Growth and Development

With Special Reference to Domestic Animals

LXII. The Specific Dynamic Action of Nutrients with Special Reference to the Effects of Vitamins and Hormones

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ABSTRACT

This bulletin reports critical discussions of the literature and presents original data with rationalizing discussions on the involved mechanisms of the specific dynamic action (SDA) of some nutrients with special reference to the effects of vitamins, enzymes, and hormones. Pyruvic and lactic acids (and glucose which is oxidized to pyruvic acid) lower the SDA of glutamic acid and tyrosine because they function as amino acid acceptors in transamination. Pyridoxine also lowers the SDA of glutamic acid and tyrosine—as well as of glucose—because it forms a prosthetic group in the co-enzyme for transaminase, which facilitates transamination. Vitamin E lowers the SDA of glucose because it facilitates synthesis of creatine from glycine. Thiamine increases the SDA of glucose by facilitating its conversion to fat. Thiouracil retards and lowers the SDA of amino acids except of tyrosine because of its already low SDA on account of its structural similarity to thyrosine. Vitamin A probably also lowers the SDA because it tends to depress thyroid activity.

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INTRODUCTION

The resting heat production in an animal in a thermoneutral environment is the sum of two components: (1) basal metabolism, which is the irreducible energy cost of maintenance during complete rest; and (2) specific dynamic action (hereafter abbreviated to SDA) which is an unavoidable energy waste, incident to food utilization. This extra heat production is analogous to $\Delta S$ in thermodynamics; it is a tax on entropy, an unavoidable free-energy loss associated with the nutritional energy conversions. This heat increment of feeding has attracted much attention since the time of Lavoisier and an enormous literature with many theories has grown up around it. Quoting Graham Lusk, the most distinguished later-day investigator of this phenomenon, "The hypotheses which have been presented on specific dynamic action transcend one's power to coordinate them."

Most of the investigators on SDA reported in the literature have been concerned with the major body fuels: carbohydrates, fats, and proteins. This dissertation, on the other hand, is concerned not so much with the SDA of the body fuels, as with the influence of the biocatalysts—the vitamins and hormones—on SDA.

The following comments summarize what is generally accepted about SDA.

The term SDA was coined by Rubner (1902) to designate the increased heat production associated with the overall nutritional process. Synonyms of SDA are: calorogenic effect of food; heat increment of feeding; thermogenic effect of food; thermal energy of food.

The Voit theory of SDA is that the intermediary nutrient fragments, such as the amino acids, raise the metabolic rate of the body cells just, as for example, dinitrophenol or thyroxine does.

The Zuntz theory of SDA is that it represents the work of digestion, assimilation, and excretion of the waste products.

The Rubner theory of SDA is that it represents the waste heat from many intermediate and side reactions and oxidations incident to the nutritive process.
An interesting aspect of this phenomenon is that different amino acids differ in respect to the heat production associated with their utilization. This suggested the comparison of the SDA of the amino acids in vivo with the theoretical heat production associated with deamination as computed from thermodynamic data. Carbohydrates and fats also undergo various intermediate reactions, with associated heat liberation, which it should be possible to estimate from thermodynamic data, when such data become available. Furthermore, physical changes such as solution, osmotic pressure, and so on involve heat liberation, all of which could, theoretically, be computed when sufficient data become available.

There is a methodologic error in SDA studies which needs emphasis in connection with the following reviews of the literature. Different conditions, exogenous and endogenous, influence profoundly the rates of digestion, assimilation, catabolism, and excretion of nutrients. Since the rates of the associated calorigenic effects are dependent on the rates of these processes, therefore the time curve of the calorigenic effects vary with the given conditions. Moreover, each nutrient is digested, assimilated, catabolized and its wastes excreted at different rates. If, then, the metabolic test is continued for an arbitrary time interval (usually four hours), it is obvious that the apparent SDA will vary with the rate of catabolism, etc., which contribute to the SDA.

For instance, digestion, assimilation, catabolism and excretion are very much slower in a hypophysectomized animal than in a normal animal; it may require eight hours instead of the usual four hours to obtain the full SDA in the hypophysectomized animal. Unless, therefore, the metabolism measurements are continued for eight hours in the hypophysectomized animal, one may conclude that the SDA in the operated animal is less. Unless observation on the heat production associated with the administration of a given nutrient is continued until the heat production rate returns to the starting base level, there is danger of underestimating the total SDA associated with a given feeding. With this background it should be possible to discuss critically the following reviews of the literature and the original data on the influence of vitamins, hormones and related substances on SDA.

The definition of SDA as used in this dissertation is that given it by Lusk. The term was originated by Rubner who called it “die spezifisch-dynamische Wirkung” which literally means both “specific dynamic action” and “specific dynamic effect.” This latter term has been used by the Forbes school for which it is especially suitable because this school is measuring SDE from the maintenance plane as reference base. As fasting metabolism was used in this work as the reference base, as has been used before by Lusk and Voit, the classical name SDA used by Lusk has been retained in this work.
Lavoisier and Laplace (1780) described the heat increment after eating. This was confirmed by Bidder and Schmidt (1852). The first and easiest explanation that necessarily came to the mind of the scientists was that this heat increment was due to the work of digestion. It is known that there is increased movement of the gastrointestinal tracts after eating. Speck (1874) put this theory to experimental test. He found that oxygen consumption and carbon dioxide output increased after eating. Mering and Zuntz (1877) found that if sodium salts of lactic acid, saturated fatty acids, glycerin or sugar are injected intravenously, there was no increase in oxygen consumption above the base level, but these substances, when fed into the stomach, increased the heat production; however, they observed that peptone does increase oxygen consumption, even when injected intravenously. But Zuntz and Mering (1883) found that pure peptone would not increase oxygen consumption if injected intravenously. So they concluded that the SDA is due entirely to the work of digestion. Loewy (1888) fed Glauber's salt (sodium sulphate) which is a purgative and increases the peristaltic movement of the intestine. This caused an increase in oxygen consumption and carbon dioxide output. A study of Loewy's protocols does not give information as to the muscular movement of the subjects during the test. Magnus-Levy (1894) also believed SDA to be due to the work of digestion. Benedict and Emmes (1912) observed and others confirmed, that after feeding agar-agar, which formed a large bulk, there was practically no increase in energy expenditure.

That the simple explanation of Verdauungarbeit does not hold is also seen by differences in SDA of different nutrients. Protein has the highest SDA, and carbohydrate, the lowest; differences in the gastrointestinal movement, cannot explain this differential action of protein and carbohydrate. If the Zuntz theory were viewed very broadly and Verdauungarbeit extended to include the physicochemical work of secretion, absorption, and excretion, this theory would be reasonable. There is more secretion of enzymes in protein digestion than in any other nutrient, but the amount of digestive secretion from glands is probably not large enough to explain the high SDA of protein. It is also known that the formation of enzymes in the gland cells does not require much energy. The so called Entwicklungsarbeit of enzymes, at first thought, seems to be very logical and has attracted the attention of a large number of workers.

But a critical examination will show that Entwicklungsarbeit is almost a misnomer, for it can be shown by thermodynamic calculation that the organizational entropy of the formation of enzymes is insignificant. To me,
it appears, that these enzyme proteins as they are synthesized in the facet of the existing enzyme patterns fit more or less automatically and therefore the expenditure of energy herein is insignificant. That SDA is not due to the increased energy expenditure of food absorption will be evident if one remembers that monosaccharides which require the active process of phosphorylation and dephosphorylation in the intestinal mucous membrane have a very low SDA. Amino acids, on the other hand, are not phosphorylated, with the probable exception of a few, yet they have a very high SDA. It is therefore clear that absorption-energy cannot explain SDA.

Much stress has been laid, however, on the excretion-energy. As carbohydrates and fats are completely oxidized and excreted as water and carbon dioxide, they involve practically no expenditure of extra energy for excretion and these have a low SDA; proteins, on the other hand, are not completely oxidized and are excreted as urea, uric acid, creatinine and sometimes creatine and a host of other products. So the next logical step was to invoke the SDA of foodstuff on an excretion-energy basis.

Borsook and coworkers (1931) defended the theory that the kidney work in excretion of the end products of protein metabolism occurs at a considerable energy cost, and this constitutes a large part of the SDA. The differential concentration of nitrogenous wastes on the urinary side of the renal tubules, as contrasted to their concentration in blood, involves work which can be calculated from the second law of thermodynamics. Under normal conditions, the human kidney expends about 0.7 gram-calories per cc. of urine secretion. By feeding urea to human subjects, they reported that the human kidney expended 6 to 11 Cal per gram of nitrogen excreted. They concluded that there is a close correlation between the SDA and extra nitrogen excretion and that the SDA of protein is due at least to two distinct processes—work of renal excretion and metabolism of nitrogen and carbon. These two processes do not proceed at parallel rates, and this difference in rates of two processes explains some of the hitherto anomalous phenomena in the SDA of protein. Borsook and Keighly (1933) estimated the oxygen-consumption of rat-liver slices during synthesis of urea from ammonia and carbon dioxide and found a definite increase in oxygen use. One additional molecule of oxygen is used for every molecule of urea synthesized. Comparison of rates of synthesis of urea leads to the belief that the fuel for this reaction is lactate or pyruvate. From this work they concluded that not more than a fraction of SDA of protein could be due to urea synthesis from ammonia. From further work, they estimated that urea synthesis is probably responsible for not over 20 percent of the SDA of protein.

In contrast to the works of Borsook, other workers could not detect any influence of kidney work on the SDA of protein. Tangl (1911) reported that protein, urea or salts can increase the oxygen consumption even if the kidney
activity is eliminated. Kocher and Torbert (1932) reported that ingestion of urea had no influence on the respiratory exchange. Di Frisco (1933) injected urea interperitoneally in pigeons and found no increase in heat production.

Dock (1934) reported that extra-work is imposed on the rat kidney by a rise in urea intake, but this extra work is so slight that the extra energy required to excrete the urea derived from amino acid would represent only 1 to 4 per cent of the total SDA. Eaton, Cordill and Gouaux (1935) fed 30 grams urea and although nitrogen excretion often increased 1-2 grams per hour over the basal output, there was no consistent rise in basal metabolic rate. Thus they concluded that the work of the kidney in excretion of urea is not a factor in the SDA of proteins. Rajzman (1936) injected rather large amounts of urea intraperitoneally to rabbits and rats placed in thermoneutrality. She concluded that there is no increase in heat production due to the excretion of urea. She also remarked that after ingestion of protein in man, heat production often precedes urea excretion. Carpenter (1938) fed 400 or 500 cc. water and compared the total metabolism change to ingestion of 30 or 40 grams urea in 400 or 500 cc. water respectively. He found no effect on total metabolism after ingestion of urea as compared to water. Thus it is clear that kidney work as such in the excretion of urea does not involve so great an amount of work as could explain the SDA of protein.

That the Verdaunungsarbeit cannot explain the SDA is remarkably shown by the works of Rapport and Katz (1927). They added glycine to perfused muscle and found that the oxygen consumption of the muscle was increased. Thus SDA of glycine could not be explained by the Verdaunungsarbeit.

Site of SDA

Regardless of the role of the kidney in the work of excretion of urea and other nitrogenous end products, it is definitely involved in a large number of chemical processes connected with the metabolism of amino acids. By using the Warburg technic, it can be shown that kidney, like liver tissue, deaminizes amino acids, as illustrated, for example, by the reaction

$$\text{RCH} \left(\text{NH}_2\right)\text{COOH} + \frac{1}{2} \text{O}_2 \longrightarrow \text{RCO-COOH} + \text{NH}_3 + \text{heat}$$

The amino acid oxidases are the enzymes involved in this type of oxidative deamination.

The kidneys, like the liver, are the site of many intermediary metabolic reactions. Glycocyamine is formed by the reaction of arginine and glycine; kidney is apparently, the chief site of this reaction. The kidney also plays a decisive role in the oxidation of some fatty acids. While liver is much more effective than kidney in forming aceto-acetic acid from fatty acids, it is much less so in carrying the oxidation further. Kidney tissue oxidize aceto-acetic acid more powerfully than liver, testes, and spleen tissue. These fatty
acids are derived not only from oxidation of fat, but also from the deaminated residues of amino acids. The kidney is also connected with the formation of citric acid by the enzyme citrogenase by the reaction of aceto-acetic acid and oxalacetic acid. (Cameron, 1945).

The principal site of SDA action is in the liver rather than kidney. Dock (1931) fed a high nitrogen diet to rats, but found no increase in oxygen consumption by the kidneys; 85 per cent of the extra heat production on high protein diets was in the liver, rather than kidneys. He measured the oxygen consumption of two groups of rats, one fed high protein diet (casein) and the other fed a protein-free diet (starch) before and after tying (a) the aorta below the renal arteries, (b) renal arteries, and (c) the splanchnic arteries. In this way he could exclude the kidneys from the system. He found that on a high protein diet, the oxygen consumption of the abdominal viscera increased by 191 per cent and of the hindquarters by 8 per cent. Rapport and Katz (1927) found increased oxygen consumption of perfused muscles after addition of glycine. It therefore seems that while the liver is the principal site of action of SDA, kidney and other viscera and also muscles do contribute to the processes associated with SDA.

