minimum work has been considered as a quantity equal to the sum of the free energy changes for the transport of each constituent, including water,

from blood to urine. It has been assumed, an assumption justifiable on practical grounds, that the substances concerned may be considered as perfect solutes.

The analysis of the urine is now so nearly complete, 95 per cent of the nitrogen being accounted for, that the total work given in table 2 may be taken as the order of magnitude of the total amount of work performed by the kidney in 24 hours. The items omitted cannot add more than a very small quantity to the present tally. This prediction is permissible on account of the form of the equation by which the work is calculated.

The value obtained for the theoretical minimum amount of work necessary to produce a solution such as urine from another such as blood is quite a small quantity; in view of the total energy used by the kidney, it is surprisingly small. Possibly because the difference between the work performed and the energy used by the kidney is so large, there has been a tendency to evade explicit statement of the obvious conclusion to be drawn regarding the efficiency of the kidney considered as a machine. The following quotation from Cushny[1] is a representative expression of this attitude: "But even if the concentration of each constituent of the plasma and urine were known, and the total work were calculated according to these formulas, this would not necessarily indicate the whole energy employed in the secretion. For this measures only the energy employed in overcoming osmotic resistance, and takes no account of that entailed in the transmission of molecules of water and solid through the cells and along the tubules. Nor until more is known of the mechanism of secretion can even a general estimate be formed of the amount of energy thus used. Now if, as one school holds, the secretion of water involves actual work on the part of the cell, more energy is required for the production of the more abundant fluid, but as it is less concentrated, the work done against osmotic resistance is smaller. The total work of the kidney in producing a dilute urine may thus be greater or less than when it is more concentrated, according as the energy required to secrete water is greater or less than that required to concentrate the solids. On the other hand, if the secretion of water does not entail the loss of energy in the kidney, as is held by some authorities, an abundant secretion of dilute urine may actually involve less work than a scantier flow containing the same amount of solids."

In the foregoing quotation there is not a clear distinction made between the work which the kidney carries out, and the energy it consumes in performing this work. In view of this quotation, it may be pointed out, that on theoretical grounds no information is required regarding the mechanism by which work is effected. The theoretical minimum quantity of work, which here is equal to the change in free energy, depends only on the initial and final states; and water may be considered in exactly

the same way as the other constituents of the urine. The uncertainty regarding the mode of calculating the work of water transport is due, in part, to the employment of the cumbersome concept of osmotic pressure.

It is also easier, with the concept of free energy change than it is with osmotic pressure, to calculate directly the work involved in such processes as the suppression of ionization, and the conversion of urea to ammonia.

Calculations.—The calculation of the work performed in the production of urine is based upon the data regarding the composition of the human blood and urine given chiefly by Cushny,[1] Mathews[3] and Hawk and Bergeim.[4] In table 1 are collected only the items which comprise the work of concentration carried out by the kidney. The free energy change, $-\Delta F$, which is equivalent to the work of concentration, is calculated by means of the equation

$$-\Delta F = NRT \ln \frac{C_{\text{plasma}}}{C_{\text{urine}}} \text{ calories.}$$

The activity coefficients have been taken as unity, because the corrections, even if adequate data were available, would be negligible in view of the low efficiency of the kidney. The volume of the urine passed in 24 hours has been taken, for convenience in calculation, as 1000 cc. N is the number of mols of any one substance excreted in 24 hours, which is here equal to the molar concentration in the urine given in table 1, R the gas constant 1.987 calories per degree, and T the absolute temperature 310.1°K.

The values of $-\Delta F$ in table 1 are computed on the basis that a finite quantity of urine is produced from an infinite quantity of blood. This amount of work may for practical purposes be taken as equal to the work involved in the actual process, arterial blood⟶urine + venous blood, because the value of $-\Delta F$ is influenced much more by the value of N than by the concentration term. If, for example, we assume that most of the urea is concentrated instead of 66.6 times, 300 times, the value of $-\Delta F$ changes only from -861 calories to -1171 calories, a negligible difference in view of the low efficiency of the kidney. And even this difference would be reduced by a corresponding correction in the opposite direction for the transport of water under these conditions. Further, as Cushny states: "The passage of the blood through the kidney does not of course completely remove the impurities it contains, because only a fraction of the plasma comes into direct contact with the cells, perhaps one-fifth or less, and even if the urea, etc., is completely removed from this fraction, 80 per cent of that brought by the artery, returns in the renal vein."

Behre and Benedict[5] expressed the opinion "that no results so far available offer definite evidence of the existence of creatinine in the blood."

They believe that there is present in the blood, not creatinine, but a "chromogenic" substance which is responsible for the color with picric acid, by which blood creatinine is estimated. Nevertheless, the kidney is able to abstract creatinine from the blood and concentrate it several hundred times in the urine, as illustrated in the findings of Rehberg.[6] We have therefore, considered the urinary creatinine as arising from creatinine in the blood.

The simplest method of computing the change in state of the phosphates is to calculate from the known total concentrations of phosphate and the hydrogen-ion concentrations, in the blood and urine, respectively, the concentration of each of the ionic forms; and then by means of the equation $-\Delta F = NRT \ln \frac{C_{\text{plasma}}}{C_{\text{urine}}}$ to compute the work for the transfer of each component from blood to urine. The value of N is so small for PO_4^{---} and H_3PO_4, that these may be ignored and the whole process of concentration of phosphate with its concomitant suppression of ionization can be considered as the transfer from one set of concentrations in the blood to another in the urine, of HPO_4^{--} and $H_2PO_4^{-}$ ions. The work involved here is given in table 1.

An alternative, longer method of computing separately the work of concentration of phosphate and the suppression of its ionization can be derived from the following hypothetical process. A quantity of phosphate equal to the amount in one liter of urine, is transferred from the blood to an infinite quantity of a hypothetical auxiliary solution in which the thermodynamic environment is the same as in the plasma, except that the total concentration of phosphate is the same as in the urine. The free energy change for this step, which is the work of concentration, is -27 calories. The suppression of ionization is calculated by considering the transfer in the form of H_3PO_4, of the total amount of phosphate which is in the urine, from the concentration of H_3PO_4 in the auxiliary solution, to its concentration in the urine where, under equilibrium conditions, it is allowed to ionize. To this must be added the difference in free energy for the neutralization by OH^- ions in the urine, instead of the plasma, of the phosphate transported; and the free energy change for the transport back to the blood of the water formed in this neutralization. The sum of all these free energy changes is only approximately -11 calories.

In calculating the change in ionization of the phosphate, the hydrogen-ion concentration of the plasma was taken as 3.98×10^{-8}, and of the urine, 1×10^{-6}, the three dissociation constants of phosphoric acid in urine and the first and third constants in plasma were calculated from the data of Sendroy and Hastings,[7] corrections being made for the ionic strengths of plasma and urine which were taken as 0.13 and 0.20, respectively; for the second dissociation constant of the phosphoric acid in plasma we have

employed the value chosen by Henderson.[8] Accordingly, the values for K_1, K_2 and K_3 in the plasma were 1.2×10^{-2}, 2.3×10^{-7} and 1.4×10^{-12}, respectively; and in the urine 1.3×10^{-2}, 2.6×10^{-7} and 2.2×10^{-12}.

The free energy change due to the suppression of ionization of the bicarbonate is almost exactly balanced by the value for the free energy of neutralization. The free energy change for suppression of dissociation and neutralization of the uric acid is similarly negligible.

In the calculation of the free energy changes for calcium the total energy change is so slight that we have neglected such factors as concentrations of complex ionic and undissociated forms.

The work involved in the transport of water from the plasma to the urine was calculated in the same way as the other constituents of the urine, by the equation

$$-\Delta F = NRT \frac{N_{\text{plasma}}}{N_{\text{urine}}}, \text{ where } N \text{ denotes the mol fraction.*}$$

From the data given by Mathews, and by Hawk and Bergeim we have taken the number of mols of solids dissolved in one liter of plasma to be 0.313, and in the urine 0.765. Mathews, quoting the data of Schmidt, gives 1.0312 as the specific gravity of plasma and 901.51 gm. as the weight of the water in 1000 gm. plasma. The weight of water in one liter of plasma is therefore 929.6 gm.; the number of mols, 51.6; and the mol fraction 0.994. A liter of urine such as that postulated in table 1 contains 36 gm. of solids and is of specific gravity 1.013; the weight of the water in one liter is 977 gm.; the number of mols 54.2; and the mol fraction 0.986. From these values, $-\Delta F$ for the transport of water is $54.2\ RT \ln \frac{.994}{.986} = +267$ calories.

We have based our calculations of the free energy change for the ammonia in the urine, upon the theory of Benedict and Nash[9] that the urinary ammonia is formed by the kidney from urea; and we have assumed that this conversion is carried out with the reacting components at the concentrations at which they exist in the blood. We have preferred this view to the alternative suggestion of Bliss;[10] the methods employed by Bliss for the determination of ammonia in the blood[11,12] must, it seems, liberate by hydrolysis of the blood proteins, amide nitrogen which is as essentially constituent of the proteins as the amino acids, and not, therefore, a factor in the acid-base equilibrium of the body.

The values employed for the standard free energies are those given by Lewis and Randall:[13] and for the solubilities, those in the International Critical Tables.

Urea ΔF_{298} (solid) = −47,280 calories.
ΔH (solid) = −78,490 calories.
ΔF_{310} (solid) = −46,020 calories.
Solubility at 37°C. = mol fraction 0.311.
Concentration in plasma = mol fraction 0.000092.
ΔF dilution = −5010 calories.
ΔF_{310} (mol fraction 0.000092) = −51,030 calories.

NH_4^+ ΔF_{298} (1.0 M.) = −18,930 calories.
ΔH = −31,700 calories.
ΔF_{310} (1.0 M.) = −18,420 calories.
ΔF dilution (to 0.000554 M. in plasma) = −4620 calories.
ΔF_{310} (0.000554 M.) = −23,040 calories.

H_2O ΔF_{298} = −56,560 calories.
ΔH = −68,270 calories.
ΔF_{310} = −56,090 calories.

H_2CO_3 ΔF_{298} CO_2 (1 Atm.) = −94,260 calories.
ΔH CO_2 = −94,300 calories.
ΔF_{310} CO_2 (1 Atm.) = −94,260 calories.
ΔF_{310} CO_2 (40 mm. Hg) = 96,070 calories.
ΔF_{310} H_2CO_3 (40 mm. Hg) = −152,200 calories.

H^+ ΔF_{310} (3.98×10^{-8} M.) = −10,500 calories.

Therefore for the reaction

$$\begin{matrix} & NH_2 & & & & & \\ & / & & & & & \\ C & = & O & + & 2H^+ & + & 2H_2O \longrightarrow 2NH_4^+ + H_2CO_3; \\ & \backslash & & & & & \\ & NH_2 & & & & & \end{matrix}$$

$-\Delta F$ = +7000 calories per mol NH_4^+ formed; from which the free energy of formation of 0.0222 mols NH_4^+ from urea is +155 calories.**

The value of each of the various forms of work performed by the kidney is set out in table 2. The total amounts to −704 gm. calories. This figure is of the same order of magnitude as that obtained in previous computations. This correspondence is due to the fact that the main work of the kidney, even when all known factors are taken into account, proves to be the work of concentration.

The processes omitted in table 2 are the free energy changes for the synthesis and excretion of 0.003 mols of hippuric acid; and the excretion of the unidentified nitrogenous constituents of the urine, comprising less than 5 per cent of the total nitrogen. These unknown quantities cannot add more than a few hundred calories at the most to the total. This prediction can be made because the value of $-\Delta F$ depends mainly upon

the value of N, the number of mols transferred; when this quantity is small, the value of $-\Delta F$ is small regardless of the magnitude of the concentration change. The total in table 2 of -704 gm. calories represents, therefore, practically all the work performed by the kidney in the production of the 24-hour urine. This value is surprisingly small, approximately 0.7 gm. calories per cc. of urine per day.