Voit's Theory of SDA

From the definition of SDA that there is an extra heat production associated with food ingestion, it must be clear that this extra heat must come from the metabolism of substances which were already present in the tissues. Hess' law of constant heat summation does not allow of any more extra heat production by intermediate chemical steps in the metabolism. Various drugs and hormones also increase the heat production. The actual mechanism of all of them is not known. Adrenaline probably activates the liver phosphatase and liberates a large amount of glucose from glucose phosphate formed from glycogen. Thyroxine probably activates the enzyme systems at the pre-dehydrogenase level. The mechanism of action of dinitrophenol and related compounds is not known. However, they all probably act by directly stimulating the metabolism of the body cells. Voit (1881) suggested that the amino acids, hexoses, and fatty acids might also stimulate the metabolism of the body cells, and that this constitutes the SDA. Johansson (1909) studied the SDA of carbohydrates and concluded that an excess of glucose raises the metabolic activities of the body cells by a mass effect. Lusk (1912-13) fed 700 grams meat to a dog and found the SDA for the second hour to be 139 and for the third hour 106. The heat production was greater during the early hours when the urinary nitrogen was only one third of its maximum. Glycine also acted as a direct stimulant to the cells, but glutamic acid had no SDA. From these Lusk concluded that the increase in metabolism after ingestion of meat was not due to the intermediary process.
of protein metabolism itself, but to a mass action effect of amino acids which acted as direct stimuli on cellular catabolism.

Schirli tz (1927) observed a parallelism between the blood sugar level and SDA after administration of carbohydrates. Rapport’s work (1927) on the direct stimulation of perfused muscle with glycine confirmed this view. Mansfeld and Horn (1928) also observed that oxygen consumption was proportional to nutrient concentration in blood. Wilhelmj, Bollman and Mann (1928) injected amino acids into hepatectomized dogs and found no SDA. This work shows that in the absence of liver there is no SDA and therefore that SDA of amino acids is not due to direct stimulation of body cell metabolism, but rather to the intermediary reactions in the process of metabolism of the nutrients. Lusk (1931) therefore discarded Voit’s theory to cell stimulation as a cause of SDA.

Reticulo-Endothelia System and SDA

Liver is necessary for exerting the SDA of amino acids. Liver is composed essentially of 2 types of cells, hepatic and reticulo endothelial (RES). It might be of interest to find out which of the cellular elements of liver tissue are concerned in the SDA. Hepatic cells are concerned mostly with the biochemical reactions, so these are probably concerned with the SDA. But cells of RES may also be concerned in the SDA.

Abel (1943) ran SDA tests on 45 subjects, using a high protein test meal of chicken. Normal SDA curves reached a peak in 3-5 hours. He found the highest peaks in those subjects who had the lowest basal metabolic rate.

He found abnormally high SDA curves in 4 patients with various diseases, all evidencing some liver damage and elevated blood cholesterol. Two of these patients showed RES proliferation on biopsy. After treatment, when the blood cholesterol decreased towards normal, the SDA curves also became more or less normal. From these observations he concluded that RES may be involved in the production of SDA of protein. It must be noted, however, that patients with high blood cholesterol will have much less tendency toward synthesis of fat from protein, and the amino acid residues may be oxidized, instead of being stored in the body. This might be responsible for greater SDA in these patients.

Rubner’s Theory of SDA

When Voit’s cell-stimulating theory of SDA was discarded following the experiments of Wilhelmj, Bollman and Mann (1928) who have shown that hepatectomized animals do not show the SDA of amino acids, it was only apparent that the SDA has to be explained not by a direct stimulating effect on the body cells but by some intermediate reaction theory. If the extra heat production is neither due to mass effect, nor due to Verdauungsarbeit; and
since the total heat production of a compound cannot be greater than the sum of the heat production of its intermediate reactions, it must be associated with the heat required to drive some of the intermediate reactions of metabolism. For example, if A goes to B and B to C with heat productions $H_1$ and $H_2$, the total heat production $H$, when A goes to C will be the sum of $H_1$ and $H_2$.

$$A \rightarrow B + H_1 \text{ Cal}; \quad B \rightarrow C + H_2 \text{ Cal}$$

$$A \rightarrow C + H \text{ Cal} \quad (\text{when } H = H_1 + H_2)$$

As, experimentally, $H$ is greater than $H_1 + H_2$, Rubner (1902) assumed that there must be other exothermic reactions interposed in the chain to drive A into B and B into C, which produces the extra heat. This is thermodynamic theory.

This theory has been discussed in detail by Aubel and Schaeffer (1932). Amino acids are deaminated and the glucogenic fatty acid radicles are converted into glucose. These endothermic reactions are irreversible and therefore cannot follow spontaneously. To make these endothermic reactions possible, there must be simultaneous exothermic reactions coupled with these endothermic reactions.

According to Wurmser (1930) the combustion of glucose might constitute the coupled exothermic reaction.

The available thermodynamic data indicate that the SDA of glycine should be different from that of alanine, but laboratory experiments in vivo show that the SDA of glycine and alanine are the same. The conversion reactions of glycine and alanine to glucose are endothermic. The endothermic reactions cannot be realized spontaneously. To make these endothermic reactions possible, they have to be coupled with simultaneous exothermic reactions, for example, with the oxidation of glucose. It so happens that the heat of the exothermic reaction of the oxidation of glucose is in excess of the endothermic reaction of conversion of glycine and alanine to glucose and there is a resultant heat increment which has been taken to be the SDA of glycine and alanine. The conversion of glutamic acid into glucose is exothermic and therefore does not need any coupled exothermic reaction and will produce more heat than the conversion of glycine and alanine to glucose.

Adams (1926) indeed calculated the change in free energy, with the aid of the third law of thermodynamics, in the conversion of glutamic acid into glucose and found that $\Delta F$ was negative; in the conversion of 1 gm. of glycine into glucose and urea, 0.871 Cal. are needed to drive the reaction; and in the conversion of 1 gm. of alanine into glucose and urea, 0.337 Cal. are needed to drive the reaction.

Gliadin, with a high glutamic acid content (over 40 per cent) exerts a high SDA.
Abelin (1927) found that the SDA of meat increases after repeated injections of phloridzin. As there is much glycosuria and as this glucose is derived from the metabolism of amino acids, he concluded that the SDA of protein is due to intermediate chemical reactions. Bornstein and Roese (1929) could not find increased oxygen consumption in surviving extremities perfused with added glycine, but oxygen consumption of surviving liver was increased 22 per cent by glycine. This he took to be due to the fact that it is the breakdown products of amino acids that act like hormones and raise the metabolism of muscle. Lusk (1932) also ascribed SDA to intermediate chemical reactions.

Kriss (1941) calculated the SDA in a different way, by using the heat production of maintenance as the base value, and found that the SDA of various amino acids are best correlated with the metabolizable energy of the administered amino acids. These results, he believed, are consistent with the theory that the SDA of amino acids and of proteins are by-products of intermediary chemical reactions and they do not support the idea that certain amino acids or some of their cleavage products act in the body as special metabolic stimulants in the pharmacodynamic sense.

Though it has been proved that the SDA is due to intermediate chemical reactions of amino acids, it is not yet clear as to which part of the molecule of the amino acids produce the SDA. Lusk and his students held that it is the metabolism of the non-nitrogenous residue; more specifically, it is the conversion of amino acids into glucose that causes SDA. The main basis for this theory appears to be based on the fact that not all amino acids exert the same SDA. Atkinson and Lusk (1918) found that asparagine and glycine, which contain the same per cent of nitrogen, behaved very differently in the metabolism in the dog. Asparagine exerted no SDA, while glycine had a high SDA; so they concluded, that the SDA was not due to the amino radicle of amino acids. Chambers and Lusk (1930) worked on the normal and phloridzinized dogs and concluded, as before, that SDA of protein and amino acids is due to conversion into glucose. Aubel and Schaffer (1932) concluded similarly.

In contrast to the school of Lusk and others, another school has ascribed the SDA to the metabolism of the nitrogen moiety of the amino acids. Grafe (1915) fed ammonium chloride, glycine, alanine, phenylalanine, asparagine and found that all of them increased the heat production, which varied from 4.6 to 40.6 Cal per gram of nitrogen. In case of asparagine, however, he did not find any heat increment in one experiment. He concluded that the nitrogen content of the amino acids and amides play an important role in SDA. Terroine, Bonnet and Zagami (1931) found a more or less constant SDA per gram of nitrogen administered. They administered glycine, alanine, aspartic
acid, glutamic acid, valine, leucine, cystine and lysine. The SDA of all of these was 8.4 Cal per gram N while for tyrosine and phenylalanine, it was 9.2 Cal and for tryptophane and histidine 10 Cal. Excluding the four higher figures, they concluded that extra heat production of different amino acids was 8.4 Cal per gram of nitrogen metabolized.

Lundsgaard (1931) found that for five hours after feeding, not only the amino acids, but also ammonium chloride caused an extra heat production to the extent of about 8 Cal per gram of nitrogen administered. He found that ammonium salts of glycollic and lactic acids, or the hydroxyacids corresponding to glycine and alanine, increased the oxygen consumption like the amino acids, but that the sodium salts did not increase the oxygen consumption. The action of ammonium chloride was like the amino acids. Brody and Procter (1933) found that the ratio of SDA to extra urinary nitrogen excretion for a period of 21 hours after feeding was about 11 Cal per gram of urinary nitrogen. The range in values of SDA per gram of nitrogen metabolized varies from 6 to 11 Cal per gram of nitrogen and is independent of the source of nitrogen.

In spite of the findings that all amino acids exert a SDA according to nitrogen content, there is no general agreement. Zummo (1933) found that glycine in 5 gram doses exerted no SDA in pigeons when fed by mouth. Stassi (1933) found the same thing true for alanine, in both normal and fasted pigeons. The more interesting results are due to Barbato (1933) that while alanine and glycine do not exert SDA in pigeons, aspartic and glutamic acids do. The curve of oxygen consumption reaches its peak in about 2 hours, remains constant for 3 hours and then returns to normal. The curve is a little prolonged for glutamic acid. Zummo (1933) found that the SDA of nitrogenous substances did not depend on conversion of ammonia to urea or uric acid.

There is also variation in the SDA according to the nature of protein used. Mark (1932) found that the SDA of muscle protein was much higher than of liver protein. Proteins of spleen, kidney, and thymus have also a lower SDA than proteins of muscle. Schlumm and Brechmann (1930) confirmed the results to some extent. Calf's liver appeared to have a lower SDA than muscle. It thus appears that though the nitrogen metabolism of amino acids may exert a large fraction of SDA, this is not the whole story. Apparent discrepancies of these findings will be discussed presently.

**Plane of Nutrition and SDA**

Many investigators believe that constitutional obesity might be due to a lower SDA and thinness to a higher SDA. Jaquet and Svenson (1900) reported that the SDA was below normal in obese individuals. A similar
view has been expressed by Wang and Strouse (1924). They found that protein showed a low SDA in the obese and a high SDA in the thin. Some workers, however, have found no difference in obese and normal persons. DuBois, Spencer, McClellan and Falk (1929) and Strang and McClugage (1931) reported that the SDA in exogenous obesity is within the normal limits.

Lauter (1926) concluded that SDA in exogenous obesity may be increased, but he did not find any marked deviation from the normal.

Some observers have found, as expected, a high SDA in overfeeding and low SDA in underfeeding. Plaut (1922) found that SDA decreased in chronic hunger and there is a corresponding increase in SDA in overfeeding. She, however, ascribed this to the pituitary gland.

Baur (1929) reported that when the body carbohydrate was low, as in fever, the SDA is low or even absent. But after a short fasting, following high carbohydrate diet, there was a high SDA.

Stassi (1933) made the interesting observation that the SDA of glutamic acid in fasting pigeons is about twice that in normally fed pigeons. That the plane of nutrition plays a role in SDA has been stressed extensively by Deighton (1929). He went to the extreme by saying that the phenomenon of SDA does not exist apart from change in the nutritive plane. In other words, the rise in metabolism following food intake is due to a change of nutritive plane, as also is the fall to the fasting level.

The fraction of the metabolizable energy which will appear as SDA in a single experiment cannot, however, be expected to approximate very closely to the average. This is because individual animals vary enormously in the level of food intake needed to attain a given nutritional plane. As regards individual nutrients, Deighton argued that proteins raise the nutrition plane more than carbohydrates and the carbohydrates more than fats. Since in low protein diets nitrogen excretion is reduced to a minimum, and with high protein diets nitrogenous end products of considerable energy content are excreted, protein must, therefore, raise the nutrition plane to a greater extent than carbohydrates and fats. This explanation does not seem to be very satisfactory because proteins or amino acids are not completely oxidized, and nitrogen products of high energy content must be excreted. Deighton's explanation seems to have taken the SDA of protein in retrospect. This paper was one of the earliest to stress the great importance of the plane of nutrition on SDA.

Forbes, Kriss and Braman (1928) investigated the energy balances on four steers at seven planes of nutrition. They took the heat production at the fourth day of fast as the basal heat level. At a very low plane of nutrition, the heat increment per unit feed was small, but this increased gradually until the maintenance plane. But at the very high planes, the rate of increase
was again low. The curve of heat production in relation to the plane of nutrition was, therefore, S-shaped. They explained the diminished rate of increase in heat production between the higher points of observation (2 and 3-maintenance levels) as due to the diminished metabolizability of the rations.

Wiegner and Ghoneim (1930-31) held that the net energy of food cannot be directly proportional to the quantity of metabolizable energy, because increase in production cannot continue indefinitely. They represented the relation by a differential equation to express the theory that successive increments of net energy decline with successive increments of food consumption. Gruenigen (1933) extended the mathematical theory.