TABLE 1

THE WORK OF CONCENTRATION PERFORMED BY THE HUMAN KIDNEY IN THE PRODUCTION OF THE 24-HOUR URINE

CONSTITUENT	CONCENTRATION IN PLASMA, MOLS PER LITER	CONCENTRATION IN URINE, MOLS PER LITER	WORK PERFORMED BY THE KIDNEY $-\Delta F$ GM. CALORIES
Na^+	0.135	0.152	− 11
K^+	0.00512	0.0384	− 48
Ca	0.00224	0.00375	− 1
Mg^{++}	0.00103	0.00247	− 1
Cl^-	0.104	0.166	− 48
HPO_4^{--}	0.000807	0.00326	− 3
$H_2PO_4^-$	0.000140	0.0125	− 35
SO_4^{--}	0.000312	0.0187	− 47
HCO_3^-	0.0266	0.00106	2
Creatinine	0.000089	0.00664	− 18
Urea	0.00500	0.333	−861
Uric acid	0.000238	0.00298	− 5
NH_4^+	0.000554	0.0222	− 50
		Total	−1126

TABLE 2

THE TOTAL WORK PERFORMED BY THE HUMAN KIDNEY IN THE PRODUCTION OF THE 24-HOUR URINE

KIND OF WORK PERFORMED	QUANTITY OF WORK $-\Delta F$, GM. CALORIES
Concentration	−1126
Transport of water	+ 267
Formation of ammonia from urea	+ 155
	− 704

The value is so small that we were led to consider the possibility that the kidney may perform other functions, in which much more work is performed, than in the production of urine. The high concentration of enzymes in the kidney, greater than in any other tissue except the liver, lends some plausibility to this view. But it seems to be eliminated by the observations on the oxygen consumption and heat production of the kidney. These point to the conclusion that the kidney carries out the

work of elaborating urine with an exceedingly low efficiency, in the neighborhood of one per cent. This is discussed in detail in the following communication.

Summary.—1. The work of the kidney in the excretion of urine is analyzed by means of the second law of thermodynamics.

2. It is shown that the work performed by the normal kidney in man in the excretion of urine, is of the order of magnitude of 0.7 gm. calories per cc. of urine; or 70 gm. calories per gram of nitrogen excreted.

* Barcroft employed the following equation for the computation of the work of formation of urine:

$$\text{work} = RT\,(a_u \log \frac{a_u}{a_b} - (a_u - a_b) + b_u \log \frac{b_u}{b_b} - (b_u - b_b) + \ldots.),$$

where a_b, b_b, ... and a_u, b_u, ... represent molecular concentrations of the constituents of the blood and urine. The work in the above equation is the minimum work in the formation of a liter of urine. The summarized form of this equation is

$$\text{work} = RT\left[\Sigma\,(c_u \log \frac{c_u}{c_b}) + \Sigma c_b - \Sigma c_u\right].$$

Essentially this equation is

$$W = -\Delta F + \Delta(PV),$$

where $\Delta(PV) = RT(\Sigma c_b - \Sigma c_u)$, P here being the osmotic pressure, and V the volume of the solution. Barcroft estimated the pressure-volume product change from calculated osmotic pressures; the quantity obtained represents an approximate computation of the work involved in the transport of water from the blood to the urine. Stated in summarized form, this equation is somewhat more correct than the expanded form. In the expanded form presumably, only those substances in the blood are taken into account which are present in the urine. As shown below, the molecular concentrations of all the substances in the blood including those absent from the urine, and similarly the molecular concentrations of all the substances in the urine including those not in the blood, should be included in Σc_b and Σc_u, respectively. The second term in the summarized form of this equation can be shown to be equivalent to the method of calculating the work of the transport of water by the equation

$$W = NRT\,ln\,\frac{P_{\text{blood}}}{P_{\text{urine}}};$$

where P represents the vapor pressure of water.

We are indebted to Professor R. G. Dickinson and to Professor R. C. Tolman for this derivation of the relationship between the two equations.

$$-\Delta F = NRT\,ln\,\frac{N_{\text{blood}}}{N_{\text{urine}}};$$

which is an approximation of the rigorous equation

$$-\Delta F = NRT\,ln\,\frac{P_{\text{blood}}}{P_{\text{urine}}};$$

where P_{blood} and P_{urine} represent the vapor pressures of water in blood and urine,

respectively. The vapor pressure, by Raoult's law, is proportional to the mol fraction of the solvent.

$$\therefore P_{\text{blood}} = K\,(I - x_a^b - x_b^b - x_c^b - \ldots\ldots);$$

$$\text{and} \quad P_{\text{urine}} = K\,(I - x_a^u - x_b^u - x_c^u - \ldots\ldots);$$

where x_a^b, x_b^b, x_c^b, and x_a^u, x_b^u, x_c^u, represent the mol fractions of the constituents other than water a, b, c, and a', b', c', in the blood and urine, respectively.

$$\therefore -\Delta F = NRT \ln (1 - \Sigma x^b) - NRT \ln (1 - \Sigma x^u).$$

The value of N is the number of mols of water transported and may be designated as $N_{H_2O}^u$.

The value of the expression log (1 + x), when x^2 is less than 1, is $x - {}^1/_2x^2 + x^3 - {}^1/_4x^4 \ldots\ldots$ In plasma the value of x is 0.007 and in urine 0.015. We may, therefore, in both cases, ignore all terms but the first, so that the value of $-\Delta F$ becomes

$$NRT\Sigma x^u - NRT\Sigma x^b.$$

Since by definition the mol fraction is $\dfrac{n_1}{n_1 + n_2 + n_3 + \ldots\ldots}$, where n_1 and n_2 are very small compared with n_3, which we shall take to represent the number of mols of water in a liter of solution, therefore, approximately

$$\Sigma x^b = \frac{\Sigma c^b}{n^b_{H_2O}},$$

where Σc^b is the sum of the concentrations of all the substances dissolved in a liter of blood, and $n^b_{H_2O}$ is the number of mols of water in a liter of blood.

$$\text{Similarly } \Sigma x^u = \frac{\Sigma c^u}{n^u_{H_2O}}.$$

$$\because N^u_{H_2O} = n^u_{H_2O} \text{ and is approximately equal to } n^b_{H_2O}.$$

$$\therefore N^u_{H_2O}\,\Sigma x^b = \Sigma c^b; \text{ and}$$

$$N^u_{H_2O}\,\Sigma x^u = \Sigma c^u.$$

$\therefore -\Delta F = RT\Sigma c^u - RT\Sigma c^b$, which, allowing for the conventional difference in sign, is the term in the summarized form of Hill's equation for the transport of water.

The following is a more rigorous thermodynamic proof. Taking $\overline{F}$, f, N, $\overline{V}$, P to represent respectively partial molal free energy, fugacity, mol fraction, partial molal volume, and the applied external pressure, and assuming throughout perfect solutions, as is the case in the Barcroft equation—

$$\left(\frac{\partial ln\, f}{\partial\, \overline{F}}\right)_{P,\,T} = \frac{1}{\overline{RT}}; \tag{1}$$

$$\therefore \overline{F} = RT\, ln\, f + C;$$

$$\therefore \overline{F} - F' = RT\, ln\, \frac{f}{f'}. \tag{2}$$

$$\left(\frac{\partial \ln f}{\partial P}\right)_{T,N} = \frac{\bar{V}}{RT}; \tag{3}$$

$$\therefore RT\int_{f'}^{f} d\ln f = \int_{P}^{P'} \bar{V}\, dP; \quad \text{where } P'$$

is the pressure that must be applied to the solution in the state where fugacity is f' to change its fugacity to f;

$$\therefore RT \ln \frac{f}{f'} = \bar{V}(P' - P). \tag{4}$$

$$f = f^{\circ}N; \tag{5}$$

where f° is the fugacity of the substance, here water, in the pure state, and is a constant,

$$\therefore \ln f = \ln f^{\circ} + \ln N;$$

$$\therefore \int d\ln f = \int d\ln N + C';$$

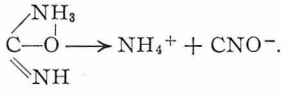
$$\therefore \ln \frac{f}{f'} = \ln \frac{N}{N'}; \tag{6}$$

$$\therefore \bar{F} - F' = RT \ln \frac{f}{f'} = RT \ln \frac{N}{N'} = \bar{V}(P' - P). \tag{7}$$

$$\bar{V}(P' - P) = RT(\Sigma C' - \Sigma C), \tag{8}$$

where ΣC is the total molar concentration of the dissolved substances.

** If the mechanism of the formation of ammonia from urea is taken to be that suggested by Werner,[14] the reaction may be written as follows:



Assuming the concentration of the cyanate ion to be of the order of magnitude of 0.0002 molar, and correcting the standard free energy of the cyanate ion for the change in temperature from 25 °C. to 37 °C., $-\Delta F$ for the reaction in the blood is +600 calories per mol of ammonium ion formed. Therefore, for 0.022 mols of ammonium ion the free energy change is +13 calories.

Since the formation of ammonia from urea appears to be a function only of the healthy kidney, it is open to question if the free energy change for this reaction, with its positive sign, is legitimately considered, as it is here, as work done upon the kidney.

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THE ENERGY COST OF THE EXCRETION OF URINE

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It has been shown that the work performed by the normal kidney in man in the excretion of urine is of the order of magnitude of 0.7 gram calories per cc. of urine; or 70 gram calories per gram of nitrogen excreted. It is instructive to compare the quantity of work performed by the kidney with the energy consumption of the kidney during the performance of this work.

The energy consumption of the kidney has been estimated by a number of experimenters and by different methods; and the values obtained are all of the same order of magnitude. The concordance of these values justifies an estimate of the efficiency of the kidney, i.e., of the ratio of the work performed, calculated from the constitution of the urine, to the energy used. The efficiency of the kidney, defined in this way, appears, even in health, to be about 1–2 per cent. So far as the authors are aware, there are no data inconsistent with this low figure. Rather the reverse, this association of the thermodynamic work with the observed energy consumption of the kidney permits the correlation of a large number of facts regarding the behavior of the kidney in health and disease.

The earliest measurements of oxygen consumption of the kidney are those of Barcroft and Brodie[15,16], and of Barcroft and Straub.[17] These show that the metabolism of the kidney is increased when it is performing work, i.e., when it is producing a urine in which the dissolved substances are present in greater concentrations than in the plasma. Barcroft and Straub found, for example, that the excretion of large quantities of chloride in the urine, in concentrations nearly the same as that in the plasma, was not accompanied by an increase in the consumption of oxygen; while the excretion of sulfate or of urea in the urine, in much greater concentration than in the plasma, was attended by large increases in the oxygen consumption. These results might have been predicted from thermodynamic theory. The association of increased oxygen consumption only with increased work, is strikingly exhibited in the behavior of the kidney damaged in various ways, as by caffeine, mercuric chloride, bleeding, or dilution of the blood or plasma with saline. Under these conditions the oxygen consumption of the kidney was not increased when diuresis was induced by sulfate or urea, and at the same time these substances were not concentrated in the urine as they were by the undamaged kidney, but were passed in nearly the same concentrations as in the plasma. In one case a larger quantity of sulfate was excreted at a low concentration,

without increased oxygen consumption, than in another where there was a large increase in the oxygen consumption, and a high concentration of the sulfate in the urine. The observations of Barcroft and Straub have been confirmed by Knowlton and Silverman[18] and by Bainbridge and Evans[19] (in one case on the isolated kidney). Winfield[20] found that the oxygen consumption of the kidney fell to $^1/_4$–$^1/_5$ of its normal value when the urine became isotonic after the intravenous injection of saline.

The dependence of the concentrating function of the kidney on concomitant oxidations is strikingly illustrated in the effects of cyanide on the kidney. Starling and Verney[21] observed, after the addition of KCN to the circulating blood in a heart-lung-kidney preparation, that the NaCl concentration in the urine rose to that in the plasma, i.e., the "concentration" of water fell to that of the serum, while the concentrations of urea and of sulfate fell markedly. On changing the circulating blood to one free from KCN, the chloride concentration fell again to a value not far from the original level, while the urea and sulfate concentrations rose a little, the sulfate more than the urea, toward their former levels. Similarly the power of the kidney to excrete and concentrate phenol red was also markedly reduced after poisoning with cyanide. The observations of Marshall and Crane[22] are essentially the same. These observers found that the urine contained approximately the same amount of water chloride, and bicarbonate after a period of temporary asphyxia induced by occlusion of the renal artery for 20 minutes, but much less urea, phosphate sulfate, creatinine and ammonia.

Three groups of workers claim to have been unable to confirm the observations described above. The first apparently contradictory results were those obtained by Tamura and Miwa.[23] It is not possible to interpret their results, or to compare them with those of Barcroft and Straub, first, because the urine analyses are not given. For example, if the excretion of urea is markedly reduced during a sulfate diuresis, it is possible that in spite of an increased excretion of sulfate, the total work performed by the kidney may be reduced; or a great change in the urinary chloride concentration may change the sign of the free energy term for the excretion of water. Tamura and Miwa found, in the rabbit, that diuresis induced by sulfate and caffeine, did not increase the oxygen consumption, while diuretin and salicylates provoked large increases. The employment of different animals by the two groups of experimenters, the rabbit in the experiments of Tamura and Miwa, the cat and dog in the experiments quoted here performed by Barcroft and Brodie, and Barcroft and Straub, is the second condition which prevents a comparison of the results obtained. The following typical observations show the distinct differences in function between the rabbit kidney on one hand, and the kidney of carnivora or omnivora on the other. After the injection of massive doses, the data of

Mayrs[45] show in the rabbit urea concentrated in the urine to only three times its concentration in the plasma, sulfate six times, phosphate four to five times and creatinine four times. On the other hand, in the urine of the dog, cat or man, urea may be concentrated sixty times, sulfate one hundred times, phosphate twenty times, and creatinine one to two hundred times the concentration in the blood. The experiments of Frandsen[24] show that the excretion of ingested sodium chloride or urea is very slow in the normal rabbit. Frandsen found in two experiments an average increased hourly excretion of only 0.016 and 0.017 gm. of nitrogen, respectively, in the four-hour period after the ingestion of 0.75 gm. of urea in addition to the usual ration. In another experiment in which a rabbit received 1.0 gm. of urea 3 hours after the routine meal, there was a uniform increased excretion of 0.052 gm. of nitrogen during the next four hours. It is possible, therefore, that in spite of the diuresis observed, the amount of the injected substances actually excreted in the experiments of Tamura and Miwa was not greatly increased. In view of the relatively slight degree to which substances are concentrated in the urine of the rabbit it seems probable, further, that the formation of urine by the rabbit's kidney entails considerably less work than is the case in carnivora or omnivora; and accordingly, the experimental error in the detection of differences is magnified.

Recently Hayman and Schmidt[25] described experiments in which "the injection of urea, caffeine, sodium sulfate, or certain drugs was not followed by any characteristic effect on apparent oxygen metabolism." Their protocols show that in four experiments out of six, caffeine was administered 10–20 minutes before sodium sulfate; here sodium sulfate did not elicit any increased consumption of oxygen, even though the flow of urine was increased. In one of the remaining experiments, in which the injection of sulfate was not preceded by caffeine, the protocol gives the largest value for oxygen consumption recorded in their experiments. The observations of Hayman and Schmidt, therefore, as described in their protocols, confirm the observations of Barcroft and Straub, "that caffeine virtually abolished the specific effect of sodium sulfate," of increasing the oxygen consumption of the kidney. Furthermore the experimental procedure of Hayman and Schmidt was rather drastic. In one experiment within a period of two hours after the completion of the operation, there were injected 150 cc. blood, 165 cc. saline, 80 mgm. caffeine, 20 cc. of 20 per cent sodium sulfate, pituitrin, adrenalin, and morphine. For this reason it is difficult to interpret the observations of Hayman and Schmidt. Hardly enough time, it seems, was allowed for the effect of one of the injected substances to manifest itself, before another was injected; much longer time was allowed in the experiments of Barcroft and Straub. In addition the data of the urine analyses are not given.

Fee[26] (1929), using a heart-lung-kidney preparation, obtained results which were taken to confirm the negative results reported by Hayman and Schmidt. Fee's statement is that "the actual excretion of large amounts of sulfate by the kidney is not attended with an increase in the renal oxygen consumption unless there is a concomitant increase in renal blood flow." It is questionable if the results of experiments on a heart-lung-kidney preparation may be compared with those obtained by Barcroft and Straub with the kidney *in situ*. Starling and Verney, and Fee and Hemingway[27] found that the urine from the heart-lung-kidney preparation is hypotonic, while the healthy mammalian kidney *in situ*, of course, excretes hypertonic urine. The production of either hypertonic or hypotonic urine is attended with an increased oxygen consumption; when the hypotonicity is reduced in the isolated kidney, by pituitrin for example, the oxygen consumption is also decreased. It is possible that the injection of sulfate under these conditions has the same effect as pituitrin, i.e., increases the concentration of salt in the hypotonic urine, rendering it more nearly isotonic, and thereby reduces the work and the oxygen consumption of the kidney. It is difficult to obtain a clear interpretation of the results reported by Fee, because the analyses of the urine, in detail are not given. The analysis of the work of secretion of hypotonic urine, below, shows that under the conditions of this experiment the result to be expected of an increased concentration of sulfate, from thermodynamic considerations, may be a reduced rather than an increased consumption of oxygen, due to a large decrease in the work of excretion of water. The results as given, therefore, may be taken to confirm, rather than to disagree with the hypothesis that increased work by the kidney is attended by a demonstrably increased consumption of oxygen. It is not certain, moreover, that the kidney in these experiments was in a "normal" state; the experiment reported in detail "was terminated by oedema" within less than an hour after the injection of the sulfate. More weight may therefore be placed on the observations on the effects of cyanide by Starling and Verney on the same kidney preparation as used by Fee.

Further confirmation of this hypothesis, that work of concentration entails increased metabolism in the kidney, is contained in the observation by Barcroft and Brodie of a large increase in renal oxygen consumption during phlorhizin glycosuria. In phlorhizin glycosuria the concentration of sugar in the urine may become very high, one hundred times that in the plasma; while the total nitrogen, urea, phosphates, and chlorides show no significant change. The observation of an increased oxygen consumption when sugar is excreted in highly concentrated solution is significant here, because the severely damaged kidney excretes approximately an isotonic urine, containing sugar in the same concentration as in the plasma; and its oxygen consumption is markedly reduced.[21]

The production of hypotonic urine, can be shown by the same method as that employed in the previous communication to entail work on the part of the kidney; and may, therefore, be expected to be attended by an increased consumption of oxygen. Only the excretion of a strictly isotonic urine, intermediate between the extremes of hyper- and hypotonicity, entails no work by the kidney. This can be illustrated by assuming an extreme case, a "urine" consisting only of a 1 per cent solution of urea; in which the mol fraction of water, therefore, is approximately 0.997. The free energy change for the transport of the water in a liter of such "urine" is now negative instead of positive, amounting to about −100 calories; $-\Delta F$ for urea is −430 calories. The total of −530 calories is of the same order of magnitude as the total free energy for the production of one liter of average hypertonic urine. This is due to the fact that a relatively large quantity of water is transported from a lower concentration in the plasma to a higher concentration in the urine. Further, the conditions under which such a urine is produced are usually those of diuresis so that the value of N for water especially is greatly increased, with the result that the work of the kidney per unit time is quite large. The tonicity of the urine depends mainly upon the sodium chloride and the urea; when the urea excretion is constant and its concentration in the urine is less than the total molecular concentration of the plasma, the concentration of sodium chloride as a rule determines whether or not work is done in the transport of water from the plasma to the urine. When the chloride concentration is low, so that the mol fraction of water in the urine is greater than in the plasma, the kidney performs work in transporting water from plasma to urine. On the other hand, when the concentration of chloride in the urine is greater than in the plasma, so that the mol fraction of water is less in the urine than in the plasma, water is "diluted" in passing from plasma to urine, and $-\Delta F$ becomes positive for the transport of water, i.e., the kidney "gains" work.

The physiological evidence bearing upon this aspect of renal function, the work of secretion of hypotonic urine, is not abundant; but as far as it goes it is completely confirmatory of the above thermodynamic predictions. The urine of the frog is always hypotonic to the plasma; and the concentration of nitrogen is practically the same as the non-protein nitrogen in the blood.[28] When the frog's kidney is poisoned with caffeine, or corrosive sublimate, the concentration of salts in the urine approximates that in Ringer's solution. After the administration of cyanide "the fluid issuing from the ureter comes to resemble the glomerular filtrate, sugar tends to appear in the urine, the chloride is less reduced than usual, potassium and calcium tend to approach the concentration of the perfusing fluid, and urea is no longer concentrated."[1] Narcotics, which Keilin[29]

recently has shown are selective poisons for one type of widely distributed oxidation mechanism, have the same effect as cyanide.

The isolated mammalian kidney, perfused by the heart-lung preparation as mentioned above, resembles a frog's kidney in that the urine secreted is hypotonic with respect to salts, although urea and sulfate are concentrated to a considerable extent. Starling and Verney found that this kidney after being poisoned with cyanide passed a urine which was isotonic with respect to the serum in every detail. Similarly, Fee and Hemingway working with the same heart-lung-kidney preparation found that the oxygen consumption increased as the urine contained less and less chloride. The significance of this observation is augmented by the fact that the oxygen consumption of the damaged or diseased kidney is less than normal.[17,39,40] When pitruitrin was added to the perfusing fluid in the experiments of Fee and Hemingway[27] the chloride concentration rose, and at the same time the oxygen consumption became less.

The evidence, therefore, seems clear that the performance of what may be called thermodynamic work by the kidney involves an expenditure of energy which is derived from oxidations within the renal cells. It is instructive, therefore, to compare the work performed by the kidney with the energy used, i.e., to determine the "efficiency" of the kidney.

The discussion of the relationship between the work performed and the energy used by the kidney is facilitated by the following explicit definition of the terms employed and the assumptions made.*

W = Minimum work necessary to produce 1000 cc. *urine* from infinite quantity of *blood* of character entering kidney.

W_1 = Minimum work necessary to produce 1000 cc. *urine* + venous blood from the actual quantity of *blood*.

$W_1 \approx W$. The justification for this assumption has been discussed in the previous communication.

Q = Heat of combustion of fuel burned while 1000 cc. urine are excreted.

Q_1 = Maximum work available from this combustion. In a previous communication[46] it was shown that the free energy of combustion of fat and of glucose in the body was, in each case, nearly equal to the heat of combustion. No data are available regarding the free energy of combustion of the proteins. But is probable that no error is involved in assuming that $Q_1 \approx Q$.

Q_m = Heat of combustion of fuel used in maintaining the kidney alive for the length of time necessary to produce 1000 cc. of urine but not connected with the production of urine.

Q_{iso} = Additional heat of combustion required to produce 1000 cc. urine of such character that $W_1 \approx W = 0$. For practical

purposes this may be taken to represent the additional heat produced by the kidney in the formation of 1000 cc. isotonic urine.

The efficiency, E, is a conventional term, which may be defined in a number of ways. Three possibilities are: $E = \frac{W}{Q}$; $E' = \frac{\Delta W}{\Delta Q}$, where Δ is the change accompanying any change in the blood and urine; and $E'' = \frac{W}{Q - (Q_m + Q_{iso})}$. It is obvious that only E' may be expected to show any degree of constancy with variation in the amount of work performed, i.e., with large variations of ΔW.

The values for the rate of metabolism of the kidney determined by a number of different methods are remarkably concordant: Barcroft and Brodie, and Barcroft and Straub observed rates of oxygen consumption per gram of "resting" kidney per minute, ranging from 0.008 to 0.075 cc. in the dog, from 0.03 to 0.12 cc. in the cat, and from 0.046 to 0.13 cc. in the rabbit. Fee and Hemingway obtained similar values for the isolated kidney (presumably of the dog) of 0.03 to 0.2 cc. per gram per minute. The term "resting" value, is to some extent a misnomer here, because the kidney even at rest is producing urine; and it is possible that the bulk of the urine excreted in a day is elaborated by the "resting" kidney i.e., not in a state of diuresis. For the purpose of estimating the order of magnitude of renal efficiency in man, we have taken as an average value for the rate of oxygen consumption of the kidney over 24 hours the relatively low figure of 0.03 cc. per gram per minute; and an average weight for the human kidney of 150 grams.[30] On this basis the renal oxygen consumption in man in 24 hours is of the order of magnitude of 13 liters. Assuming an R. Q. of 0.80, the caloric equivalent of this quantity of oxygen is 62 kg. calories.[31] Since the total work performed by the kidney as set out in table 2 of the previous communication is 704 gram calories, the "efficiency" of the kidney, i.e., W/Q, is approximately 1 per cent. Barcroft and Brodie calculated from their experiments ratios of work performed to energy consumed during periods of diuresis, i.e., $\Delta W/\Delta Q$, and obtained values for urea excretion of 1.75 and 0.14 per cent; it should be mentioned that, because these values for renal efficiency are so low, Barcroft and Brodie hesitated to accept them as correct. Nevertheless, as pointed out before, the total of 704 gram calories represents probably all but a negligible quantity of the work performed by the kidney, and the values employed for oxygen consumption are low rather than high. During diuresis Barcroft and Brodie found that the oxygen consumption of the kidney may be relatively 5–10 times greater than the oxygen consumption of the body as a whole.