Mitchell, Hamilton, et al (1932), believed that from the maintenance level upward, heat production is a linear function of the dry matter consumed. They reported that the highest digestibility occurs at the lowest feeding level. They found that the metabolizable energy increased from high to low levels of nutrition. But when the metabolizable energy is computed per kilogram of digestible nutrients all effects of the level of feeding disappear. They explained that the increase in percentage availability of metabolizable energy at low levels is due to the increased digestibility and decreased SDA.

Brody and Procter (1933) analyzed the data of Forbes, Mitchell and Wiegner and concluded that, among other factors, energy losses due to SDA vary enormously with the plane of nutrition. SDA loss accounts from 3 per cent of the gross energy at about one-half maintenance to about 20 per cent of gross energy at maximum feed intake.

Kriss, Forbes, and Miller (1934) used a new system of reference bases for expressing SDA. They expressed the SDA as percentages of the metabolizable energy of the food supplements to a maintenance ration. They found that the values of SDA as expressed in this way were greater than when using fasting rather than maintenance metabolism as a reference base and when using the gross energy rather than metabolizable energy of casein, starch and olive oil. They concluded that the heat produced by catabolism of body protein includes a factor of waste heat of utilization. The heat increment values of rations, therefore, determined directly with reference to fasting heat production (uncorrected for sparing of body tissue) are lower than the true energy expense of utilization by the amounts of dynamic effects of body substances spared.

Differences in SDA reported by different observers thus appear to be due to the use of different food energy categories (metabolizable energies; gross energy).
SDA of Mixed Diets

The SDA of mixed diets is usually different from the sum of the SDA's of the constituent nutrients when fed separately. For instance, as noted elsewhere with respect to hexoses galactose has a higher SDA when combined with other hexoses than the sum of the SDA's of galactose and of the other hexoses when fed separately. The mechanism has been discussed there.

Mitchell (1934) believed that utilization of any food nutrient for any purpose in the animal body requires simultaneous presence of all other nutrients required for that purpose. In that case, the net energy value of all perfectly balanced rations would be the same under the same conditions of feeding. He remarked that the SDA of various nutrients are not characteristic except when they are fed singly. When the nutrients are fed in combinations, the SDA of the mixture will be less than the weighted mean of the individual effects of the constituent nutrients when fed separately. This decrease in the SDA would be expected to continue as the feed combination approaches a perfect balance when the SDA will be minimum.

Murfin, Burton and Barrows (1936) fed fat and sugar and found two types of SDA. In some cases there was a complete summation of the SDA of fat and sugar; in others the increase in the heat increment of the mix was greater than sugar when fed alone. Wachholder and Frenz (1944) fed a mixed diet to a large number of subjects and found individual differences in SDA. They believed that these differences were due to differences in the intensity of the primary SDA-raising factors as also to secondary factors.

Forbes and Swift (1944) have made an extensive study of the SDA of mixed diets. They fed beef protein, cerelose (corn sugar), and lard individually and in various combinations. It must be noted, however, that all these experiments were conducted at planes of nutrition above maintenance; SDA was measured by the difference between heat production on the basal plane and on the basal plus the supplement. The SDA of the gross energy of the beef protein was 32 per cent, cerelose 20 per cent, lard 16 per cent. These SDA values, it will be noted, are higher than those reported by other workers. The reason has been explained before. They found that the dynamic effects of mixed supplements of protein and fat, and of carbohydrate and fat, were even lower than the dynamic effect of fat alone. They concluded that fat must be more potent than protein and carbohydrate in determining the SDA of diets.

Ring (1946) determined the SDA of diets high in carbohydrate or fat and the results were different from those of Forbes and Swift; the latter workers found that fat was more effective in reducing the SDA of protein than was carbohydrate. They measured the SDA, as already described, by superimposing protein on a diet high in carbohydrate. Ring explained the
results of Forbes and Swift by assuming that on a high carbohydrate diet, protein will have little tendency to be converted into carbohydrate and addition of carbohydrate should not lower SDA of protein very greatly. In Ring's fasted animals, when the R.Q. was about 0.72, protein was converted to carbohydrate and if carbohydrate is given with protein, the tendency of protein to carbohydrate conversion will be reduced. Similarly, in such fasting animals which are already burning fat, the addition of fat is not expected to reduce the SDA of protein.

These explanations, as is clear from the context, are based on the assumption that certain nutrients tend to be converted into other nutrients, and that these intermediate products somehow influence the SDA. As these hypotheses have not been put to crucial experimental test, we tested them.

**SDA in Children**

Rubner (1902) believed that there is no SDA for retained protein; as there is a tendency to protein storage in children they should not show appreciable SDA. Howland (1911) used Lusk's calorimeter to investigate the SDA of protein in 2 infants, 3 and 7 months old. Daily additions of 2.14 gm and 4.27 gm of protein nitrogen to the infant's diet raised their metabolism 10 and 26 per cents respectively above the basal figures in the 3 hours following the last meal. He calculated the calories derived from the combustion of protein and found them to be 14 to 20 per cent and 16 to 26 per cent respectively. These values are lower than the accepted ones in adults, which is 30 per cent. Murlin and Hoobler (1915) measured the heat production of a markedly marantic infant, aged 3 months, following fat, carbohydrate and protein meals of the same energy content. The heat production after the fat meal was 6.01 Cal. per hour, while after the carbohydrate meal, it was 7.25 to 8.12 Cal per hour. An increase in protein content of the last carbohydrate diet from 11 to 30 per cent produced an additional rise in metabolism of 12 Cal per hour. This latter figure indicates an increase of 25 per cent above the value obtained following the fat meal. In adults, the expected increase in heat production following protein meal should be much more comparable to fat. Hoobler (1915) raised the protein content to 43.3 gm on the marantic child and obtained similar results.

Murlin, Conklin and Marsh (1925) reported a 6.8 per cent metabolism increase in newborn infants following breast feeding. They measured the metabolism only for 2 hours (the SDA of lactose persists longer).

Levine, Wilson, Berliner, and Rivkin (1927) measured the metabolism of two infants after feeding fat, protein, and milk meals. The rise in metabolism during the first 3 hours after feeding was 15 to 17 per cent above the basal level for fat. The SDA of milk and protein, however, persisted un-
checked at the end of 6 hours. Levine, Wilson, and Gottschall (1928) found no significant SDA difference between normal and marantic infants.

Dann, Kelly, McNamara, and Curtis (1942) measured the SDA of protein in infants as previously noted, and reported that the SDA of high protein feeding is slight in premature infants. Moreover, when premature infants are fed 5 grams or more of protein per kilogram body weight per day, derivatives of aromatic amino acids, as p-hydroxy-phenyl-acetic and p-hydroxyphenyl pyruvic acids are excreted in the urine. The authors fed 1 gram glycine per kilogram body weight and found 6 to 16 per cent increase in heat production above basal, while phenylalanine and tyrosine in 0.4-2 gram per kilogram body weight showed no rise in heat production above the control level.

They concluded that aromatic amino acids might undergo different metabolic stages in infants from those which normally occur in adults. Presence or absence in the urine of infants of deaminized intermediary products of aromatic amino acid metabolism did not influence the SDA of these amino acids.

Lower SDA of protein in childhood is due to its greater retention and synthesis. As discussed elsewhere, this protein synthesis may involve transamination reactions. Tyrosine may be active transaminatically, although it is a monocarboxylic amino acid, because of the fact that the para-hydroxyl group, like phenolic hydroxyl, is acidic. As discussed in the section on transamination, all dicarboxylic or pseudo-dicarboxylic (i.e., with another acidic group at the other end of the molecule) acids are active transaminatically.

Work and SDA

It is generally agreed that the SDA of protein cannot be utilized for work. Some evidence however indicates that the SDA of carbohydrates and fats may be depressed by work.

For instance, Rapport (1929) measured the heat production of a well-trained dog running for 10 minutes at the rate of 13/4 miles per hour. The metabolic measurements were made during post-absorptive state (18 hours after feeding); after feeding fat; after glucose; and after meat. The results indicate that the SDA of glucose and fat was depressed, or it could be thought of as utilized as free energy in muscular work. Similar results were published by Anderson and Lusk (1917) and later by Meyer (1930).

Meyer (1930) fed 500 gm. raw lean beef to a 10 kilogram dog at rest and found the increase in heat production over rest to be 47 Cal.; but after an 8 hour run, the same amount of meat ingestion was followed by an increase in heat production of 72 per cent. This greater SDA after work, he believed, is due to the extra chemical work which the body has to perform to
convert the ingested protein into materials suitable for the metabolism of muscles.

The difference between the SDA of carbohydrate and of fat on the one hand and of protein on the other, led to the conclusion that SDA of protein is due to some irreversible reactions corresponding to the entropy of thermodynamics.

It must be remembered that the site of SDA is mostly in the liver and not in the muscle. As physical work is performed in the muscle and not in the liver, it is conceivable that the SDA of protein will not be utilized as work. Fat and carbohydrate can be used by muscle itself and therefore their SDA may be convertible into work.

It may be noted, incidentally, that the calorigenic action of epinephrine and of thyroxine like that of protein, cannot be used in muscular work.

The question is not why the SDA of protein is not convertible into work, but what are the irreversible reactions that are responsible for the SDA. But as it is now known that some of the amino acids can be used by muscles, it might be worthwhile outlining the mechanism of work in muscle.

The initiation of muscular work is believed to be due to the breakdown of adenosinetriphosphate into adenosinediphosphate and phosphoric acid. This is under the control of the enzyme adenosine triphosphatase which has been identified with myosine, the principal contractile element of muscle (Engelhardt and Lyubimova 1939; Needham 1942). Normal stimulation of muscle is believed to be due to the liberation of acetylcholine at the neuromuscular junction and acetylcholine accelerates decomposition of adenosine-triphosphate (DuBois and Potter 1943), apparently by activating the adenosine-triphosphatase. Since muscular work is associated with acetylcholine and since fatigue of muscle can be decreased by reducing the acetylcholine concentration, it was of importance to find out if the acetylcholine concentration could be reduced. Thiamine has been found to neutralize acetylcholine and decrease the muscle fatigue (Sadhu 1946). Thiamine may thus help to utilize part of SDA as work.

Transamination and SDA

It was generally believed that the nitrogenous part of amino acids was deaminated and converted into urea until a group of Russian workers, including Braunstein and Kritzmann (1937-38), reported that some of the amino acids can transfer their amino groups to the keto acids, thereby saving the nitrogenous part from being converted into urea. They found the reaction to be most marked with dicarboxylic amino acids and their corresponding keto acids as follows:
1 (+) \( \alpha \)-glutamic acid + oxaloacetic acid \( \rightleftharpoons \)
\( \alpha \)-keto-glutaric acid + 1 (−) aspartic acid.
1 (+) \( \alpha \)-glutamic acid + pyruvic acid \( \rightleftharpoons \)
\( \alpha \)-keto-glutaric acid + 1 (+) = alanine.

They reported similar reactions with other amino acids. In fact any
\( \alpha \)-amino acid, with the possible exception of glycine, is active in transamination
with either of \( \alpha \)-keto glutaric or oxaloacetic acid. Cohen (1939) confirmed the findings of the Russian workers with glutamic and oxaloacetic or
pyruvic acid, but failed to confirm the reaction between aspartic and pyruvic
acids.

Kritzmann (1939) reported two different enzymes for glutamic and
aspartic acids. She found that the enzyme for glutamic acid, glutamic acid
aminopherase, has an active prosthetic group which is similar to the coenzyme of aspartic acid aminopherase, but less readily dissociable. This could
explain why Cohen did not find transamination between aspartic and pyruvic
acids. Coren (1940) could not find two different aminophorases, one enzyme
could transaminate both glutamic and aspartic acids. Zorn (1940) found that
transamination was restricted to glutamic acid, aspartic acid, and alanine.
The apparent contradictions were cleared by Braunstein and Bychkov
(1940). They pointed out that the oxidative deamination of monocarboxylic
\( \alpha \)-amino acids is, in all probability, indirect. Members of the dicarboxylic
amino acid group, such as glutamic acid, can transfer amino groups to either
monocarboxylic-ketonic acids as pyruvic, or to dicarboxylic \( \alpha \)-ketonic acids
such as oxaloacetic acid. Monocarboxylic amino acids, however, cannot
transfer amino groups directly to monocarboxylic \( \alpha \)-ketonic acids except
through the intermediation of catalytic amounts of some dicarboxylic \( \alpha \)-
ketonic acids. This is how dicarboxylic amino acids came to be called primary
amino donators, while monocarboxylic amino acids are considered as secondary amino donators.

This explains why the dicarboxylic amino acids have higher transamination
coefficients than the monocarboxylic amino acids.

The availability for enzymatic amino-transfer is due to a specific electro-
static configuration (polarity) of \( \alpha \)-amino acids bearing 2 acidic groups at
the ends of the molecule rather than to special chemical properties depending
on the presence of 2 carboxyl radicals. Thus phosphoserine, homocysteic
acid and cysteic acid (pseudo-dicarboxylic amino acids in which phosphoric
and sulfuric acids provide the second acidic group) can act as amino-donators
in transamination. This explains how tyrosine may function in transamination. Tyrosine has a parahydroxy group which is acidic, like phenolic
hydroxyl, and therefore behaves as a pseudo-dicarboxylic acid.
Transamination not only occurs in liver, but also in other tissue proteins. Agren (1940) reported that \( \alpha \)-keto groups in peptide linkage to amino acids can function as acceptors of amino groups from glutamic acid with the formation of dipeptides. Transamination is thus intimately related to protein synthesis in the organism. Cohen and Hekhuis (1941), however, found an inverse relation between protein and transaminase activity in neoplastic, fetal, and regenerating liver tissues, in which synthesis of protein is known to occur at a very rapid rate. They found a high transaminase activity in adult tissue. But Albaum and Cohen (1943) found a high transaminase activity in germinating oat seedlings where protein synthesis is rapid. Lichtenstein and Cohen (1945) found a higher transamination rate in bacteria than in the tissues of higher animal; and as bacteria have a rapid protein synthesis, they concluded that transamination is directly concerned with the protein synthesis. The inverse relation between transaminase activity and protein synthesis as reported above may be due to nondevelopment of the cytoarchitectonics of the rapidly growing tissues. However, it is directly concerned with protein synthesis.