Janssen and Rein[32] recently attempted to determine the metabolism of the kidney by measuring the difference in temperature between the

arterial and venous blood. They obtained values for the metabolism of the kidney of a similar order of magnitude as those above.

In view of these low calculated values of renal efficiency, it was desirable to obtain, if possible, some estimate of renal efficiency in man. A number of such experiments are on record in the literature; but the results given are so discordant that it was decided to carry out the experiments again.

The experiments described below were performed on young men. These came into the hospital immediately after the evening meal, between 7 and 8 P.M., on the night before the experiment. From then until the end of the experiment at 11–12 A.M. of the next day, they remained in bed and received no food or drink, except the urea solution or water as indicated in table 1. The subjects voided during the night, the following morning on waking, and then during the experiment at the times indicated. The metabolism was determined by measuring the oxygen consumption over a period of five or six minutes by means of the McKesson "Metabolor." In order to accustom the subjects to the procedure of determining the metabolic rate, a number of determinations were made, after the subject was in bed the night before. The practice during the experiment was to obtain, at intervals of one-half hour, three consecutive values for the metabolism before administering the urea solution or water; and then for four or six half-hourly intervals afterward. Urine was collected immediately after each estimation of the metabolism. The subjects experienced no discomfort, and only brief transient sensations of any sort after the ingestion of the urea. In order to minimize the effects of water *per se*, the urea was given in very concentrated solution. Since, according to Dubois[33] it is not possible to maintain a human subject in a steady basal state for more than a few hours, the experiments as a rule began at 8:00 A.M., and all values for the metabolism taken after 12:00 noon have not been used. The urea solution was warmed to 37°C. before it was given to the subject to drink. Chlorides were determined by the Volhard-Arnold method,[4] urea, with the use of urease.

The results obtained are summarized in table 1. They show an unequivocal rise in metabolism accompanying increased excretion of urea. The calculated values given in the last column of table 1 indicate the low order of magnitude of $\Delta W/\Delta Q$. This ratio is a better measure of the working efficiency of the kidney than either of the other two efficiency ratios. The fact that the value of this ratio is of the same order of magnitude as W/Q and $W/\Delta Q$ obtained from estimations of the oxygen consumption of the isolated kidney, indicates that the sum of Q_m and Q_{iso} probably is small compared with the additional energy expended in the performance of work by the kidney. This is substantiated by the findings of Winfield, and by the oxygen consumption of the poisoned as compared with the unpoisoned kidney.

The data are not sufficiently comprehensive, however, to throw any light on the problem of the variation of the value of the ratio $\Delta W/\Delta Q$ with the quantity of work performed. It seems probable that this quotient is not a constant, but that it will vary with the amount of work performed and with the condition of the kidney, and of the animal as a whole. On the other hand, the value of two per cent for $\Delta W/\Delta Q$ seems to be a maximal rather than a minimal value.

It is obvious that the explanation for the low efficiency of renal function is to be sought in the mechanism of the production of urine. It is possible that the reabsorption of large quantities of water, according to Rehberg several hundred times the volume of the urine, with the corresponding amounts of sodium chloride and sugar, may be the process which incurs a large expenditure of energy by the kidney, and for which there is nothing to show in the urine. Nevertheless, the figures computed for the work of reabsorption of even 200 liters of water, with the corresponding amount of electrolytes and sugar, during the formation of 1000 cc. of urine, calculated as in table 1 in the previous communication, amount to only a few per cent of the energy used. It would appear that the work of such a hypothetical reabsorption is performed with a low efficiency similar to that obtained for the final formation of urine.

A low mechanical efficiency appears to be characteristic of glandular function. For example, the data quoted in Bayliss[47] give 1.5 mg. sugar, or six gram calories, as the amount of energy consumed in the secretion of one cc. of saliva, and the calculated work for the formation of saliva from blood is much less than that for urine.

These are not the first observations of an increase in metabolism following the ingestion of urea. Zuntz,[34] quoting from experiments carried out by Steck, records that in dogs there was a rise in metabolism of 0.49–0.53 calories per gm. of nitrogen ingested and in man 0.94–1.37 calories. The rise in metabolism occurred only during the first two hours, and in man only one-sixth of the ingested urea was excreted. If the values obtained by Steck in men are accordingly multiplied by six the results are the same as those recorded in table 1. Recently Lublin[35] measured the change in metabolism in men following the ingestion of large quantities of urea solution, and observed a rise in the metabolic rate in one case of 8 per cent, and in another of 12 per cent of the basal rate; the high point coinciding with the time of greatest nitrogen excretion, and not of greatest urine volume.

A rise in metabolism following the ingestion of urea has been observed also by Lusk[36] and by Grafe,[37] on whose authority, nevertheless, the conclusion has been accepted generally that the excretion of urea is not accompanied by any demonstrable increase in metabolism. This conclusion has passed unchallenged in spite of the work on the oxygen consumption

TABLE 1

THE INCREASE IN METABOLISM IN MEN DURING THE EXCRETION OF UREA

SUBJECT			TIME			EXCRETION OF CHLORIDE			EXCRETION OF UREA			
NAME	BODY SURFACE	YEARS, AGE	OF INGESTION OF UREA SOLUTION OR OF WATER	OF DETERMINATION OF METABOLISM	OF URINE PRODUCTION	VOLUME OF URINE	CONCENTRATION AS NaCl	AMOUNT PER HOUR AS NaCl	CONCENTRATION	AMOUNT PER HOUR	METABOLISM, KG. CAL. PER SQUARE METER PER HOUR	INCREASE IN METABOLISM FOR ADDITIONAL NITROGEN EXCRETION, KG. CAL./GM.
	SQUARE METERS					CC.	GM./100 CC.	GM./HR.	GM./100 CC.	GM./HR.	KG. CAL.	KG. CAL.
R. L. B.	1.77	27	at 9.38,		8.02– 8.32	25	1.08	0.54				
			37.5 cc.	8.55– 9.00	8.32– 9.02	26	1.06	0.55	0.80	0.42	36.3	
			of 40	9.25– 9.30	9.02– 9.34	30	0.98	0.55	0.78	0.44	36.4	
			per cent	9.55–10.00	9.34–10.02	35	0.82	0.83	0.96	0.97	39.8	
			Urea	10.25–10.30	10.02–10.33	64					39.0	10
			solution	10.55–11.00	10.33–11.05	41	0.94	0.69	1.19	0.87	38.0	
				11.35–11.40	11.05–11.42	43					41.3	
B. B.	1.58	26	at 9.36,	8.00– 8.05							37.5	
			37.5 cc.	8.25– 8.30	8.06– 8.31	30	1.26	0.91	0.77	0.55	36.7	
			of 40	8.55– 9.00	8.31– 9.03	27	1.24	0.63	0.65	0.33	..	
			per cent	9.25– 9.31	9.03– 9.32	28	1.24	0.72	0.60	0.35	37.3	
			Urea	9.55–10.00	9.32–10.02	31	1.08	0.74	0.81	0.55	38.6	11
			solution	10.25–10.31	10.02–10.31	36					40.0	
				10.55–11.00							40.0	
				11.05–11.12	10.31–11.05	39	0.90	0.68	1.10	0.83	39.7	
				11.25–11.30	11.05–11.32	38					41.3	
G. H. P.	1.90	19	at 7.50,		6.43– 7.49	46	1.39	0.58	0.96	0.40		
			20 gm.	7.10– 7.15							41.8	
			Urea	7.40– 7.45							42.4	
			in 35 cc.	8.10– 8.15	7.49– 8.23	39	1.18	0.81	0.73	0.50	44.0	
			water	8.45– 8.50	8.23– 8.52	40	1.00	0.83	1.08	0.90	44.0	7
				9.00– 9.05							44.1	
				9.20– 9.25	8.52– 9.32	67	0.94	0.94	1.22	1.22	43.6	

R. C. H.	1.77	21 at 8.59,	8.15– 8.22	7.53– 8.54	34.5	1.08	0.37	0.83	0.28	39.0	
		37.5 cc.	8.45– 8.52							38.5	
		of 40	9.15– 9.20	8.54– 9.27	22.5	1.02	0.42	0.87	0.36	39.0	
		per cent	9.45– 9.55	9.27– 9.56	28.0	0.79	0.46	1.16	0.68	41.5	
		Urea	10.15–10.21	9.56–10.22	25.5					40.3	6
		solution	10.45–10.52	10.22–10.53	28.0	0.74	0.36	1.38	0.67	38.9	
			11.15–11.22	10.53–11.24	22.5					40.3	
O. P. J.	1.99	21 at 9.47,	8.00– 8.06	6.30– 9.26	164	0.96	0.54	0.66	0.37	38.3	
		50 cc.	8.50– 8.55							38.3	
		of 40	9.45– 9.50	9.26– 9.47	20	0.96	0.55	0.66	0.38	38.3	
		per cent	10.00–10.06	9.47–10.06	122	0.70	2.70	0.48	1.85	47.1	
		Urea	10.30–10.36	10.06–10.38	132	0.56	1.39	0.47	1.16	45.8	
		solution	11.00–11.06	10.38–11.07	85	0.68	1.20	0.76	1.34	43.4	11
			11.30–11.36	11.07–11.39	54	0.89	0.90	1.13	1.14	38.2	
			12.00–12.15	11.39–12.45	73	1.03	0.68	1.36	0.90	39.7	
			12.37–12.44							40.0	
O. P. J.	1.99	21 at 8.06,	7.05– 7.12	7.18– 8.06	65	1.46	1.19	0.59	0.48	38.2	
		35 cc.	7.30– 7.35							37.2	
		water	7.55– 8.00							38.4	
			8.25– 8.30	8.06– 8.35	59	0.93	1.14	0.40	0.49	39.2	
			8.55– 9.00	8.35– 9.05	73	0.73	1.07	0.31	0.45	38.7	
			9.25– 9.30	9.05– 9.35	57	0.98	1.12	0.42	0.48	38.1	
R. D. M.	1.84	18 at 8.57,	8.15– 8.20							40.8	
		30 cc.	8.45– 8.50	8.23– 8.54	15	1.39	0.40	0.67	0.19	40.3	
		water	9.15– 9.20	8.54– 9.24	18	1.43	0.51	0.68	0.24	39.7	
			9.45– 9.51	9.24– 9.53	20	1.56	0.63	0.62	0.25	38.3	
			10.15–10.20	9.53–10.23	20					38.7	
J.	1.66	18 at 9.02,	7.55– 8.00	7.34– 8.04	23					42.7	
		35 cc.	8.20– 8.25	8.04– 8.31	16.5	1.34	0.53	1.00	0.39	44.8	
		water	8.50– 8.55	8.31– 9.00	17					43.6	
			9.30– 9.35	9.00– 9.39	20					43.1	
			9.55–10.00	9.39–10.04	10	1.38	0.39	1.17	0.33	38.4	
			10.20–10.25	10.04–10.31	13					43.2	

of the isolated kidney, and in spite of the fact that the conclusions both of Lusk and of Grafe rest in each case upon only two experiments, of which the results do not agree with each other.