Transamination reactions are reversible. When these reactions occur, there is very little development of heat, as contrasted to the oxidative deamination reactions. Again the reversible transamination reactions are more likely to be utilized as work compared to the irreversible deamination reactions which give out a large amount of heat.

These considerations led to the belief that the greater part of SDA of protein is due to the deamination reactions, which is decreased by the transamination reactions. Glutamic and aspartic acids which are transaminatically very active are expected to have low SDA provided they have the amino acceptors. The findings of Lusk and co-workers that glutamic and aspartic acids had no SDA is due to the fact that their dog, which had been gaining weight throughout the experimental period, apparently had a large amount of pyruvic and \( \alpha \)-keto glutaric and other dicarboxylic acids in the tissues which helped transamination of glutamic and aspartic acids. Lundsgaard and others failed to confirm the absence of SDA of dicarboxylic amino acids, apparently because of the fact that their experimental subjects had not enough amino acceptors which can help in transamination. Interesting in this connection is the report of Stassi (1933) to the effect that the SDA of glutamic acid was twice as great in fasting subjects as in the controls. The amino acceptor level is reduced in fasting. The finding of Forbes and his group that the ration of protein to fat SDA is much less than the classical value of 30:6 by their method of measuring SDA above the maintenance plane also become clear. In the supermaintenance plane, a large amount of amino acceptors are expected to be present. This subject has been discussed in further detail under the heading of “Plane of Nutrition.”
The Pituitary Gland and SDA

Most workers in this field have attributed an effect of the anterior lobe of the pituitary gland on the SDA. Foster and Smith (1926), however, believed that the SDA is influenced by both the anterior and posterior lobes of the pituitary. They found that the SDA of glycine is abolished by hypophysectomy in rats and that it could be restored by replacement of both anterior and posterior lobes, but not by either alone. They also found that basal metabolism of the hypophysectomized rats was about 35% below normal. Evans, Lusk, Pencharz and Storrer (1938), however, found that hypophysectomy in rats did not abolish the calorogenic action of glycine. They, like Foster and Smith, noted that the metabolic rate of hypophysectomized rats was 40-45% below that of normal animals. There is evident disagreement concerning the role of the pituitary in SDA.

Fulton and Cushing (1932) reported that the metabolism is lowered after hypophysectomy and increased in hyperpituitarism as in acromegaly, but that disturbances of pituitary function as observed clinically in man have no influence on the SDA of protein; that, indeed, the SDA of food is not related to endocrine functions. Staehelin and Gigon (1923) found that the SDA of food was normal in cases of nanosomia (pituitary deficiency).

Rahel Plaut (1922) believed that the SDA depends on the pituitary, and pituitary deficiency decreases SDA and promotes obesity (Fettsucht). Liebesny (1924) found that the SDA of food decreases in adiposogenital dystrophy and can be increased after treatment with anterior pituitary extract. Kestner (1925) confirmed the observation of Liebesny that the SDA of food is low in cases of adiposo-genital dystrophy (pituitary hypofunction), and that it can be increased by injection of hypophyseal extract. Since, however, the SDA was measured for only 4 hours after feeding and as SDA continues for 8 hours or more the decrease in SDA may have been only apparent, an effect of delayed absorption associated with hypopituitarism. Moreover, the pituitary extracts used were rather crude. Peters (1930) also confirmed the observations of Kestner by injecting anterior pituitary extracts in hypopituitary patients. Serejski and Jislin (1930) found that SDA of protein decreased in pituitary hypofunction. Goldzieher and Gordon (1933) also noted low SDA of protein in cases of adiposogenital dystrophy. Schere and Pellerano (1944) showed that while the SDA of egg protein was 16.3 percent on the average in normal humans, it is as low as 4.47 percent in hypopituitary subjects. They found that the SDA is not increased by injection of thyroxine, but injection of chorionic or hypophyseal gonadotrophins.

Reiss (1941) tried relatively pure pituitary fractions. He found that in adult rats the SDA of egg albumen begins to decrease 6 days after complete hypophysectomy; 30 days after hypophysectomy the animals showed no
signs of SDA after administration of egg albumen. He could re-establish SDA by injection of extracts containing growth hormone. This is, however, in contrast to the immense number of clinical findings that the SDA of food is less in growing children. Aub and DuBois (1919) observed an increased SDA in an achondroplastic dwarf i.e., in the absence of growth hormone; this is in contrast to the findings of Reiss. Samuels and coworkers (1943) noted that hypophysectomized rats deaminated much more protein than controls.

As will be presently noted, deamination without transamination-acceptor leads to more heat production, that is to a higher SDA. It must be remembered that the growth hormone extract used by Reiss was not pure and therefore the effect cannot be ascribed to the growth hormone alone. If the extract contains some diabetogenic or ketogenic hormone, the SDA would be expected to increase due to increased deamination and ketogenesis.

Reiss found that injecting thyrotrophic hormone increased the rate of $O_2$ consumption in hypophysectomized animals, and increased the SDA of egg albumen. Schittenhelm and Eisler (1932) found that SDA of food decreased, rather than increased, by injection of thyrotrophic hormone. Feuling (1933) confirmed the findings of Schittenhelm and Eisler by injection of thyrotrophic hormone. Reiss also found that corticotrophic and gonadotrophic hormones do not influence the SDA of protein. Reiss believed that contradictions in the literature on the SDA in patients of hyper- and hypothyroidism could be explained solely by the involvement of the pituitary body. In other words, the SDA of food does not depend directly on the function of the thyroid, but rather on the way the anterior pituitary function is affected by disturbances in thyroid function.

The above review indicates that the pituitary does not seem to have a unique role in SDA. It may affect it in diverse ways by its numerous hormones. Growth hormone, by causing greater nitrogen retention, will cause a lower SDA. The observation of Reiss that the SDA is increased by administration of growth hormone, as already discussed, may be due to factors in the extract other than growth hormone. The fall in basal metabolism following hypophysectomy indicates that the activity of oxidative tissue enzymes is also low. Injection of growth hormone for the short experimental period did not help any in increasing tissue anabolism and the impure hormones actually raised the SDA instead of lowering as expected. The target organs cannot be completely excluded.

The whole SDA situation is complicated by the fact that hypophysectomy reduces the rate of digestion, absorption and assimilation; these delays also cause delay in the SDA, which delay may be mistaken for a decrease in SDA.
The Adrenal Glands and SDA

The adrenal cortex is associated with certain phases of protein metabolism. For instance, it is involved in forming glucose from the amino acids (gluconeogenesis). Similarly, the adrenal medulla is connected with the conversion of glycogen into glucose. As the SDA is connected with the metabolism of amino acids, rather than with the direct stimulation of tissue cells, it is pertinent to ask if the adrenal cortex has any influence on SDA.

Nord and Deuel (1928) measured the SDA of glycine administered both orally and intravenously to dogs before and after adrenalectomy; they found no significant difference. They concluded that the adrenals do not exert any significant role in the SDA of glycine. Krauss, Bruni and Rettig (1929) found essentially normal SDA of amino acids in Addison's disease, with the associated hyoadrenocorticism.

Brownell and Hartman (1941) adrenalectomized dogs and kept them in good condition by injection of the unfractionated adrenal extract, cortin, the sodium factor. They found that these animals showed a marked delay in the rate of development of SDA of protein, carbohydrate and fat. However, when they were fed 25 or 15 grams dextrose, instead of 40 grams, the rate of SDA approached normal values. Delayed rate of development of SDA is due to a large extent to the delayed absorption of the nutrients. Here the adrenal cortex apparently has no direct effect on the SDA, but it is associated indirectly by accelerating general cellular activity, such as the rate of absorption by intestinal epithelia.

Parent (1941-1943) tested the influence of the adrenal medulla on SDA. He found that the augmentation of oxidation by administration of adrenaline is reinforced or prolonged by simultaneous administration of amino acids in amounts which have no definite effect on metabolism. Schere and Pellerano (1944) have referred to the observations of Bauer where the SDA was increased by injection of adrenaline by stimulation of the sympathetic system. Ederer and Wallerstein (1929) injected ergotamine subcutaneously in children and found increased SDA and this has confirmed the work of Abeline (1923). This might be due to stimulation of sympathetic system by small doses of ergotamine.

That a relation might exist between the autonomic nervous system and SDA was reported by Abelin (1923). He found that both basal metabolism and SDA, increased after administration of sympathomimetic drugs as phenylethylamine, adrenaline, and tyramine. Lami (1929) also found that the SDA was affected by the administration of atropine and adrenaline. Schaeffer and Le Breton (1938) postulated excitation by amino acid split-products by liberation into the blood of one or more hormones.
(epinephrine, sympathin) which are responsible for increased heat production following the ingestion of protein. In extension of the work of Ederer and Wallerstein (1929) it has been found that ergotamine tartarate in doses which paralyze the sympathetic nervous system diminish the SDA of proteins so long as its paralyzing action lasts. Rothschild (1938) injected piperidine-methylbenzo-dioxane which blocks the sympathetic nervous system, thus preventing a central stimulation reaching the adrenal gland. When this drug was injected into dialized cats and protein fed, there was no SDA due to the protein.

The Thyroid Gland and SDA

As thyroxine is the most powerful metabolic accelerator, a great deal of attention has been paid to its influence on SDA. This has been studied clinically in both hypo- and hyper-thyroidism and experimentally by surgical removal of thyroid. No definite conclusion has yet been reached on the nature of the influence of thyroid on SDA as indicated by the following citations.

Magnus-Levy (1897) reported that the SDA was essentially normal in hyperthyroid individuals. Eckstein and Grafe (1919) believe that SDA occurs only in the presence of the normal thyroid gland; Aub and Means (1921), on the other hand, concluded from their clinical studies that SDA is increased in cases of hyperthyroidism. Biedl (1926) observed increased SDA in hyperthyroidism, confirming thereby the findings of Aub and Means. Montmillon (1929), however, concluded that SDA is decreased in hyperthyroidism.

Plaut (1923) reported a normal SDA in myxedema. Krauss et al, (1929) observed a normal SDA in myxedema, which is a hypothyroid condition. Lichtwitz (1941) found a low SDA both in hyper- and hypothyroidism.

Mahoux (1937) found a low SDA in myxedema in contrast to previous workers who found no essential change.

At this stage of confusion of clinical studies, Schittenhelm and Eisler (1932) resorted to experimental hyperthyroidism. They injected anterior pituitary thyrotrophic hormone, thereby producing a condition of hyperthyroidism. In this condition, SDA was found to be decreased, thus confirming the findings of Montmillon. However, Landowns (1935) measured SDA before and after removal of thyroid gland of humans and found no significant change in SDA.

Horejsi and Hloucal (1941) found that thyrotrophic hormone injection lowers the SDA of glycine.

Guns and Calseyde (1938) measured the SDA in Basedow's disease
before and after removal of thyroid of these patients and concluded that thyroid increases the SDA.

Boenheim (1945) concluded that SDA lasts longer than usual in myxedema. Patients with Grave’s disease showed a decrease of basal metabolic rate and a rise of SDA after treatment with di-iodotyrosine.

The present knowledge can be summarized as follows: Hypothyroidism—SDA unchanged (Landowne, Krauss, et al., and Plaut) and decreased (Lichwitz, Mahaux).

Hyperthyroidism—SDA low (Lichwitz, de Montmillon), normal (Aub and Means, Magnus-Levy), and increased (Biedl and Krauss).

The difference in conclusion of different observers is due to several factors:

1) Clinical disorders of hypo-and hyper-thyroidism are not always exclusively thyroidal, they may be associated with disorders of other endocrines. In many cases it is my contention that the organism may reach a new equilibrium state, when low tissue metabolism due to thyroid-deficiency may be compensated by increased activity of tissue enzyme systems.

2) Surgical removal of the thyroid, if total, usually cuts off the thyroid secretion, all of a sudden, causing a disequilibrium of the whole organism.

Pyridoxine and SDA

So far no one has demonstrated an effect of pyridoxine on SDA. The known functions of pyridoxine in animal organism are in connection with the metabolism of unsaturated fatty acids (Birch, 1938) and carbohydrates (Masanozi, 1939); proteins and creatine (Beard and Pizzolato, 1941).

While injection of pyridoxine in normal guinea pigs does not affect the blood sugar level, hyperglycemia was reduced by simultaneous administration of pyridoxine and glucose, which led to the conclusion that pyridoxine influences carbohydrate metabolism. Evidence is available as to the relation of pyridoxine to protein metabolism (Richter and Hawkes, 1940). Beard and Pizzolato (1940) found that pyridoxine in 20-mgm. doses for 3 days, or one 40-mgm. dose, gave in normal rate a 21-35 per cent increase in muscle creatine which remained high. From this work, he concluded that pyridoxine is necessary for the maintenance of normal muscle metabolism and creatine content of muscle tissue.

Gammon, Harvey and Masland (1941) found that pyridoxine improves patients suffering from progressive muscular dystrophy, that is when the muscles are deficient in the formation of creatine.

McHenry and Gavin (1941) demonstrated that pyridoxine is needed for the synthesis of fat from protein. The mechanisms thereof are discussed below.
Supplee and Bender (1942) and Peretti (1942) reported that pyridoxine increases liver glycogen. Voris and Moore (1943) reported that pyridoxine is concerned not only with catabolism of proteins but also with its anabolism. They showed, by paired feeding experiments, that rats receiving pyridoxine gained more weight than their pyridoxine-deficient pair mates. The gain in weight was confined to increase in water and protein in the tissues, not to fat. Influence of pyridoxine on endogenous nitrogen metabolism was also noted by Peretti (1942).

Lepkovsky and coworkers (1943-44) have shown that pyridoxine is necessary for the normal metabolism of tryptophane. Pyridoxine-deficient rats excrete a large quantity of xanthurenic acid (4,8-dihydroxy-quinoline-2-carboxylic acid), which originates from dietary tryptophane. This work has been confirmed by Sarma (1945) in studies with rice moth larvae, by Miller and Baumann (1945) in mice, and by Axelrod, Morgan and Lepkovsky (1945) in dogs.

Pyridoxine also increases the output of work by the perfused frog muscles during the final stages of fatigue (Shock and Serbell, 1944). That pyridoxine is intimately related with protein metabolism is also shown by the fact that the time of appearance, and severity, of symptoms of pyridoxine deficiency are influenced by the protein intake level. Cercedo and Fay (1944) have shown that in rats which received diets high in casein, acrodynia (pyridoxine deficiency) developed earlier and was more severe than in rats which were fed diets low in casein. Miller and Baumann (1945) also found that pyridoxine-deficient rats fed 60 per cent casein lived only one-third as long as those fed 10 per cent casein. Though pyridoxine restored growth on both diets, about three times as much pyridoxine was required on a sixty percent casein as on a twenty percent casein diet.

Mechanism of Pyridoxine action:

The mechanism of pyridoxine action was first suggested by the discovery that pyridoxine occurs in tissues partly bound to proteins (Kuhn and Wendt, 1938). It was thought that pyridoxine might act as the prosthetic part of some enzyme system, like some of the other members of the vitamin B group. This suggestion has been confirmed. Pyridoxine has been found to be connected with two different phases of protein metabolism. It functions as a codecarboxylase and cotransaminase. Amino acids (R-CHNH-COOH) may be either decarboxylated by amino-acid decarboxylase or de-amminated by deaminases. By decarboxylation, amino acids are converted into amines (R-CHNHCOOM → RCH₂NH₂ + CO₂). Pyridoxine is converted into pyridoxal in the body which is phosphorylated on its primary hydroxyl group and forms pyridoxal phosphate. This latter acts as codecarboxylase of amino acids (Baddiley and Gale 1945). This
function was first noted with respect to tyrosine which is decarboxylated into tyramine. Later works have shown that it acts as a decarboxylase not only to tyrosine, but also to lysine, arginine, ornithine, glutamic acid and "dopa" (dihydroxy-phenylalanine).

Deamination of amino acids involves removal of amino groups, usually by oxidative process, whereby the amino acids are converted into keto-acids.

\[
R\cdot CH_2NH\cdot COOH + \frac{1}{2} O_2 \rightarrow RCOCOOH + NH_3
\]

This process is irreversible, but recent works have shown that amino acids may undergo transamination, which is reversible, and whereby the amino group can react with a keto-acid and thus forms a new amino acid. This requires an enzyme called transaminase or aminopherase. Pyridoxine is supposed to function as a coenzyme to transaminase. Schlenk and Snell (1945) reported that the ability of tissues to catalyze the transamination reaction (Glutamic acid or oxalacetic acid \(\text{-----} a\)-keto glutaric acid + aspartic acid) is dependent on the dietary pyridoxine level. The transamination rate of deficient tissues can be stimulated by the administration of pyridoxal and adenosine-triphosphate. So they concluded that pyridoxine promotes biological transamination. Snell (1945) concluded that pyridoxal itself may react with an amino-acid and form pyridoxamine. This pyridoxamine may now react with a keto-acid, forming an amino acid.

This was confirmed by Lichstein, Gunsalus and Umbreit (1945). Hawkins, MacFarland and McHenry (1946) found that in rats fed a high protein diet, there is increase in blood urea and non-protein nitrogen. Administration of further protein as casein hydrolysate or alanine to pyridoxine-deficient rats produced a sustained increase in blood urea. They conclude that pyridoxine insufficiency interferes with nitrogen utilization but does not interfere with the urea formation. This, they hold, is concordant with the transaminase explanation of pyridoxine action. Schlenk and Fisher (1947) have identified the prosthetic group of transaminase as pyridoxal phosphate. The enzyme is supposed to act by pyridoxal \(\text{-----}\) pyridoxamine change in the prosthetic group.

**Vitamin E and SDA**

Vitamin E or \(\alpha\)-tocopherol has a profound influence on protein metabolism especially in muscle. Goettsch and Pappenheimer (1931) first described nutritional muscular dystrophy in guinea pigs and rabbits, but its relation to vitamin E was first demonstrated by MacKenzie, McCollum, and associates (1940, 1941). In vitamin E deficiency, there is necrosis of the striated muscle fibers. Vitamin E has been found to be important not only in homeotherms but also in poikilotherms. Cunings (1942) observed
that the guppy fish developed typical muscle lesion when fed a vitamin E free diet. This is preventable by adding the vitamin to the diet. This shows a rather universal need for vitamin E in the maintenance of muscle structure and function.

In vitamin E deficiency, there is a profound change in the chemical pattern of muscle structure. There is a change in the electrolyte pattern, leading to an increase in sodium and chloride with corresponding decrease in potassium, phosphorus, and magnesium which indicate a loss of cellular elements into the interstitial fluid and the composition of the cell approaches that of the surrounding fluid.

There is an increase in cholesterol and total lipids (Heinrich and Mattill, 1943). Muscle glycogen is reduced (Goettsch, Lonstein and Hutchinson, 1939). The most significant change is the striking loss of muscle creatine with associated creatinuria, which is proportional to muscle damage. Goettsch and Brown (1932) found that white muscles are more extensively altered than red muscles. The white muscles are more active and contain more active contractile protein, myosin. These active muscles again contain more creatine, which is responsible for the contraction of the muscle. It thus appears that vitamin E has a profound effect on protein metabolism, especially endogenous nitrogen metabolism of creatine.

More significant from our viewpoint is the effect of vitamin E on energy metabolism. Victor (1934), Madsen (1936) and Friedman and Mattill (1941) have shown that three is a significant increase in O₂ consumption in the dystrophic muscle of rabbits. The same has been noted in guinea pigs (Madsen, 1936) and rats (Houchin and Mattill, 1942 and Kehler, 1943). As the increased oxygen uptake occurs even in muscles without anatomical change, this is attributed to changes in metabolic activity of the muscle fibers. As the O₂ uptake of liver of deficient rats does not show an increase, the changes in metabolic activity may be characteristic of muscle tissue alone (Kehler 1943). The oxidative changes are highest during the more acute stage of the disease and are not so pronounced as the muscle tissue becomes seriously damaged. Kunitz and Pappenheimer (1943) have found that changes in total O₂ consumption of the intact rat parallel those observed in isolated muscles.

Houchin and Mattill (1942) have shown that hamsters are very susceptible to muscular dystrophy and show a greatly increased oxygen consumption on vitamin E depletion (240-250% of the normal). This high oxygen use is reduced to about normal in 27 hours after oral administration of a-tocopherol acetate.

Hottinger (1941) was probably the first to show that vitamin E administration may lower the basal metabolism in humans. He did not find
the basal metabolic lowering effect in all cases. This may have been due to difference in body saturation with vitamin E. Houchin (1942) has shown that though vitamin E reduces the $O_2$ consumption of rabbit and hamster muscle to normal, vitamin E addition has no effect on the $O_2$ uptake of muscles which already contain optimal amounts. The patients who were not fully saturated with vitamin E might be the ones whose basal metabolism fell by administration of tocopherol. He did not test the vitamin E saturation of the patients.

The increased $O_2$ uptake on vitamin E depletion is characteristic only of intact dystrophic muscle; minced or homogenized muscle tissues do not show it. When a tissue is homogenized, the active components of tissue become dispersed in a larger volume of material; that is, its concentration is reduced. Houchin (1942) has therefore inferred that some water soluble component, probably an enzyme-system, is affected by the vitamin E. He investigated the succinoxidase system of dystrophic muscle. Succinoxidase activity was 162% above normal and was proportional to the degree of dystrophy. This activity was diminished by administration of $\alpha$-tocopherol phosphate, but not by $\alpha$-tocopherol alone.

Apparently nothing is definitely known concerning the mechanism of action of vitamin E on energy metabolism. Houchin (1942) suggested that tocopherols, probably in phosphorylated form and acting through some unknown systems, may inhibit or regulate oxidative processes of skeletal muscle. In their absence, these mechanisms get out of control and there is enhanced oxidation in the muscle. This hypothesis is very similar to the popular belief that tocopherols may act as antioxidants. But there is an inverse relation between antioxidant and biological activity of the tocopherols, the activity of vitamin E in the body can not be due to its simple antioxidant powers alone.

Hottinger (1941) found that the physiological creatinuria induced by administration of glycine in children is diminished or prevented by administration of $\alpha$-tocopherol. As glycogen and creatine metabolism are intimately related, vitamin E may be in some way connected with creatine phosphate in the regulatory mechanism of glycogen metabolism. On the above basis, Hottinger suggested that vitamin E may have some effect on the SDA of glycine. He found that in some humans, the SDA of glycine was slightly lowered by administration of 90 mgm. of $\alpha$-tocopherol daily for 3 days.

**Vitamin A and SDA**

The thyroid gland influences the SDA and is itself affected by the vitamin A. Vitamin A may thus be indirectly related to SDA. The following discussions and results indicate that vitamin A neutralizes part of the action
of the thyroxine and may thereby reduce the SDA of food stuffs. It also lowers the basal metabolism.

Thyroxine is a powerful catalyst of energy metabolism. The thyroid function is thus related to SDA (see section on the thyroid). What is particularly interesting in this connection is that excess vitamin A administration depresses the effect of thyroxine, modifies the activity level of the thyroid, and thus influences indirectly the SDA.

**Thiamine and SDA**

The role of thiamine in carbohydrate metabolism is generally known. Thiamine pyrophosphate acts as a coenzyme of decarboxylase and thus helps oxidation of pyruvic acid. Thiamine deficiency interferes with glycogen storage with a corresponding hyperglycemia. There is, moreover, an increase in bisulfite-binding substances in blood (BBS). The BBS consist mostly of pyruvic and other keto acids. Thiamine is thus vitally involved in the oxidation of carbohydrates.

In the synthesis of fat from carbohydrates, a large number of decarboxylating reactions are involved, and it is usually assumed that thiamine helps the synthesis of fat from carbohydrate.

The SDA of carbohydrate is believed to be due to the intermediary reactions in which glucose is converted into glycogen and into fat. As thiamine accelerates these reactions, it would appear that thiamine would increase the SDA of glucose. Ring (1943) measured the SDA of glucose with and without added thiamine. He also tested the effect of oleic acid with and without added glucose and thiamine. He found that the SDA of glucose was 4.2% while that of glucose with added thiamine (50 mcgm.) was 8%. He also found a higher R.Q. From these he concluded that the added thiamine apparently increased the rate of heat production involved in converting sugar into fat. He did not find any effect of thiamine on the SDA of oleic acid.

Ring (1947) produced thiamine deficiency in rats and measured the SDA of glucose in normal and thiamine deficient rats. The rats on thiamine deficient diets ate less food than the normal controls and also produced less heat per unit glucose consumed. Forbes and others (1934) have shown that the SDA per unit mixed food is greater for a large intake of food than for a small one. But as Ring found no correlation between the amount of food ingested and the SDA he concluded that differences in food intake were apparently not large enough to produce measurable changes in SDA. Baur (1929) believed that the SDA of carbohydrates is due to its conversion to and storage as glycogen. Harper (1942) has shown that in thiamine deficiency there is decrease in glycogenesis. It thus appears that in thiamine deficiency there is a decrease of SDA of glucose due to defects in the mechanisms of synthesis of glycogen and fat.
The liver is the main site of glycogen and fat synthesis from glucose. It would therefore be expected that, as in the SDA of amino acids, removal of the liver should reduce the SDA of carbohydrate. Mann and Boothby (1928), on the contrary, found that after removal of the liver, the SDA of glucose increased. It appears from this result that the SDA of glucose cannot be explained by the glycogen and fat synthesis reactions alone. We are inclined to offer the following explanations of these observations. Glucose is oxidized to pyruvic acid; the pyruvic acid acts as an ammonia acceptor, that is, it is transaminated in the liver into amino acid. This is a reversible reaction involving but little increase in oxygen consumption. Thus normally the SDA of glucose is reduced by transamination. Thiamine has no effect on this reaction, but pyridoxine, as it forms a cotransaminase as pyridoxal phosphate, it helps this transamination reaction and lowers the SDA of glucose. This has been discussed in detail in the section on pyridoxine.

It might be of interest to review in this connection the SDA of different carbohydrates. The most striking difference is between the SDA of lactose and glucose. Lactose has a much lower SDA than glucose.

A related unexpected observation is that of Carpenter and Lee (1932) that there was more heat production following feeding galactose and other monosaccharides together than by feeding them separately. While the sum of glucose and galactose was expected to produce 1.5 Cal. when fed separately, actually 8.0 Cal. of heat was produced.