The experiments of Lusk were carried out on dogs. In one, 17 grams of urea dissolved in 150 cc. of water were given at 12:00 noon. At 12:45 the dog vomited. Nevertheless, a large quantity of urea must have been absorbed, because the nitrogen elimination was increased from 0.154 to 0.424 gm. per hour after the administration of the urea. The basal metabolism of this dog varied from 14.7 to 18.3 calories; the general average given is 16.2 calories. In the interval designated 1–2 (which presumably was the second hour after administration of the urea) the metabolism rose to 20.42 calories per hour, and in the third hour was 18.8 calories per hour. The results of this experiment were rejected by Lusk because the animal vomited during the first hour after administration of the urea. Benedict and Emmes[38] have shown that intestinal movements have a negligible effect on the metabolic rate; and the rise in metabolism observed here occurred over a period of two and a quarter hours after the vomiting. It is important that an increase in metabolism in this experiment was obtained both by direct and indirect calorimetry. In the second experiment a dog was given 12 gm. urea in 150 cc. water at noon, and the recorded observations on the metabolic rate were made during the third and fourth hours after the administration of the urea. No increase in metabolism was observed in this interval. On this one result is based the conclusion that the excretion of urea is not accompanied by an increase in metabolism. Our observations on human beings, and those of Zuntz, indicate that the greatest excretion of urea generally occurs in the first two hours after the ingestion of urea; after this time the excretion of urea may be little more than in the pre-administration period. It was precisely in this last period that Lusk failed to observe any increase in metabolism; the large increase observed in the first experiment, on the other hand, was in the second hour. These results are not, therefore, antithetical to those obtained by Steck, Lublin, or those given in table 1.

In Grafe's experiments, both carried out on the same human subject, 22 gm. urea were administered in 750 cc. bouillon. In one case there was a decline of 2 per cent over a period of six hours; and in the other, over a period of $3^1/_2$ hours there was a rise in metabolism of 8 per cent. Apart from the discordance of the results, these experiments of Grafe are open to the criticism, based upon the observations of Dubois, that it is not possible to maintain a human subject in a steady basal state for more than three or four hours, and results obtained after such a period cannot be considered as reliable.

The question of the energy cost of the elimination of nitrogen is obviously an important one in the elucidation of the specific dynamic action

of protein. The experiments of Lusk and of Grafe were carried out ostensibly for this purpose, and their conclusion seems justified, in spite of the discrepancies in their data, that the whole of the specific dynamic action of protein cannot be due to the work of the kidney in the elimination of nitrogen. On the other hand, their conclusion that the excretion of nitrogen by the kidney incurs no expenditure of energy is not warranted by their own data. Moreover, it is improbable from the observations on the oxygen consumption of the isolated kidney, and it is contradicted by the observations of Steck, of Lublin, and those summarized in table 1. The significance of the work of the kidney in the specific dynamic action of protein is discussed elsewhere.

The conception of the normal kidney as a chemical machine which possesses a great working capacity, but which performs its work with a very low efficiency readily lends itself to the explanation of the effects of disease of the kidney on the composition of the urine and blood.

In general there may be said to be two possible effects of disease on the kidney: one, that the diseased kidney tends to perform its normal quantity of work, but with a lower efficiency; the other, that the effect of disease is to reduce the capacity of the kidney for work. It may be expected *a priori*, since "loss of function" is one of the classical signs of disease, that the second rather than the first of the two alternative reactions to injury is the case in the kidney. The observations of Barcroft and Straub mentioned above, to the effect that caffeine, mercuric chloride, and anaemia reduce the oxygen consumption of the kidney, and at the same time change the composition of the urine so that it approximates the plasma, support this surmise. Its correctness is sustained further by the findings of Tribe, Hopkins and Barcroft,[39] and of Tribe, Harvey and Barcroft,[40] that the oxygen consumption of the kidney damaged by uranium or by diphtheria toxin was always lower, and was frequently less than half the average value obtained for the normal. The effects of cyanide and of narcotics also support this view.

The human kidney when it is diseased fails in a like manner. This is the basis of the various concentration tests for kidney function. The changes in the excretion of the inorganic constituents by the failing kidney have been emphasized less, but are more instructive here than the excretion of the organic constituents. In general the findings are that the inorganic constituents whose relative concentrations are greatest in normal urine, as compared with the blood, suffer the greatest reductions in the rates of excretion when the kidney is damaged. It is this and not the amount in the blood which appears to be the determining factor. For instance, Denis and Hobson's summary[41] of the results of their analysis of a fairly large number of cases of nephritis is: "The relative rarity of abnormally high figures for the sodium and chlorides in the blood would

therefore appear to show that these two ions are excreted by the kidney with great ease, whereas the retention of inorganic phosphates may amount to 400 per cent, and of sulfate to as much as 3000 per cent of the average normal value." They found that though the normal molar concentration of phosphates in the plasma is six times that of sulfate, in nephritis, the sulfate ion in the plasma became not only relatively, but, in some extreme cases, absolutely more concentrated than the phosphates. The normal ratios of the concentrations of the three anions, chloride, phosphate and sulfate in the urine and in the blood, is least for the chloride and greatest for the sulfate.**

It is unfortunate that the concentrations of the inorganic constituents of the urine in disease are not often given. In the experiments of Hendrix and Bodansky[42] on uranium nephritis in dogs, the data given show that the concentration of phosphates in the urine in all the animals that died fell to considerably below 0.1 per cent P_2O_5, and in the two animals that survived the concentrations, though lowered somewhat, returned to 0.1 per cent or more. Frandsen found in rabbits with uranium nephritis no such lowering of the concentration of chloride in the urine. Rather the reverse, the ability to retain chloride seemed to be impaired. This may have been due to the acidosis which is engendered in uranium poisoning, resulting in an increased excretion of chloride in compensation for the failure to excrete sulfate and phosphate and the inability to form ammonia.

To test more thoroughly this view of kidney function, it is planned to obtain more data on the concomitant variations in concentration of the constituents of the blood and the urine in health and disease.

It is possible that the efficiency of the kidney varies somewhat for each substance excreted, and further that the relationship between work performed and oxygen consumed is not linear. The available data does not seem to throw any light on this interesting problem which may be related to the problem of thresholds in renal excretion.

Summary.—1. The work of the kidney in the excretion of urine is analyzed by means of the second law of thermodynamics. It is shown that the production of either hypertonic or hypotonic urine entails work on the part of the kidney. The excretion of a urine which is the same as the plasma in all details incurs no work by the kidney.

2. The energy consumed by the kidney in man in the production of urine was found to be 6–11 kg. calories per gram of nitrogen excreted.

3. It is shown that the normal healthy kidney considered as a chemical machine possesses a great capacity for work; but performs its work with an "efficiency" probably not greater than 1–2 per cent.

4. The effect of disease is to reduce markedly the capacity of the kidney for work.

This conception of renal function is supported by the evidence in the literature of the constitution of the urine in health and disease; of the effects of drugs, metallic poisons, anaemia, cyanide and narcotics on the oxygen consumption of the kidney *in situ* and isolated, and on the constitution of the urine; and by the observations of the authors, confirming earlier observations, of the increase in metabolism in man during increased excretion of urea.

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* These definitions were suggested by Professor R. C. Tolman.

** From the observations of Davies, Haldane and Peskett[43] and of Harvard and Reay[44] it seems that the concentration of chloride in the urine in health varies between 3 times that in the plasma to only a trace. Harvard and Reay, by the ingestion of large quantities of water, reduced the concentration of phosphate in the urine to as low as $^1/_5$ that in the blood. This apparently is the lowest concentration of phosphate in the urine of healthy man on record. Even under these conditions of extreme dilution of the urine, urea and sulfate were found to be more concentrated in the urine than in the blood. Ignoring the question of threshold substances and the mechanism of renal excretion it may be stated that in the urine, chlorides normally are concentrated less and may be diluted more than phosphates. The relative concentration of sulfate is always greater than either of these.

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ON THE SPECIFIC DYNAMIC ACTION OF PROTEIN

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In a previous communication[1] evidence was presented that the normal excretion of nitrogen by the mammalian kidney is attended by a relatively great expenditure of energy, which in man amounts to 6–11 kg. calories per gram of nitrogen. The work of renal excretion is, therefore, an important element in the specific dynamic action of protein.

Current views on the specific dynamic action of protein, whether derived from Rubner or Voit, look to one process only as responsible for the increased metabolism following the ingestion of protein. In this Zuntz,[2] who first ascribed the specific dynamic action of protein to the work of digestion and absorption, and later to the work of excretion of nitrogen, held in this respect essentially a similar position.

The purpose of the present communication is to show that the course of the specific dynamic action of protein parallels the course of nitrogen excretion; and in the well-nourished animal is the result of at least two processes, of which one is the work imposed upon the kidney, and the other is what may be called the "specific dynamic action proper," due to the metabolism other than excretion of the nitrogen, and of the carbon.

In the majority of studies of the specific dynamic action of protein, the recorded observations, upon which the stated conclusions are based, cover a period in which a fraction only of the ingested nitrogen was accounted for, and the magnitudes of the increases in metabolism obtained have been referred to the total weight, or amount of nitrogen ingested. If the specific dynamic effects of two substances, glycine and glutamic acid for example, are compared, one of which is absorbed and metabolized quickly, the other very slowly, and the data upon which the comparison is based are derived from observations during the first few hours, only, and if the comparison is referred also to the amount ingested, not metabolized, it is obvious that the substance metabolized more quickly, all other factors being the same, will appear to exert the larger specific dynamic effect. Nevertheless, differences in rate of metabolism often have

not been taken into account. When this factor is not omitted from consideration, the explanation of some hitherto anomalous phenomena becomes evident, viz.: the neutralization of the specific dynamic effect of glycine or alanine when administered with protein; the apparently low specific dynamic effect of glutamic acid; and the reduction of the specific dynamic action of protein in cases of endocrine disturbance.

A correlation between the amount of additional urea and ammonia excreted and the extent of the specific dynamic action has been controverted by Lusk and his pupils. Accordingly we have set out in figures 1, 2 and 3, and tables 1, 2 and 3, the data obtained on the dog, by Rapport,[3,8] Weiss and Rapport,[4] and Rapport and Beard[5] which, we believe,

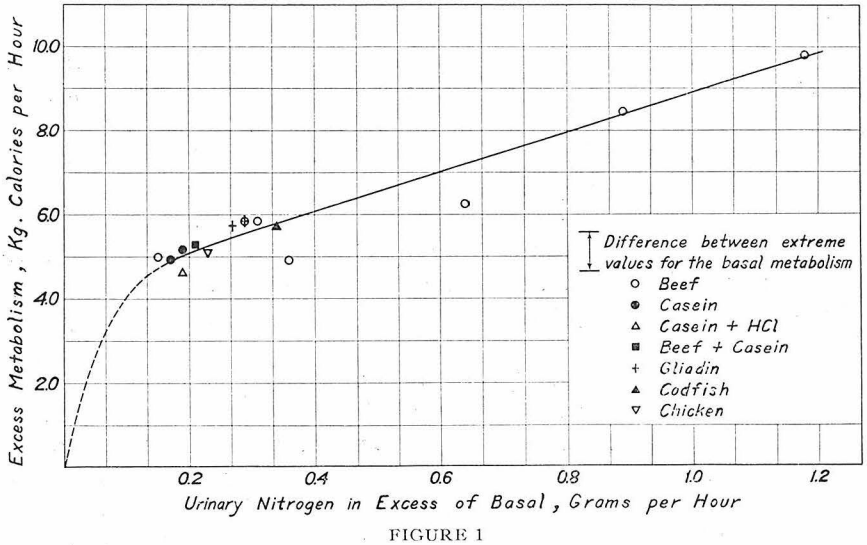

FIGURE 1

clearly demonstrate this correlation, although Rapport, and Weiss and Rapport stated that there is no clearly demonstrable relation between the nitrogen eliminated and the increase in metabolism.

The data have been arranged in three groups: one containing the results obtained with proteins only; another, the data on glycine, alanine and gelatin; and one containing the results with glycine, gelatin, and total and fractionated hydrolysates of gelatin. The later observations of Rapport and Beard[5] show the necessity for not including gelatin with the other proteins if just comparisons are to be made. Since a number of amino acids exert a greater specific dynamic effect, per gram of nitrogen excreted, than glycine or alanine, it is to be expected that the effect of most proteins in general will be greater than that of these two simple amino acids.

Gelatin, on the other hand, contains 26 per cent glycine, 9 per cent

alanine, and 25 per cent of the slowly absorbed dicarboxylic amino acids and leucine. It is low in phenylalanine and is lacking in tyrosine; both of these amino acids have higher specific dynamic effects per mol metabolized than glycine or alanine. It belongs, therefore, in respect of its specific dynamic action in the first few hours, more properly with glycine and alanine than with the proteins. The results given in tables 1 and 2 and figures 1 and 2 were obtained on the same dog; those in table 3, and figure 3, were obtained some time later on a different dog.