This may perhaps be explained by assuming a competitive inhibition of glucose by galactose. If galactose inhibits glycogen formation from glucose more glucose may be oxidized with consequently greater heat production. That a competitive inhibition exists may be proved in various ways. Handler (1947) has shown that rats on diets containing excess lactose or galactose die long before they were expected to die from simple inanition. Galactose must then exert some toxic effect. Blood glucose levels varied from 26 to 73 mgm. per cent, but blood galactose varied from 210 to 640 mgm. per cent. Simultaneously there was almost complete depletion of liver glycogen. Handler suggested that galactose may interfere with glucose metabolism.

Another series of observations lead to the same point of view. Bell (1936) showed their liver glycogen after galactose feeding in rabbits is built of 18 glucose units rather than the 12 units found in liver glycogen after glucose feeding. Though Bacon, Baldwin, and Bell (1944) found that sucrose under certain conditions may also give rise to 18 unit glycogen, these observations tend to point to the fact that galactose competes with glucose for enzyme centers in glycogenesis. It is well known from colloid chemistry that in slow reaction velocities there is a tendency for larger crystal formation than in rapid reaction velocities. Galactose slows down the reaction velocity
of glucose by substrate competition and thus help the formation of longer
glycogen units. Thus galactose may increase the SDA of other hexoses by
preventing their deposition as glycogen and helping oxidation to pyruvic
acid and more synthesis of fat. It appears that more reversible glycogenesis
will involve less energy expenditure than oxidation to pyruvic acid and decar-
boxylation reactions of fat synthesis.

EXPERIMENTAL PROCEDURE

Choice of Animal

The albino rat was selected for the experiments because of its known
metabolic history. It can be reared on a synthetic diet for generations; it
is omnivorous; it can be used for any type of experimental ration. It is less
active during the day than at night which is an advantage in metabolism
measurements.

Choice of Metabolism Apparatus

The metabolism of rats has been measured in a modified Regnault-Reiset
apparatus (Brody 1945). The chamber cover has a glass window, through
which the movement of the animals can be observed. The bottom of the
chamber is connected with carbon dioxide absorbers which contain a solution
of barium hydroxide (equal volumes of barium chloride and potassium
hydroxide solutions) rocked by a motor to help in the absorption of carbon
dioxide. The barium hydroxide solution contains two indicators, cresol red
and thymol blue. The change of color to yellow indicates that the solution
will not absorb more carbon dioxide. After the experiment is over the absorbers
are removed and the solution titrated against standard hydrochloric acid
solution to estimate the carbon dioxide absorbed. To guard against loss of
carbon dioxide, titration is done slowly with the use of a special device
(Winchester 1940).

The bottom of the chamber is also connected with the oxygen source
which consists of two large glass cylinders. These glass cylinders are connect-
ed at the bottom with graduated burettes on which is read the amount of
oxygen used, as water displaces the oxygen when it is used up in the chamber.
These burettes have been calibrated and the correction factors employed in
the final calculations.

The metabolism chambers are housed in a constant temperature cabinet
regulated by a thermostat set to 26° C. The oxygen pressure is maintained
at constant atmospheric pressure by the use of a Mariotte bottle connected
with an oxygen reservoir. The oxygen from this reservoir is used for displac-
ing the water which fills the machine when its oxygen has been used up by
the animals. The regular oxygen bubbling from the oxygen reservoir into
the Mariotte bottle serves as a guide to the proper working of the oxygen supply.

Training of the Animals

Before the rats were used for the experiments, they were conditioned to the metabolism chamber by being placed there under the condition of the experiment to serve as blank trials. Usually the animals are excited for several trials and their oxygen consumption runs very high. Some of the experiments reported in literature have been faulty because of the omission of training. Janes (1942) tried to show the calorigenic action of sex hormones. The curves presented there will show how the oxygen consumption gradually decreased for several subsequent occasions, before it became steady.

To guard against such errors, the animals were given preliminary trial runs until they showed a relatively constant oxygen consumption rate. This required 2 to 3 weeks.

The Diets

The animals were placed on a low protein diet containing just enough nitrogen to maintain equilibrium. Vitamins were supplied in the form of yeast (strain G) and a mixture of pure fat-soluble vitamins ADEK in lard. Wood pulp was added to maintain bulk.

<table>
<thead>
<tr>
<th>RATION No. 1200</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gr.</strong></td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Corn starch</td>
</tr>
<tr>
<td>Lard</td>
</tr>
<tr>
<td>Wood pulp</td>
</tr>
<tr>
<td>Mineral salts (5)</td>
</tr>
<tr>
<td>ADEK (7022)</td>
</tr>
<tr>
<td>Yeast (strain G)</td>
</tr>
</tbody>
</table>

Experiments on pyridoxine deficiency were carried out with a different ration. Controls of the pyridoxine deficiency group were fed the deficient diet supplemented with pyridoxine.

**Pyridoxine-Deficient Ration**

| **Gr.** | **Gr.** |
|-----------------|
| Casein (alcohol washed) | 10 gm. |
| Corn starch | 72 gm. |
| Lard | 8 gm. |
| Wood pulp | 3 gm. |
| Mineral salts (5) | 5 gm. |
| ADEK (7022) | 2 gm. |
| Thiamine | 0.4 mg. |
| Riboflavin | 0.4 mg. |
| Niacin | 0.4 mg. |
| Calcium pantothenate | 2.0 mg. |
| Choline | 200 mg. |

Para-aminobenzoic acid, inositol, folic acid were not supplied, as the rat can do without them.

Mode of Administration of Nutrients

Pure proteins were administered in feeding cups individually to the rats and the cups were weighed before and after feeding. The difference is
the feed eaten. The time allowed to eat was half an hour. The time of feeding was counted as the middle of feeding time, 15 minutes after starting of feeding.

In case of pure amino acids, they are converted into the sodium salts, neutralized to phenolphthalein, and fed by a blunt needle and record syringe by mouth. Soluble carbohydrates have been fed similarly by dissolving in water. The solutions have been made by dissolving the nutrients in warm water and bringing to body temperature before feeding. The amount of solution fed was 3.5 ml. per rat, as this was found to be the optimal amount. Too much of solution fed causes distension of the stomach and the rats get restless. Too little solution also makes them restless after 3 to 4 hours.

Reference Bases

The classical fasting reference metabolism base of Rubner and Lusk has been used throughout the experiments. The maintenance reference base of Forbes and his group was used in some experiments, but no special advantage was found. Forbes has been measuring SDA in rats above maintenance plane by feeding nutrients over and above the basal. In this method, SDA is found to be higher than the classical method where fasting basal metabolism is taken as the reference base. The difference between these bases has been attributed by Forbes to the SDA of tissue protein metabolism.

This, however, is not necessarily the case. The reactions that the nutrients undergo in fasting animals are not the same as in well-fed animals. In the latter plane, amino acids have a greater tendency to be deaminated and lost than in the fasting state where they help more in the building of the tissue proteins. These considerations led us to use the classical fasting reference base.

EXPERIMENTS, RESULTS AND INTERPRETATIONS

SDA of Mixed Diets

The SDA of various amino acids were measured with and without the addition of pyruvic acid. It is well known that most nutrients pass through the stage of pyruvic acid, and pyruvic acid forms the center of many cycles such as Szent Gyorgy's dicarboxylic acid cycle and Kreb's citric acid cycle. Pyruvic acid and the amino acids were neutralized to their sodium salts, fed and the oxygen consumption measured up to 8 hours.

It will be seen from the Figures (1-5) and Table 1 that pyruvic acid lowers the SDA of glutamic acid very markedly, but not much that of glycine. Pyruvic acid also lowers the SDA of cystine and phenylalanine. The results may be explained on the basis of the fact that transamination occurs more rapidly with dicarboxylic acids as glutamic, than with mono-
Carboxylic acids as glycine. Pyruvic acid acts as an amine acceptor and is transaminated to alanine. These reactions of transamination are reversible and do not lead to much heat production. So in case of glutamic acid, the SDA is reduced to a great extent by pyruvic acid. However, in the case of glycine, as it is not very active transaminatically, its SDA is not reduced significantly. Similar, though not so marked, results will be noted with lactic acid which is oxidized to pyruvic acid in the body. (Table 2, Figures 6-7.)

Table 1.—Effect of Feeding Pyruvic Acid on the Heat Increments of Some Amino Acids.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Nutrients</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. O₂ consumed per hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>16</td>
<td>Pyruvic acid 0.5 gm.</td>
<td>281</td>
<td>304</td>
<td>304</td>
<td>304</td>
<td>301</td>
<td>295</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>Cystine 0.5 gm.</td>
<td>281</td>
<td>318</td>
<td>301</td>
<td>333</td>
<td>330</td>
<td>218</td>
<td>348</td>
<td>343</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>Cystine 0.5 gm. + Pyruvic acid 0.5</td>
<td>281</td>
<td>284</td>
<td>312</td>
<td>309</td>
<td>285</td>
<td>304</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>Phenylalanine 0.5 gm.</td>
<td>281</td>
<td>324</td>
<td>340</td>
<td>343</td>
<td>354</td>
<td>329</td>
<td>334</td>
<td>351</td>
<td>22</td>
</tr>
<tr>
<td>16</td>
<td>Phenylalanine 0.5 gm. + pyruvic acid 0.5 gm.</td>
<td>281</td>
<td>323</td>
<td>329</td>
<td>318</td>
<td>306</td>
<td>332</td>
<td>323</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>Glutamic acid 0.5 gm.</td>
<td>281</td>
<td>357</td>
<td>365</td>
<td>374</td>
<td>382</td>
<td>383</td>
<td>388</td>
<td>379</td>
<td>34</td>
</tr>
<tr>
<td>16</td>
<td>Glutamic acid 0.5 gm. + pyruvic acid 0.5 gm.</td>
<td>281</td>
<td>298</td>
<td>300</td>
<td>309</td>
<td>306</td>
<td>287</td>
<td>306</td>
<td>306</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>Glycine 0.5 gm.</td>
<td>281</td>
<td>337</td>
<td>354</td>
<td>337</td>
<td>346</td>
<td>329</td>
<td>348</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Glycine 0.5 gm. + pyruvic acid 0.5 gm.</td>
<td>281</td>
<td>298</td>
<td>322</td>
<td>220</td>
<td>306</td>
<td>348</td>
<td>343</td>
<td></td>
<td>16</td>
</tr>
</tbody>
</table>

Fig. 1. The effect of feeding 0.5 gm. pyruvic acid on the heat increment of feeding 1 gm. glutamic acid. Note the marked depression of heat increment instead of the expected heat summation.
Fig. 2. The effect of feeding 0.5 gm. pyruvic acid on the heat increment of feeding 1.0 glycine. There is but little depression of the heat increment of glycine.

Fig. 3. The effect of feeding 0.5 gm. pyruvic acid on the heat increment of feeding 0.5 gm. cystine. There is some depression of the heat increment of cystine.
Fig. 4. The effect of feeding 0.5 gm. pyruvic acid on the heat increment of feeding 0.5 gm. phenylalanine. There is some depression of the heat increment of phenylalanine.

Fig. 5. The effect of feeding 1 gm. lactic acid on the heat increment of feeding 1 gm. glutamic acid. The heat increment of the mixture is much less than of the theoretical value.
### Table 2.—Effect of Feeding Lactic Acid on the Heat Increment of Some Amino Acids.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Nutrients</th>
<th>Hours after Feeding</th>
<th>Heat increment above basal percentage</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>0 1 2 3 4 5 6 7 8</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Lactic acid 1 gm.</td>
<td>281 323 334 323 320</td>
<td>300 326 15</td>
</tr>
<tr>
<td>16</td>
<td>Glycine 1 gm. + lactic acid 1 gm.</td>
<td>281 323 334 327 337</td>
<td>322 337 19</td>
</tr>
<tr>
<td>16</td>
<td>Glycine 1 gm.</td>
<td>281 337 354 337 346</td>
<td>329 348 22</td>
</tr>
<tr>
<td>16</td>
<td>Glutamic acid 1 gm.</td>
<td>281 351 365 374 382</td>
<td>380 379 34</td>
</tr>
<tr>
<td>16</td>
<td>Glutamic acid 1 gm. + lactic acid 1 gm.</td>
<td>281 565 392 300 351</td>
<td>348 374 29</td>
</tr>
</tbody>
</table>

Fig. 6. The effect of feeding 1 gm. lactic acid on the heat increment of feeding 1 gm. glycine.

Fig. 7. The time course in the change of resting metabolism following feeding 0.1 percent thiouracil.
Transamination and SDA

As transamination reactions are tested in vitro on a cellular level, while SDA is tested on the organismic level with intact animals, the works reported here have been mostly indirect. The SDA has been tested with nutrients with or without pyruvic acid which is expected to help in transamination. Similarly experiments have been conducted with pyridoxine which acts as a co-transaminase. Results and discussions have been presented under the respective headings. The major fact is that transamination reactions lower the SDA to a great extent.

Thyroid Gland and SDA

Thyroid Inactivation—In this experiment, secretion of the thyroid gland was gradually abolished by the administration of goitrogens. Kennedy and Purves (1941) observed that a Brassica seed diet caused enlargement of the thyroid gland. This effect was found to be due to the presence of allyl-thiourea in Brassica seeds (Kennedy, 1942). Since then, a large number of investigators, especially Astwood and Dempsey (1943) have made extensive studies of synthetic goitrogens. It is now established that there are two types of goitrogens, one whose action is inhibited by iodine injection and the other not inhibited by iodine. Thiouracil belongs to the latter group and has been widely used for its goitrogenic effect. These drugs act by preventing the formation of thyroxine in the thyroid gland and not by neutralizing the action of thyroxine on the metabolically active tissues in the periphery. The immediate action is by way of the pituitary by increasing the production of thyrotrophic hormone. MacKenzie (1947) has found that thiouracil action is inhibited by iodide, while sulfaguanidine is not.

A group of 8 albino rats were used in this experiment. Their SDA was tested with respect to glucose, glycine, glutamic acid and tyrosine. Then they were put on a diet containing 0.1 per cent of thiouracil and their resting metabolism studied daily. When the metabolism decline was at its lowest, the rats were taken to be comparable to those which had been completely thyroidectomized. SDA was again measured on these rats with respect to glucose, glycine, glutamic acid and tyrosine.

Figures 8-10 and Table 3 indicate that the SDA is markedly less in thiouracil-fed rats. The time course of development of SDA is prolonged. This delayed development of SDA perhaps cannot be ascribed entirely to delayed absorption, for absorption of pure amino acids is rather rapid; moreover, the total SDA for the period of 8 hours was lower in the thiouracil-fed than the control group of rats. As a matter of fact, after feeding glycine and glutamic acid the heat production seemed to fall below the level of the controls.
### Table 3.—Effect of Feeding Thiouracil on Heat Increment of Some Amino Acids.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Nutrients</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Heat increment above basal percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Glutamic acid 0.5 gm.</td>
<td>222</td>
<td>286</td>
<td>310</td>
<td>316</td>
<td>305</td>
<td>305</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiouracil fed</td>
<td>Glutamic acid 0.5 gm.</td>
<td>232</td>
<td>233</td>
<td>240</td>
<td>249</td>
<td>258</td>
<td>278</td>
<td>292</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Glycine 0.5 gm.</td>
<td>272</td>
<td>294</td>
<td>318</td>
<td>326</td>
<td>325</td>
<td>325</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiouracil fed</td>
<td>Glycine 0.5 gm.</td>
<td>233</td>
<td>233</td>
<td>247</td>
<td>247</td>
<td>263</td>
<td>266</td>
<td>273</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Tyrosine 0.5 gm.</td>
<td>272</td>
<td>307</td>
<td>307</td>
<td>291</td>
<td>296</td>
<td>296</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiouracil fed</td>
<td>Tyrosine 0.5 gm.</td>
<td>233</td>
<td>247</td>
<td>254</td>
<td>265</td>
<td>268</td>
<td>247</td>
<td>256</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8. The SDA of 0.5 glutamic acid in control animals as compared to that of the thiouracil-fed animals. There is some depression of the SDA in the latter animals.

![Graph showing SDA of glutamic acid](image)

Fig. 9. The SDA of 0.5 gm. glycine in control animals as compared to that in thiouracil-fed animals. There is some depression of the SDA in the latter animals.

![Graph showing SDA of glycine](image)
It is well known that in hypothyroid conditions there is a tendency to increased deposition of proteins under the skin and in some other tissues. Under the conditions of greater anabolism, more amino acids are deposited with decrease in the SDA.

The figures indicate that SDA's of glutamic acid and of glycine but not of tyrosine are reduced by feeding thiouracil. The difference in SDA of tyrosine may be explained by assuming that it competes with thyroxine and may displace it in the tissue cells, with consequent reduction of heat production. The SDA of tyrosine might be expected to diminish by the presence of thyroxine in the metabolically active centers of tissue cells. But in thiouracil-fed rats, this inhibitory action of thyroxine is absent and the SDA of tyrosine is increased compared to other nutrients.

To test this hypothesis of competition between tyrosine and thyroxine several anatomic and metabolic experiments were conducted. Anatomic experiments consisted in feeding a diet containing 0.1 per cent thiouracil to deplete the thyroxine stores of body and also to prevent the secretion of thyroxine by the thyroid gland. These rats were then divided into two groups, one group getting 10 per cent tyrosine in the diet, the other tyrosine plus thiouracil. Half of the thiouracil-fed and half of the thiouracil-tyrosine fed rats were then injected with 5 mcgm. thyroxine per 100 gm. body weight daily for 3 weeks. All the rats were then sacrificed and their thyroids weighed. The thyroids of the tyrosine-thiouracil fed rats were 12 per cent heavier than the thiouracil-fed rats. This indicates that tyrosine partly inhibited the action of thyroxine.
Abeline (1935) fed thyroid extract to rats; and when the metabolism was increased, he fed tyrosine and observed a lowering of heat production in the tyrosine-fed rats. He also found that tyrosine reduced the basal metabolism of guinea pigs which were injected with anterior pituitary thyrotrophic hormone, but he did not find any histological change in the thyroid. Woolley (1947) has found that the metabolic action of thyroxine can be reduced by derivatives of tyrosine.

The inhibitory action of thyroxine might perhaps be as nonspecific as that of any other amino acid. To test this possibility, 5 mcgm. of thyroxine were injected daily to two groups of rats fed 0.1 per cent thiouracil; and one of these groups was getting in addition 10 per cent glycine. It was found that the glycine-fed rats had 20 per cent smaller thyroids than the controls. These results indicate that, with the exception of tyrosine, amino acids augment the action of thyroxine; but that tyrosine exerts a significant inhibitory action on the thyrotrophic hormone inhibitory effect of thyroxine on the anterior pituitary.

The metabolic experiments consisted in feeding tyrosine (10 per cent) to a group of rats and injecting 100 mcgm. thyroxine per 100 gm. body weight to these and to the controls, and measuring their resting oxygen consumption for 7 days. It was found that the metabolism of the tyrosine-fed rats increased to a smaller extent than of the control rats.

These experiments tend to prove the hypothesis that the SDA of tyrosine is depressed by the competitive action of thyroxine at the metabolically active centers of tissue cells; they also explain why the SDA of tyrosine is not reduced significantly in thiouracil fed rats.

**Pyridoxine and SDA**

As pyridoxine is intimately related to transamination, it was expected that it might increase the content of coenzyme that would combine with apo-transaminase and thus might increase the transaminase activity. As discussed in the section on transamination and SDA it was thought desirable to compare the SDA of different foodstuffs with and without pyridoxine. Glucose, in the form of cerelose was used. Four rats received 0.5 gm. glucose alone and the other 4 rats received the same amount of glucose together with 5 mg. pyridoxine each. In the same way the SDA of glycine, glutamic acid and tyrosine were tested. The results as shown in the following Figures 11-15 and Tables 4-5, indicate that pyridoxine definitely lowered the SDA of glucose, glutamic acid and tyrosine, but not of glycine.

Transamination is more marked in dicarboxylic acids such as glutamic than in monocarboxylic such as glycine. Glucose when oxidized gives pyruvic acid which acts as a receptor for the ammonia formed from amino
Table 4.—Effect of Feeding Pyridoxine on Heat Increment of Some Amino Acids.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Nutrients</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. O2 consumed per hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Glutamic acid 0.5 gm.</td>
<td>169</td>
<td>206</td>
<td>228</td>
<td>211</td>
<td>203</td>
<td>230</td>
<td>232</td>
<td>222</td>
<td>82</td>
</tr>
<tr>
<td>16</td>
<td>Glutamic acid 0.5 gm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Pyridoxine 5 mgm.</td>
<td></td>
<td>169</td>
<td>174</td>
<td>177</td>
<td>211</td>
<td>206</td>
<td>198</td>
<td>196</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Glycine 0.5 gm.</td>
<td>169</td>
<td>174</td>
<td>194</td>
<td>213</td>
<td>188</td>
<td>188</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Glycine 0.5 gm.</td>
<td></td>
<td>169</td>
<td>172</td>
<td>178</td>
<td>196</td>
<td>198</td>
<td>193</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Tyrosine 0.5 gm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Pyridoxine 5 mgm.</td>
<td></td>
<td>169</td>
<td>215</td>
<td>201</td>
<td>193</td>
<td>208</td>
<td>203</td>
<td>194</td>
<td>204</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>Glucose 0.5 gm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Pyridoxine 5 mgm.</td>
<td></td>
<td>169</td>
<td>210</td>
<td>250</td>
<td>250</td>
<td>220</td>
<td>201</td>
<td>194</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.—Effect of Feeding Pyridoxine Deficient Diets on Heat Increment of Some Amino Acids.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Nutrients</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. O2 consumed per hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 16</td>
<td>Glutamic acid 0.5 gm.</td>
<td>196</td>
<td>212</td>
<td>220</td>
<td>227</td>
<td>220</td>
<td>214</td>
<td>210</td>
<td>225</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Pyridoxine 12</td>
<td>Glutamic acid 0.5 gm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>Glutamic acid 0.5 gm.</td>
<td>200</td>
<td>220</td>
<td>239</td>
<td>231</td>
<td>237</td>
<td>219</td>
<td>224</td>
<td>247</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Control 16</td>
<td>Glycine 0.5 gm.</td>
<td>196</td>
<td>221</td>
<td>202</td>
<td>204</td>
<td>200</td>
<td>210</td>
<td>206</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxine 12</td>
<td>Glycine 0.5 gm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>Glycine 0.5 gm.</td>
<td>200</td>
<td>213</td>
<td>214</td>
<td>211</td>
<td>210</td>
<td>222</td>
<td>228</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 11. The effect of feeding 5 mgm. pyridoxine on the heat increment of 0.5 glutamic acid. There is a significant depression of the heat increment in the pyridoxine-treated animals.
Fig. 12. The effect of feeding 5 mgm. pyridoxine on the heat increment of feeding 0.5 gm. tyrosine. There is a significant depression of the heat increment in the pyridoxine treated animals.

Fig. 13. The effect of feeding 5 mgm. pyridoxine on the heat increment of feeding 0.5 gm. glycine. There is no significant depression of the heat increment.
Fig. 14. The effect of feeding 5 mgm. pyridoxine on the heat increment of feeding of 0.5 gm. glucose. There is a significant depression of the heat increment in the pyridoxine-treated animals.

Fig. 15. The SDA of 0.5 gm. glutamic acid in the control rats as compared to that of the pyridoxine-deficient rats. There is some increase in the SDA in the deficient rats.
acid, i.e., it becomes active in transamination. Pyridoxal phosphate acts as a codecarboxylase for tyrosine decarboxylase and thus may be helpful in the metabolism of tyrosine and in lowering its SDA. Glycine, however, is but slightly active transaminationally, unless it is associated with other active substances, such as dicarboxylic acid. This harmonizes with the observations that pyridoxine did not lower the SDA of glycine.

These discussions together with figures make it clear how pyridoxine lowers the SDA of many foodstuffs. In case of glycine or other monocarboxylic amino acids, it is likely that if there is enough pyruvic acid or related compounds in the liver, pyridoxine will lower the SDA of glycine also. In case of a mixed diet, therefore, sufficient amount of pyridoxine will lower the SDA of food stuff in general.

**Vitamin E and SDA**

A group of rats was fed 5 mgms. of tocopherol in corn oil base on the day previous to experiment. The basal metabolism of the treated rats fell slightly below the control untreated rats. The difference, however was not significant.

The SDA of glycine and glutamic acid were estimated in similar manner.

The Figures 16-17 and Table 6 will show that the SDA of glycine in tocopherol-treated rats is lower than that in the control rats. The SDA of glutamic acid was, however, unaffected. This shows that vitamin E does not effect the transamination process. It appears that Hottinger's hypothesis is essentially correct. Vitamin E is known to help in creatine metabolism. So it is likely that tocopherols help the conversion of glycine into creatine, part of which process is reversible and thereby lowers the SDA.

**Vitamin A and SDA**

Metabolic and anatomic observations were made on the following groups of white rats: controls; fed percomorph oil containing about 30,000 I.U. vitamin A per day; injected 1 mgm. thyroxine per kgm. body weight

**Table 6.—Effect of Feeding α-Tocopherol on Heat Increment of Some Amino Acids.**

<table>
<thead>
<tr>
<th>Observations</th>
<th>Nutrients</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Heat increment above basal percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 Glycine 0.5 gm.</td>
<td>208</td>
<td>238</td>
<td>258</td>
<td>270</td>
<td>281</td>
<td>229</td>
<td>241</td>
<td>229</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Glycine 0.5 gm. + α-tocopherol 5 mgm.</td>
<td>197</td>
<td>213</td>
<td>230</td>
<td>217</td>
<td>225</td>
<td>207</td>
<td>217</td>
<td>201</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Glutamic acid 0.5 gm.</td>
<td>208</td>
<td>215</td>
<td>220</td>
<td>229</td>
<td>243</td>
<td>243</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Glutamic acid 0.5 gm. + α-tocopherol 5 mgm.</td>
<td>197</td>
<td>203</td>
<td>208</td>
<td>216</td>
<td>223</td>
<td>221</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 16. The effect of feeding 5 mgm. α-tocopherol on the heat increment of feeding 0.5 gm. glycine. There is a significant depression in the heat increment in the treated animals.

Fig. 17. The effect of feeding 5 mgm. α-tocopherol on the heat increment of feeding 0.5 gm. glutamic acid. There is no depression of the heat increment in the treated animals.
at the beginning of each weekly experiment; injected thyroxine and fed percomorph oil in the above dosages; fed thio uracil (feed contained 0.1 per cent thio uracil); thio uracil plus vitamin A; dinitro phenol 2.5 mgm. per kgm. body weight; dinitrophenol plus vitamin A; partly and completely oxidized vitamin A.