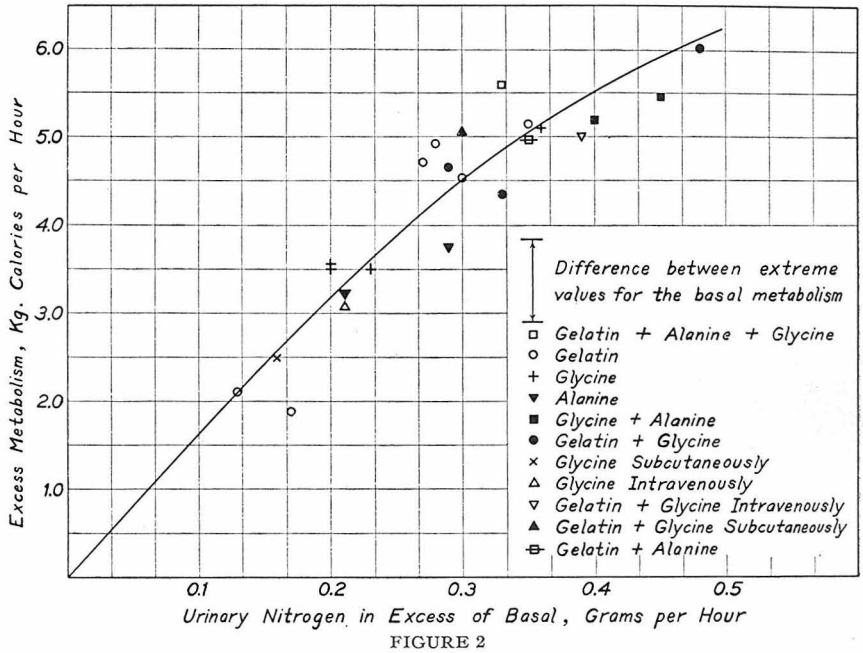

FIGURE 2

Figures 1, 2 and 3, and tables 1, 2 and 3 show the correlation between nitrogen excretion and excess metabolism. In view of the difficulty of the experimental technique, and of the variability of animals, the concordances of the results obtained by Rapport, Weiss and Rapport, and Rapport and Beard, is remarkably good. Figure 1, which is the most extended of the three curves, shows that the relationship between excess metabolism and excess nitrogen excreted is not defined by a straight line passing through the origin. The curve seems, rather, to begin from the origin as a steep curve, and to pass later into a straight line of distinctly lesser slope. This changing slope expresses in part the neutralization postulated by Weiss and Rapport[4] of the specific dynamic action of amino acids when these were added to relatively large quantities of protein. Weiss and Rapport compared the specific dynamic effects over the first

four hours of mixtures of gelatin and casein, and an amino acid, glycine or alanine, with the specific dynamic effects, over the same period of time of the same quantity of each of the substances when administered separately. The results observed were that the increase in metabolism was distinctly less than the linear sum of the effects of the protein and of the amino acid when administered separately. Weiss and Rapport concluded from these results that the specific dynamic effects of the amino acids were neutralized when administered with protein.

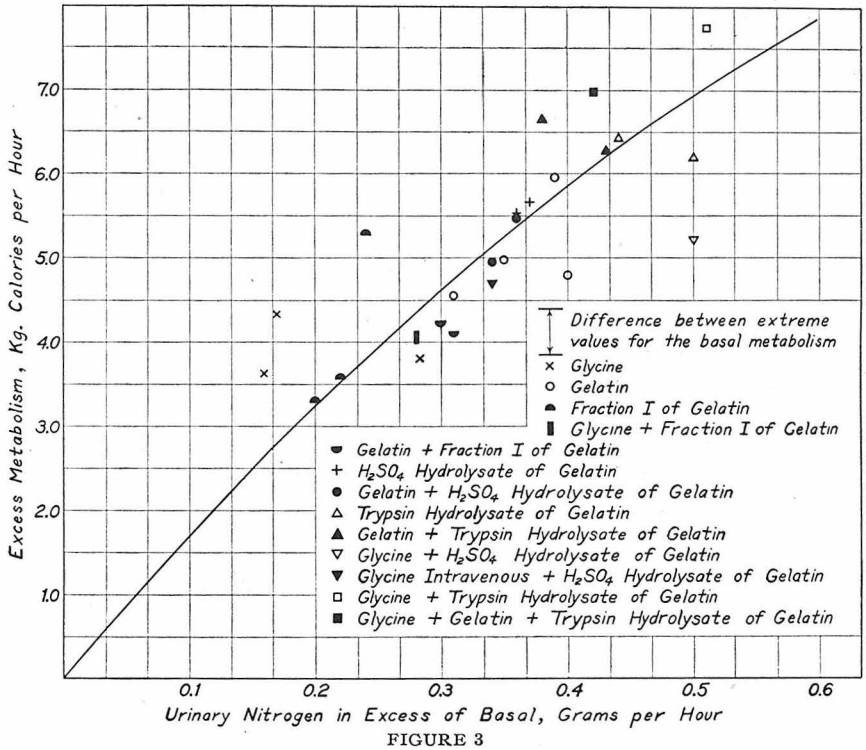

FIGURE 3

Figures 1, 2, and 3 show that an apparent neutralization of the specific dynamic action was obtained during the first four hours, whenever the quantity of nitrogen metabolized in this period was so large that it fell on the portion of the curve which does not pass through the origin when extended. This begins to be indicated when rather large quantities of amino acids were given, for which no neutralization phenomenon was postulated, and is clearly shown also when increasing quantities of beef were administered.

The data of Weiss and Rapport show an increase in metabolism, after the ingestion of 1.87 gm. of glycine, of 21.4 per cent; when an additional

1.87 gm. of nitrogen in the form of glycine were given, the increase over the basal was 30.9 per cent; and when 1.55 gm. of nitrogen in the form of gelatin were given with 1.87 gm. of glycine nitrogen, the increase in metabolism over the basal was 26.5 per cent. The increase in metabolism for this amount of gelatin alone was 12 per cent. Therefore, when the bolus ingested was increased by additional glycine or gelatin, the effect obtained was less than a linear sum of the separate specific dynamic effects; and the effect of the glycine-gelatin mixture was substantially the same as when the same amount of nitrogen was given in the form of glycine. Here, therefore, glycine exerted a neutralizing effect upon itself, similar to that

TABLE 1—(See Figure 1)

THE RELATION BETWEEN THE EXCESS NITROGEN EXCRETED AND THE SPECIFIC DYNAMIC ACTION OF PROTEIN (3) (4)

SUBSTANCES INGESTED	NITROGEN EXCRETION IN EXCESS OF BASAL PER HOUR	METABOLISM INCREASE OVER BASAL, PER HOUR	METABOLISM INCREASE OVER BASAL
	GM.	CALORIES	PER CENT
Beef	0.15	4.99	30.3
	0.29	5.86	35.6
	0.31	5.86	35.6
	0.37	4.92	29.8
	0.64	6.27	38.1
	0.89	8.43	51.2
	1.18	9.81	59.5
Casein	0.17	4.93	29.9
	0.19	5.18	31.4
Casein + HCl	0.19	4.65	28.2
Beef + Casein	0.21	5.29	32.1
Gliadin	0.27	5.76	35.0
	0.29	5.88	35.7
Codfish	0.34	5.73	34.8
Chicken	0.23	5.11	31.0

postulated when larger amounts of gelatin were given with glycine. When the amounts of nitrogen ingested and metabolized in the first four hours were larger, the neutralization was more marked.

A second factor in the phenomenon of neutralization is that the relationship between nitrogen ingested and nitrogen metabolized, i.e., excreted, is similarly not represented by a straight line, but by a curve of diminishing slope. For instance, the excess nitrogen excreted during the four-hour period after the ingestion of 10 gm. of glycine was 0.21 gm. per hour; after 20 gm. of glycine, 0.36 gm.; after 10 gm. of gelatin the excess urinary nitrogen was 0.15 gm.; after 40 gm. of gelatin, 32 gm.

When the exciting substances are similar, though not necessarily identical, the same increase in metabolism was obtained when the amount of nitrogen metabolized was the same. This is shown in the following

experiments of Weiss and Rapport. When 6 gm. of nitrogen were administered in the form of gelatin, there was an increase in the urinary nitrogen of 0.30 gm., and a 29.3 per cent increase in metabolism. In another experiment, when 6 gm. of nitrogen in the form of gelatin and 1.87 gm. of nitrogen as glycine were given together, the increased urinary nitrogen was 0.29 gm. and the increase in metabolism, 28.2 per cent. The apparent neutralization in this case of the specific dynamic effect of glycine is obviously due to some slowing of the rate of metabolism of

TABLE 2—(See Figure 2)

THE RELATION BETWEEN THE EXCESS NITROGEN EXCRETED AND THE SPECIFIC DYNAMIC ACTION OF ALANINE, GLYCINE, AND GELATIN (3) (4)

SUBSTANCES INGESTED	NITROGEN EXCRETION IN EXCESS OF BASAL PER HOUR	METABOLISM INCREASE OVER BASAL PER HOUR	METABOLISM INCREASE OVER BASAL
	GM.	CALORIES	PER CENT
Gelatin + Glycine + Alanine	0.33	5.58	33.9
Gelatin	0.13	2.09	12.7
	0.17	1.88	11.4
	0.27	4.71	28.6
	0.28	4.93	29.9
	0.30	4.53	27.5
	0.35	5.14	31.2
Glycine	0.20	3.51	21.4
	0.20	3.57	21.7
	0.23	3.50	21.2
	0.36	5.09	30.9
Alanine	0.21	3.21	19.5
	0.29	3.76	22.8
Glycine + Alanine	0.40	5.19	31.5
	0.45	5.46	33.1
Gelatin + Glycine	0.29	4.64	28.2
	0.33	4.36	26.5
	0.48	6.03	36.5
Glycine (subcutaneous)	0.16	2.48	15.1
Glycine (intravenous)	0.21	3.07	18.6
Gelatin + Glycine (intravenous)	0.39	5.01	30.4
Gelatin + Glycine (subcutaneous)	0.30	5.07	30.8
Gelatin + Alanine	0.35	4.98	30.2

the mixture, and not to a neutralization of the specific dynamic effect of glycine, *per se*. When large amounts of protein or amino acids or mixtures of the two are ingested there is a superposition of two non-linear effects; one is that the relationship between nitrogen ingested and nitrogen metabolized is not a linear function; the other, that the relationship between nitrogen metabolized and the increase in energy metabolism, over the first four hours, is also less than linear.

The term neutralization for this phenomenon implies that the specific

dynamic effects of these amino acids would be absent even when all the nitrogen ingested was accounted for in the urine. In the experiment in which glycine was administered with casein, during the first four-hour period of the experiment, only 14 per cent of the ingested nitrogen was excreted; in the case of casein and alanine, 12 per cent; gelatin and alanine,

TABLE 3—(See Figure 3)

THE RELATION BETWEEN THE EXCESS NITROGEN EXCRETED AND THE SPECIFIC DYNAMIC ACTION OF GLYCINE, GELATIN AND GELATIN HYDROLYSATES (5) (8)

SUBSTANCES INGESTED	NITROGEN EXCRETION IN EXCESS OF BASAL PER HOUR GM.	METABOLISM INCREASE OVER BASAL PER HOUR CALORIES	METABOLISM INCREASE OVER BASAL PER CENT
Glycine	0.16	3.62	29.2
	0.17	4.33	34.9
	0.28	3.79	27.7
Gelatin	0.31	4.56	36.8
	0.35	4.98	40.2
	0.39	5.96	43.6
	0.40	4.81	35.2
Fraction I of Gelatin	0.20	3.31	26.7
	0.22	3.60	29.0
	0.24	5.31	42.8
Glycine + Fraction I of Gelatin	0.28	4.06	32.7
Gelatin + Fraction I of Gelatin	0.30	4.21	34.0
	0.31	4.08	32.9
H_2SO_4 Hydrolysate of Gelatin	0.36	5.53	40.5
	0.37	5.67	41.6
Gelatin + H_2SO_4 Hydrolysate of Gelatin	0.34	4.96	36.3
	0.36	5.48	40.1
Trypsin Hydrolysate of Gelatin	0.44	6.42	47.0
	0.50	6.20	45.4
Gelatin + Trypsin Hydrolysate of Gelatin	0.38	6.64	48.6
	0.43	6.27	45.9
Glycine + H_2SO_4 Hydrolysate of Gelatin	0.50	5.22	38.2
Glycine (intravenous) + H_2SO_4 Hydrolysate of Gelatin	0.34	4.71	34.5
Glycine + Trypsin Hydrolysate of Gelatin	0.51	7.75	56.7
Glycine + Gelatin + Trypsin Hydrolysate of Gelatin	0.42	6.98	51.1

16 per cent; gelatin and glycine, 10 gm. of each, 39 per cent; and of a mixture of gelatin, glycine and alanine, 13 per cent. The effects observed obviously relate only to rates of absorption and metabolism; the data do not permit of any conclusions regarding absolute effects.