The effect of vitamin A, of thyroxine, and of combinations of the two on basal metabolism are graphed in Figure 18 and the effects on thyroid size are listed in Table 7.

Figure 18 shows that vitamin A in large doses depresses the rate of oxygen consumption of normally-fed rats by about 10 per cent; and it depresses the metabolism of the thyroxine-injected rats by about 20 per cent. These results have been confirmed by repeating the experiments several times. Feeding potassium iodide lowered slightly the metabolism in the normal but not in the thyroxine-treated rats.

Table 7 shows that the rats that received the excess vitamin A supplement had smaller thyroids than those that did not get the vitamin A supplement. The males were affected somewhat less by the vitamin A than females or castrated males. The sex difference, however, was not great.

"Completely oxidized" vitamin A (air bubbled through the hot oil for 6 hrs.) had no effect; but "partly oxidized" vitamin A (air bubbled 1 to 2 hrs.) exerted the thyroid-reducing effect.

Table 7 and Figure 18 demonstrate in a quantitative manner that excess vitamin A depresses the metabolic rate and reduces the thyroid size of normal, thio uracil-treated and thyroxine-treated rats. What are the mechanisms of these effects?

Belasco and Murlin (1940) suggested that since vitamin A has a double bond, it may become iodinated and relieve certain hyperthyroid conditions,

### Table 7.—The Influence of Vitamin A, Thyroxine, Thio uracil and Dinitrophenol on Thyroid Weight.

<table>
<thead>
<tr>
<th>Subject</th>
<th>No. of rats</th>
<th>Average body weight gm.</th>
<th>Thyroid weight mgm. per 100 gm. body wt.</th>
<th>Decrease in thyroid weight percentage</th>
<th>Statist. Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, female</td>
<td>10</td>
<td>148.7</td>
<td>10.65</td>
<td>1.920</td>
<td>7.84-11.85</td>
</tr>
<tr>
<td>Vitamin A, female</td>
<td>10</td>
<td>147.3</td>
<td>6.37</td>
<td>1.030</td>
<td>4.87-8.61</td>
</tr>
<tr>
<td>Thio uracil, female</td>
<td>10</td>
<td>105.7</td>
<td>31.29</td>
<td>4.651</td>
<td>28.37-40.49</td>
</tr>
<tr>
<td>Thio uracil plus vitamin A, female</td>
<td>10</td>
<td>170.4</td>
<td>27.48</td>
<td>4.125</td>
<td>23.11-37.19</td>
</tr>
<tr>
<td>Control, male</td>
<td>8</td>
<td>208.5</td>
<td>8.18</td>
<td>0.248</td>
<td>7.21-9.31</td>
</tr>
<tr>
<td>Vitamin A, male</td>
<td>8</td>
<td>228.0</td>
<td>5.72</td>
<td>0.102</td>
<td>5.53-6.27</td>
</tr>
<tr>
<td>Dinitrophenol, male</td>
<td>8</td>
<td>170.1</td>
<td>5.22</td>
<td>0.171</td>
<td>4.45-6.50</td>
</tr>
<tr>
<td>Dinitrophenol plus vitamin A, male</td>
<td>6</td>
<td>183.3</td>
<td>4.81</td>
<td>0.122</td>
<td>4.45-5.62</td>
</tr>
<tr>
<td>Control, castrated</td>
<td>10</td>
<td>158.4</td>
<td>7.42</td>
<td>0.070</td>
<td>6.96-7.56</td>
</tr>
<tr>
<td>Vitamin A, castrated</td>
<td>7</td>
<td>208.6</td>
<td>4.58</td>
<td>0.117</td>
<td>4.43-5.50</td>
</tr>
</tbody>
</table>

*There is less than one chance in one hundred trials that the differences between thyroid weights could have arisen by chance. The tests for significance were made by means of Snedecor's Tables (see Snedecor, G. W., Statistical Methods, p. 174, 1937).

N.S.—Not significant.
such as increased metabolic rate, as do some other iodine compounds. This suggestion was tested by feeding potassium iodide to the thyroxine-treated rats. The potassium iodide depressed slightly the metabolic rate of the controls but not of those treated with thyroxine.

We should like to suggest that when vitamin A is fed in excess, its double bond takes up the iodine from the thyroxine, thus rendering the thyroxine ineffective and thereby reducing the metabolic rate. The iodinated vitamin A so formed may, however, depress the secretion of the anterior pituitary thyrotrophic hormone as thyroxine does, and thus diminish thyroid size. This seems to explain quite simply how excess vitamin A reduces thyroid size (Table 7) by depressing the production of thyrotrophic hormone; and how it depresses the metabolic rate (Figure 18) by inactivating the thyroxine.

Other simple explanations may be suggested which, however, may be criticised. For instance, the recent dramatic developments in the field of anti-vitamins, as illustrated by the effectiveness of the sulfonamides and related substances is displacing essential vitamins, suggests that excess vitamin A may act analogously as an anti-thyroid. Vitamin A in excess

---

**Fig. 18.** The effect of excess vitamin A feeding on the basal metabolism and on the thyroxine-induced hypermetabolism of rats.
may displace thyroxine from metabolically essential systems and thus reduce the metabolic rate. The thyroxine thus set free will depress the production of thyrotrophic hormone and therefore reduce thyroid size. The objection to this theory is that excess vitamin A also decreases the thyroid size of thiouracil-fed rats (Table 7), that is when the thyroxine stores were presumably exhausted. This objection does not hold in the preceding theory in which it is assumed that the iodinated vitamin A, not the thyroxine, depresses the secretion of thyrotrophic hormone and therefore reduces the thyroid size. We are attempting to determine the effects of iodinated vitamin A on thyrotrophic hormone secretion, or thyroid size.

Other, more far-fetched, explanations come to mind. For instance, since vitamin A has an unsaturated linkage, it may act as a redox system perhaps as a part of an electron-transfer system and compete with some oxidative enzyme. The vitamin A may thus depress tissue oxidation indirectly and reduce the metabolic rate, just as if the thyroid function were depressed. This hypothesis was tested, rather crudely, by partly oxidizing the vitamin A as previously explained, when it continued to depress the thyroid size; but if the vitamin A was completely oxidized, the thyroid-depressing effect was lost.

Having established the fact that vitamin A depresses the metabolism-stimulating effect of thyroxine, one wonders whether vitamin A might not similarly depress the metabolism-stimulating effect of dinitrophenol. This idea was subjected to experimental test but could not be demonstrated perhaps because, unlike thyroxine, dinitrophenol acts very rapidly. Moreover, dinitrophenol is toxic, it blocks normal glycogen synthesis and upsets the normal metabolic sequence (Clifton 1923, 1937; Doudoroff 1940).

**Thiamine and SDA**

SDA was measured by feeding glucose with and without added thiamine. The following Figure 19 and Table 8 show that the SDA of glucose is increased when thiamine is added. Experiments were undertaken to include galactose, sucrose, and lactose. The results do not appear to be conclusive. The present work confirms the results of Ring (1943, 1947) and leads to the conclusion that thiamine increases the SDA of glucose by accelerating the synthesis of fat from glucose.

**Table 8.—Effect of Feeding Thiamine on Heat Increment of Some Amino Acids.**

<table>
<thead>
<tr>
<th>Observations</th>
<th>Nutrients</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Heat increment above basal percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>Glucose 0.5 gm.</td>
<td>180</td>
<td>295</td>
<td>265</td>
<td>265</td>
<td>230</td>
<td>274</td>
<td>248</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Glucose 0.5 gm. + Thiamine 200 megm.</td>
<td>180</td>
<td>302</td>
<td>281</td>
<td>266</td>
<td>275</td>
<td>293</td>
<td>248</td>
<td>58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 19. The effect of feeding 200 mcgm. thiamine on the heat increment of feeding 0.5 gm. glucose. There is some increase of heat increment in the treated animals.

**SUMMARY AND CONCLUSIONS**

1. This dissertation is concerned with the problem of the heat increment of feeding, usually referred to as specific dynamic action or specific dynamic effect. The latter term is used by Forbes and his group who employed the maintenance plane as the reference base. In this work the reference base has been the classical fasting plane as used by Rubner and Lusk, and therefore the term specific dynamic action (SDA) has been retained. The literature on SDA has been reviewed up to date.

2. The SDA of pure carbohydrates and various amino acids has been measured on albino rats in a modified Regnault-Reiset apparatus. Carbohydrates have been administered in the form of 3.5 ml. water solutions. The amino acids were neutralized with sodium hydroxide to phenolphthalein and fed in 3.5 ml. water solutions. All solutions were brought to 26° C. before feeding. The metabolism measurements were made at about 26° C.

3. The animals were habituated to live in the metabolism apparatus for 2 hours daily during a period of 3 to 4 weeks, until their oxygen consumption attained a steady level. This pretreatment was found effective in eliminating daily variations. Variations were also eliminated by running two control rats.

4. The SDA of mixed diets is usually lower than the sum of the individual constituents when fed separately, except in case of hexoses. The
observations of Carpenter and Lee (1932) that there is more heat production following feeding a mixture of galactose and other monosaccharides than feeding them separately are believed to be due to competitive inhibition of glucose to be oxidized and to be converted into fat. This may cause higher SDA of glucose and galactose mixture than when each is fed separately.

5. Pyruvic acid has been found to lower the SDA of glutamic acid and tyrosine but not of glycine. This is thought to be due to the fact that pyruvic acid acts as an amino-acceptor of glutamic acid and tyrosine which are active transaminatically. Tyrosine is believed to act as a pseudodicarboxylic amino acid due to its parahydroxy group which may behave as a phenolic acid group. Glycine cannot be active transaminatically unless there is a primary amino-donator.

6. The effect of lactic acid on lowering the SDA of glutamic acid and tyrosine is similar to that of pyruvic acid though less markedly so. Lactic acid lowers the SDA of glutamic acid more than of glycine. Lactic acid is oxidized in the liver and in other body tissues to pyruvic acid which may act as an amino acceptor. The SDA of the glutamic and lactic acids mixture is much lower than of the two taken separately.

7. The thyroid was inactivated by feeding thiouracil. The resting metabolism fell on the second day by 10 per cent and gradually declined by the 18th day to 62 per cent minimum. Thiouracil prevents the secretion of thyroxine by the thyroid gland. When the metabolism was at its lowest, the SDA of several nutrients was tested again. It was found that the SDA of all nutrients decreased significantly except that of tyrosine.

8. The SDA of tyrosine appears to be different from that of other amino acids. Tyrosine has a structural similarity to thyroxine and is expected to behave like an antithyroxine. It was thought that the normal SDA of tyrosine is lowered by displacement of tyrosine from the metabolic centers of tissue cells by thyroxine. In the thiouracil-fed rats there was a general depression of SDA of nutrients but in the case of tyrosine the general depression of SDA is counteracted by the absence of competitive action of thyroxine, which normally displaces tyrosine from tissue cells. This hypothesis was tested by anatomical and metabolic experiments. While the 10 per cent glycine-fed rats had 20 per cent smaller thyroids than the controls, the 10 per cent tyrosine-fed rats had thyroids which were 12 per cent heavier than the controls.

The metabolic experiments consisted in measuring the resting oxygen consumption of rats injected with thyroxine with and without 10 per cent tyrosine in the diet. It was found that the metabolism of tyrosine-fed rats increased to a smaller extent than of the control rats. These experiments
explain why the SDA of tyrosine is not reduced significantly in thiouracil-fed rats.

9. Pyridoxine administration lowered the SDA of glucose, glutamic acid, and tyrosine, but not of glycine. As pyridoxine forms the coenzyme for transaminase, these results lead to the belief that the SDA is reduced by transamination. The SDA of glycine is not affected by pyridoxine because it is not active transaminationally.

The lowering of the SDA of glucose by pyridoxine is believed to be due to transamination of pyruvic acid formed from oxidation of glucose. The SDA of glucose is probably reduced normally by transamination of some of its oxidation products such as pyruvic acid.

10. Pyridoxine deficiency raises the SDA of glutamic acid, but not of glycine. Pyridoxine deficiency leads to lowered level of cotransaminase and this explains the observed results.

11. Vitamin E has been found to lower the SDA of glycine, but not of glutamic acid. This is believed to be due to the fact that vitamin E does not influence transamination, but it somehow helps in the synthesis of creatine from glycine, as suggested by Hottinger. Vitamin E fed by mouth slightly lowers the basal metabolism.

12. Vitamin A in heavy doses lowers the basal metabolism by 10 per cent. It decreases the thyroid weight. It also lowers the metabolism-stimulating properties of thyroxine. This is thought to be due to the combination of double bonds of the vitamin A with the iodine of the thyroxine. This vitamin A-iodide compound depresses the secretion of thyrotrophic hormone by the anterior pituitary just as thyroxine does and therefore reduces the thyroid size as well as the metabolism.

Vitamin A may also act as an antihormone by competing with thyroxine at the metabolically active centers of the tissue cells. Vitamin A is therefore expected to lower the SDA of nutrients, but indirectly by way of the thyroid.

13. Thiamine increases the SDA of glucose probably by helping the conversion of glucose into fat. Thiamine has not been found to affect the SDA of other carbohydrates significantly.

14. The SDA of amino acids appears to be due largely to deamination in the liver. This deamination is counterbalanced by the simultaneous transamination in which the ammonia is accepted by an α-keto acid, the latter being converted into amino acid. Transamination as a reversible process, reduces the irreversible steps of deamination which cause the energy loss as heat, and thereby reduces the SDA of amino acids. The role of transamination in SDA is our contribution to a new concept in SDA and has therefore been discussed in detail.
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