If the observations during the first four hours are confined to quantities

of nitrogen not more than 0.3 or 0.4 gm. per hour greater than the basal, the curvature is such that there is not a wide departure from a linear relationship between excess metabolism and nitrogen excreted in excess of the basal. For instance, the results in tables 2 and 3 depicted in figures 2 and 3 yield a relatively constant value of approximately 16 kg. calories per gram of nitrogen excreted in excess of the basal. The results of Wilhelmj and Bollman[6] confirm this conclusion. These authors concluded from their observations on the effects on metabolism of injected glycine, alanine and phenylalanine that "The relationship between the specific dynamic action may be expressed in at least six different ways, and reasons are given for believing that the most suitable manner of expressing this relationship is as calories and extra heat produced by each millimol of the amino acid deaminized." In a later paper Wilhelmj and Mann[7] present further experimental evidence in support of this correlation.

The results plotted in figures 1, 2 and 3 are the individual observations, when these are given; the alignment of points is distinctly better if averages only are used; the widest divergence from the mean position of the points is usually little greater than the difference between the extreme values for the basal metabolism.

It is interesting that the slope of the straight line portion of figure 1 yields a value for the increase in metabolism per gram of excess urinary nitrogen of 5 kg. calories, which is of the same order of magnitude as the energy consumption of the kidney observed in man, per gram of urinary nitrogen. This coincidence suggests the following possible interpretation of the results given above. The specific dynamic action of protein or amino acids, at any given time, is an expression of the resultant of two rates, of which one is the excretion of nitrogen, and the other, the metabolism and probably also the formation of the deaminized residues. The first steep part of figure 1, which corresponds to nearly the whole of figures 2 and 3, represents these combined effects. The flattening of the curve is due to the attainment of a maximum rate of deamination and metabolism of the deaminized fragments. Once this maximum intensity of metabolism is attained, this factor no longer contributes to the slope of the curve. The continuing straight line portion expresses the metabolism of the kidney. The persistence of the slope unchanged indicates that the rate of urea excretion can be increased by the ingestion of increasing amounts of protein, long after the maximum rate of metabolism of the deaminized residues has been attained; and that the excretion of nitrogen lags behind deamination.

The hypothesis advanced here is based chiefly on observations made over the first four hours following the ingestion of proteins or amino acids. The fact that even in this short period processes with different rates are discernible indicates that if a sufficiently large number of observations

over a longer period were available, further complications would be observed, due, for example, to the completion of some processes and the continuance of others.

Further evidence of the association of the specific dynamic action of protein with nitrogen metabolism is contained in the observations of Wishart[9] on the variations in the basal metabolism with changes in the nitrogen excretion, resulting from the variations in the daily protein intake. During the period of observation the daily nitrogen excretion varied in one subject from 5.3 gm. to 23.2 gm., and in another from 3.8 gm. to 16.5 gm. The results obtained could be expressed by the equations $x = 28.456 + 0.449y$ in the one case, and in the other by $x = 28.269 + 0.448y$; where x is the basal metabolism in calories per square meter per hour, and y the urinary total nitrogen in g. per day. The correlation coefficients were, $+0.84 \pm 0.036$, 0.70 ± 0.05. These results show a linear relationship between nitrogen excretion and increase in metabolism. The coefficient of y is of the same order of magnitude as that for the first part of figure 1, or for figures 2 and 3, which includes the effects of the metabolism of nitrogen and the deaminized fragments. This is in accord with the results obtained on dogs because even the largest amount of nitrogen metabolized here corresponds to points on the lower portions of figure 1, or figures 2 and 3. For instance, 23.2 gm. of nitrogen per day would correspond in a 20-kg. dog to about 0.3 gm. per hour. As pointed out above, in this range of excess nitrogen excreted the specific dynamic effects observed on the dog are defined approximately by a linear function of the amount of nitrogen excreted in excess of the basal.

The observations in cases of endocrine disturbance, also show the relationship between nitrogen metabolism and the specific dynamic action of protein. Liebeschütz-Plaut[10] noted a reduction in the specific dynamic action of protein in cases of *dystrophia adiposogenitalis*. This was confirmed by Liebesny[11] on men, and by Foster and Smith[12] on hypophysectomized rats. Gaebler[13] in Lusk's laboratory compared the specific dynamic effects of meat, in the dog, before and after hypophysectomy; and found before the operation an average increase in excretion of nitrogen per hour, after a meat meal, of 0.16 gm., and an increase in metabolism of 4.4 calories per hour; after the hypophysectomy the average increase in nitrogen excretion (over the normal basal value, which is probably too high in this case) was 0.09 gm. per hour, and the increase in metabolism 3.2 calories per hour. Gaebler's conclusion was that "discrepancies in the amount of protein catabolized probably account for the difference in the extent to which the heat production was increased in the two animals." Lusk's summary of the findings in this condition,[24] shows that the general rate of metabolism is lowered after hypophysectomy or pituitary deficiency. Foster and Smith found that the metabolism in these animals

may be restored to normal by anterior pituitary homotransplants, or by daily injections of thyroid extract.

Taken in conjunction with Gaebler's experiments, the findings in case of hypophyseal deficiency indicate no real reduction in the specific dynamic action, but rather a slowing of the rate of metabolism. Per gram of nitrogen metabolized, the specific dynamic action is the same as in the normal. Lauter[14] showed that in the case of obese individuals the specific dynamic action of protein is less, during the first two hours, than in normal individuals, but is the same as in the normal over a period of six hours.

The correctness of the association of the extent of the specific dynamic action with the nitrogen metabolism, i.e., with the increase in urinary nitrogen, is consistent with the time relationships. Lusk reproduces in "The Science of Nutrition" a curve from the data obtained by Aub and Dubois on human beings, which shows the concomitant increases in urinary nitrogen, urinary sulfate, and in the rate of metabolism. Another similar curve is reproduced from the data of Williams, Riche and Lusk. It may be added that, following the ingestion of some proteins, there is an increase also in urinary phosphate. The excretion of sulfate and of phosphate imposes work upon the kidney, in much the same manner, though to a lesser extent in the case of protein, as in the excretion of urea; and therefore, also figures in the specific dynamic action of protein.

The observations of Wilhelmj, Bollmann and Mann[15] that injected amino acids do not exert any specific dynamic effect in hepatectomized dogs, definitely eliminates the view that the specific dynamic action of amino acids is due to a general stimulation of the cells of the body by the amino acids *per se*. The earlier observations of Rapport and Katz[16] of an increase in metabolism following the addition of glycine to the circulating blood of an isolated hind leg preparation is open to the criticism that the concentration of glycine in the circulating blood was relatively enormous, about one per cent. The difficulty of interpreting this observation of Rapport and Katz is further increased by the recently reported observations of Needham[17] that neither glycine nor alanine increased the respiration of a minced muscle preparation, though a marked increase was observed with glutamic acid.

The observations of Grafe[18] that ammonium chloride and acetamide exert a large specific dynamic effect, with the failure of the amino acids to provoke an increase in metabolism in the hepatectomized animal, and the general correlation of the increase in metabolism with the increase in urinary nitrogen, indicate that either the whole or part of the composite process of urea formation and excretion are responsible for a large fraction of the specific dynamic action of protein.

The view that the specific dynamic action of protein is due to the

formation of glucose from the deaminized fragments chiefly of glycine and alanine is again put forward by Chambers and Lusk in a recent paper.[22] This proposal meets with the difficulty of accounting for the important observation of Rapport[3] that the specific dynamic action of a variety of proteins is nearly the same, and was lowest among the proteins tried for gelatin, which contains the most glycine and alanine, and was highest for gliadin which contains over 40 per cent glutamic acid and very small amounts of glycine and alanine.

In this paper, Chambers and Lusk reassert the contention that no specific dynamic action is exerted by glutamic acid. Grafe, Rapport and Beard, and Terroine and his collaborators[19,20] have reported that glutamic acid exerts a considerable specific dynamic action. Johnston and Lewis[23] observed that glutamic acid, administered to rabbits *per os*, was absorbed very slowly compared to alanine and glycine. On the other hand, injected intravenously glutamic acid was deaminized as quickly as alanine or glycine. Rapport and Beard, from their own observations, suggested that the apparently low specific dynamic effect of glutamic acid is due to the slowness of its absorption. The data in the protocols of Chambers and Lusk suggest that this discrepancy may be resolved by considering the amount of glutamic acid deaminized during the period of observation. In one experiment, the data of Chambers and Lusk show that in the third hour after the ingestion of 20 gm. glutamic acid by a normal dog, with an increase in urinary nitrogen of only 0.03 gm. per hour, the metabolic rate was 1.8 per cent lower than the average basal value. In three other experiments, when the same amount of glutamic acid was given with 10 gm. lard (the specific dynamic action of which is only 3.5 per cent and which alone induced no increased excretion of nitrogen), during the second and third hours there was an increase in the hourly urinary nitrogen of 0.1 gm., accompanied by an increase in the metabolic rate of 15 per cent. The difference between the rate of absorption and metabolism of glutamic acid as compared with other amino acids is illustrated in the effect on the same dog of 300 gm. lean meat. The hourly urinary nitrogen was increased eight-fold, and the metabolic rate 52.3 per cent.

After these observations were made the animal was phlorhizinized and glutamic acid again administered; from the results obtained, Chambers and Lusk concluded that there was no specific dynamic action of this amino acid in the phlorhizinized animal. The values of the basal metabolism, 26.5 calories per hour, with which the glutamic acid figures were compared, is an average of the following values obtained on the days of the experiments: 23.4, 29.4 and 26.7. It is not possible from the urinary nitrogen figures, which were also irregular here, to obtain a reliable estimate of the amount of glutamic acid deaminized, except that it was small.

The strongest evidence upon which the theory suggested by Lusk is

based, that the specific dynamic action is due to the conversion of the deaminized residues of the amino acids into glucose, is derived from the observations that the specific dynamic action of glycine and alanine is the same in the phlorhizinized dog, as in the normal animal. In the interpretation of this result, Lusk assumes that the phlorhizinized dog is unable to burn glucose, and that the glycine and alanine ingested are converted quantitatively into glucose. In Lusk's summary[24] of the effects of phlorhizin, the observations of Richardson and Shorr are quoted that the excised tissues of phlorhizinized rats burn glucose as freely as the normal; and further, that a variable quantity of ingested glucose is oxidized by the phlorhizinized animal. As Terroine and Bonnet[25] have pointed out in their discussion of this problem, even in the normal animal glucose probably is not oxidized as such, but in the form of some degradation product such as lactic acid. An alternative and equally tenable interpretation, therefore, of the observations on the phlorhizinized dog is that the deaminized fragments of the amino acids are oxidized by the phlorhizinized dog in place of degradation products derived from glucose, with the result that an amount of glucose is spared from combustion, and is thereby excreted in the urine, which is approximately equivalent to the ingested amino acids. This would tend to lessen the total metabolic rate in the phlorhizinized as compared with a normal animal. On the other hand this is compensated for by the increase in the renal work, and hence the total metabolism, by the excretion of an increased amount of glucose.[1] The magnitude of this increase will be of the same order as the energy consumed by the kidney in the excretion of the nitrogen.

In their paper, Chambers and Lusk return to a discussion of the energy changes in the hypothetical conversion of glycine, alanine and glutamic acid into glucose and urea. The following equations are written:

for alanine: $2C_3H_7NO_2 + H_2O + CO_2 \longrightarrow C_6H_{12}O_6 + CON_2H_4$;

for glycine: $3C_2H_5NO_2 + {}^3/_2CO_2 + {}^3/_2H_2O \longrightarrow C_6H_{12}O_6 + {}^3/_2O_2 + {}^3/_2CON_2H_4$;

and for glutamic acid:

$$2C_5H_9NO_4 + 3O_2 \longrightarrow C_6H_{12}O_6 + H_2O + CON_2H_4 + 3CO_2.$$

The calculations of Adams[26] are referred to, who, with the aid of the Third Law of Thermodynamics, estimated that approximately 60,000 calories would be required to effect the change of state indicated above for alanine. The specific heat data at present available are too scanty to permit of reliable estimates of the entropies of alanine and of glycine. Accordingly, calculations of the free energy change incurred in the conversion of glycine to glucose and urea, although the standard free energies of glucose and urea are now known, are at present uncertain. An estimate

of the direction and magnitude of the energy change can be obtained by means of the First Law of Thermodynamics. From the data in the International Critical Tables, the following values have been calculated for the molar heats of formation: water, −68,000 calories; carbon dioxide, −94,000 calories; glucose (solid), −303,000 calories; urea (solid) −79,000 calories; glycine (solid), −126,000 calories; alanine (solid) −134,000 calories; and glutamic acid (solid) −237,000 calories. The value of $-\Delta H$ in the reaction postulated above for alanine is −24,000 calories per mol; for glycine, −66,000 calories; and for glutamic acid +129,000 calories. The values for the alanine and glycine reactions are nearly the same as those given by Adams.

Assuming for a moment the reality of these reactions, it may be concluded from the above values of $-\Delta H$, that the conversion of glycine and alanine into glucose and urea are endothermic processes requiring the driving force of another exothermic process. And if it is assumed that the relationship is not perfectly reversible between the endothermic and exothermic processes, the specific dynamic action will be the difference between the amount of energy produced and the amount used in the endothermic reaction. There are no data for estimating the degree of reversibility of such a reaction in the body. If a 25 per cent efficiency is assumed, then the specific dynamic action per mol of glycine would be approximately 200,000 calories. This is the order of magnitude of the specific dynamic action of glycine. But on the same basis, the specific dynamic action of alanine should be one-third that of glycine. The experiments of Lusk, and of Wilhelmj and Bollman, and of Wilhelmj and Mann are in accord that the specific dynamic action of alanine per mol of amino acid deaminized is practically the same as that of glycine. $-\Delta H$ for glutamic acid is positive; the reaction is, therefore, exothermic. The value of $-\Delta H$, moreover, is quite large, equivalent to the heat of combustion of $^1/_5$ mol glucose. There is no obvious justification for assuming, as do Lusk, and Aubel,[30] that because this reaction is itself exothermic, that it is not responsible for any specific dynamic action. Rather the reverse, it seems that this is the one reaction of the three which would certainly exert a specific dynamic effect. Even in the cases postulated for glycine and alanine the specific dynamic action is due to exothermic processes. As pointed out above, gliadin, which contains over 40 per cent of glutamic acid, exerts a large specific dynamic effect. In this connection reference may be made to the observation of Terroine and Bonnet that the whole of the heat of combustion of ingested alcohol can be recovered as heat evolved by the organism.[27]

Furthermore, the values of $-\Delta H$ calculated for the above reactions include the energy changes for deamination and urea formation. There is no *a priori* reason for considering that the latter reactions and the excre-

tion of urea are negligible in the energy balance. The failure of sodium lactate and sodium glycollate to exert a large specific dynamic action[21] might be taken to indicate that the formation of glucose from the deaminized residues of the amino acids is a negligible element. In the case of glycine and alanine, also, the mechanism postulated for the formation of urea (the dehydration of ammonium carbamate) has been severely criticized on chemical grounds by Werner.[28] If urea normally appears by way of cyanic acid from the concomitant oxidation of the amino acids and glucose,[29] the metabolism of glycine and alanine probably are exothermic instead of endothermic processes. This criticism of the view of Lusk and Aubel[30] regarding the cause of the specific dynamic action of protein has many points in common with the views of Terroine and Bonnet.

The values obtained for the specific dynamic action of the amino acids show that the work of the kidney in eliminating nitrogen cannot be responsible for the whole of the increase in metabolism. It was shown that the excretion of nitrogen in man incurs an expenditure of 6–11 kg. calories per gram of nitrogen.[1] In experiments in which most of the injected amino acid was accounted for, Wilhelmj and Bollman obtained in dogs the following values, expressed as kg. calories per gram of amino acid deaminized: for glycine, 35, 29 and 21; for alanine, 22, 28 and 20; and for phenylalanine, 56 and 44. In the later experiments of Wilhelmj and Mann, the values obtained for glycine were 16 and 18; and for alanine, 16 and 19. The few observations on the effect of tyrosine suggest that the specific dynamic action of this amino acid, per gram of nitrogen metabolized, is greater than that of phenylalanine. The data of Rapport, Weiss and Rapport, and of Rapport and Beard give somewhat low values for glycine, in accord with the lower values for glycine and alanine obtained by Wilhelmj and Mann. The values for glutamic acid obtained by Rapport and Beard are nearly as great as those of phenylalanine; but in these experiments only a small fraction of the ingested amino acid was accounted for.

If the specific dynamic action of protein were due only to the energy released in the deamination, formation, and excretion of urea, the specific dynamic action would be practically the same per mol for all the monoamino acids. Terroine and Bonnet found in the frog that, per gram of nitrogen injected, the specific dynamic action of glycine, alanine, aspartic acid, glutamic acid, valine, leucine, cystine and lysine was approximately 8.4 calories; for tyrosine and phenylalanine, 9.2 calories; and for tryptophane and histidine, 10 calories.[31] It is interesting and possibly significant, in view of these low values, that in the urine of the frog the concentration of nitrogen is practically the same as the non-protein nitrogen of the blood,[32] so that no additional work is imposed upon the kidney by the ingestion of proteins or amino acids, and hence in the frog the

specific dynamic action of amino acids might be predicted to be 6–11 kg. calories per gram of nitrogen less than in the mammal. Terroine and Bonnet[25] observed similar values in the rabbit. But the values obtained by the French observers on the rabbit may be too low if they are taken to signify the specific dynamic action per gram of nitrogen excreted in the dog or man. For instance, 16 gm. of glycocoll were injected into a rabbit weighing 2.7 kilos. The observations were made over a period of six hours, and it is improbable that in this time such a relatively very large quantity of the nitrogen was completely metabolized and excreted. On the other hand the energy consumption during renal excretion in the rabbit is probably less than in the dog or man.[1] The evidence favors acceptance of the higher values, in the case of the higher animals, obtained by Lusk, Weiss and Rapport and by Wilhelmj and Mann.

It is possible that the difference in the specific dynamic effects of different monoamino acids may be due to the metabolism of the varying number of carbon atoms which they contain. This would account for the greater specific dynamic effect, per mol metabolized, of phenylalanine, tyrosine and glutamic acid. But the data available are too incomplete for analysis.

An estimate of the value of the specific dynamic action of protein, as distinguished from that of an amino acid, can be derived from the observations of Wishart on man, referred to above. The equations of Wishart expressed as calories per hour, instead of calories per square meter per hour, are:

$$x = 52.65 + 0.83y,$$

and

$$x = 48.0 + 0.76y.$$

Taking y as gm. of nitrogen excreted per hour, the equations become:

$$x = 52.65 + 20y,$$

and

$$x = 48.0 + 18y.$$

The coefficients of y, 20 and 18, represent the increase in metabolism per hour per gram of nitrogen excreted. It is confirmatory of the explanation of the specific dynamic action of protein proposed here, that these values for the increase in metabolism induced by protein, calculated from changes in the basal rate with changes in the nitrogen excretion, are of the same order of magnitude as those calculated from the directly observed specific dynamic action of protein. These figures carry additional weight because the errors due to long periods of observation or to incomplete metabolism of the ingested protein could not occur here.

The results obtained indicate that the additional work imposed upon the kidney is responsible for 25–60 per cent of the specific dynamic action of protein and amino acids. The remainder is due to the "specific dynamic action proper" resulting from the metabolism of the nitrogen and of the

carbon. In this estimate it is assumed that the energy consumed by the kidney in the excretion of nitrogen is approximately the same in the dog as in man, in spite of the lower values of the dog obtained by Steck.[2] No details are given in the quotation of Zuntz regarding Steck's experiments; while the oxygen consumption values for the dog's kidney during urea diuresis indicate that the work of the kidney in the excretion of nitrogen is approximately the same in the dog as in man.[1]

The values obtained for the increased energy consumption by the kidney in man, per gram of nitrogen excreted indicate that increased renal function may be responsible for as much as 40 per cent of the specific dynamic action of protein observed in man by Aub and Dubois.

Summary.—Taken in conjunction with the data on the efficiency of the process of renal excretion, analyses of the data bearing upon the relation of the increase in metabolism following the ingestion of protein or amino acids reveals:

(1) A close correlation between the specific dynamic action of proteins or of amino acids and the increase, over the basal level of excretion, in the urinary nitrogen;

(2) That neither the direct experimental evidence nor the considerations of the energy relations support the view that the specific dynamic action of protein is necessarily due to the conversion of the deaminized fractions into glucose.

(3) The values of the specific dynamic action of amino acids and of proteins indicate that 25–60 per cent is due to the work imposed upon the kidney. The remainder of the specific dynamic action of protein or amino acids is due to the metabolism of the nitrogen and the carbon, though it is not possible, from the evidence, to estimate the proportion for which each is responsible.

(4) This hypothesis, that the specific dynamic action of protein is due to at least two distinct processes, which do not proceed at similar rates, provides an explanation for some hitherto anomalous phenomena in the specific dynamic action of protein.

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GENERAL SUMMARY

A study has been made of the application of the laws of thermodynamics to physiological processes. In the consideration of fuels for muscular work, the theoretical maximum amount of work obtainable from the oxidation, under physiological conditions, of glucose and of tripalmitin has been computed from the standard free energies and heats of combustion of these compounds. Such calculations show that the ratio of the theoretical maximum work obtainable to the total energy change, is little different for glucose and tripalmitin. It is also shown that the conversion of tripalmitin into glucose involves a release of energy approximately equivalent to 20 per cent of the theoretical maximum work available from the oxidation of tripalmitin. Since the observed difference in the efficiency of fat as a fuel for muscular work, as compared with carbohydrate, is much less than 20 per cent, it follows either that fat is burned as such in the provision of energy for muscular work, or that the energy released in the hypothetical conversion of fat into carbohydrate is not dissipated as heat, but is used for work; and the efficiency of utilization of this energy is little different from that in the consumption of carbohydrate. The process of the conversion of glucose into tripalmitin probably exerts a considerable "specific dynamic action" on metabolism.

In the study of renal function, the work of the kidney in the excretion of urine is analyzed by means of the second law of thermodynamics. The work performed by the normal kidney in man in the excretion of urine is of the order of magnitude of 0.7 gm. calories per cc. of urine; or 70 gm. calories per gram of nitrogen excreted. On the other hand, the energy consumed by the kidney in man, in the production of urine, was found to be 6-11 kg. calories per gram of nitorgen excreted. The production of either hypertonic or hypotonic urine entails work on the part of the kidney; the excretion of a urine which is the same as the plasma in all details incurs no work by the kidney. The normal, healthy kidney considered as a machine possesses a great capacity for work, but performs its work with an "efficiency" probably not greater than 1 to 2 per cent. In disease, the capacity fo the kidney for work is markedly reduced.

This conception of renal function permits a correlation of observations on the constitution of amphibian and mammalian urine, in situ and isolated, in health and disease; of the effects of drugs, metallic poisons, anemia, cyanide and narcotics on the oxygen consumption of the kidney; and of

the observations on the differences in renal oxygen consumption during the excretion of increased amounts of chlorides, sulfates and urea. There is a close correlation between the specific dynamic action of proteins or amino acids, and the increase, over the basal level, in the urinary nitrogen. The values of the specific dynamic action of amino acids and of proteins indicate that 25 to 60 per cent is due to the work imposed upon the kidney; the remainder is due to the metabolism of the constituent nitrogen and carbon, though it is not possible, at present, to estimate the proportion for which each is responsible. This hypothesis accounts for hitherto anomalous phenomena in the specific dynamic action of protein.