The effect of the extent of hydrolysis in casein on its specific dynamic action in the rat

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1. Adult female rats given a diet containing 200 g casein/kg showed an increase in heat production which reached a maximum in 10–20 min after completion of food intake.

2. Replacement of casein in the diet by enzymic hydrolysates of casein of different extents of hydrolysis (pepsin for 1 or 3 h and pancreatin for 6, 12 or 24 h) resulted in a decrease in metabolic rate (stimulation) in the rat, reaching a maximum of 61·8 % of the control value (non-hydrolysed casein).

3. The specific dynamic action of casein and casein hydrolysate was inversely proportional to the amount of amino-nitrogen released.

4. On the basis of the experimental findings it may be concluded that the synthesis and secretion of digestive enzymes were the main causes of the increase in metabolic rate after protein ingestion.

The increase in the metabolic rate after food intake, referred to not quite correctly as the specific dynamic action (SDA), has been known to physiologists for a long time. However, the nature of this phenomenon has not yet been definitely clarified. The primary view that the digestion process is the reason for SDA was questioned by Rubner (1902) and Borsook (1936). In other studies, attention has been focused on proteins in respect of their high heat-producing effect, and a positive correlation was found between SDA and the quantity and kind of the ingested amino-acids (Terroine & Bonnet, 1926; Borsook, 1936), the quantity of amino-nitrogen undergoing deamination, as well as N excretion in the urea (Borsook & Winegarden, 1931). Recent studies (Garrow & Hawes, 1972; Gawęcki, Jeszka, Urbanowicz & Gardyś-Modrzyńska, 1978) have suggested SDA to be a reflexion of protein synthesis rather than of protein catabolism. In other reports the variation in SDA was attributed to nutritional status (Mitchell, 1943), endocrine gland secretion (Magnus-Levy, 1894; Rubner, 1902), carbon metabolism (Borsook, 1936), body thermo-regulation (Śimek, 1975), as well as the influence of sensory stimuli (Tanaka & Yano, 1975).

Since the highest stimulation of metabolic rate has been found normally within the first hour after protein intake (Tanaka & Yano, 1975; Gawęcki *et al.* 1978) it is assumed that the functioning of, and the processes occurring in the digestive tract can be of some influence on the magnitude of SDA, even though the intravenous administration of an amino acid mixture has been shown to increase the metabolic rate (Benedict & Emmes, 1912).

This paper reports the results of studies concerning the effect of the extent of hydrolysis in casein on its SDA in the rat.

MATERIALS AND METHODS

Experimental animals and conditions

Wistar albino female rats of average body-weight 210 g (± 10 g) were used in the experiments. In the initial period they were fed on a pelleted breeding diet (LSM; OHZD, Ustanów, Poland) containing (/kg) 180 g protein and 12·977 MJ metabolizable energy (ME). The rats had access to food twice daily (09.00–11.00 hours and 15.00–16.00 hours).

Experimental animals were fasted for 12 h before they were placed in metabolism cages (Simax; Liberec, Czechoslovakia), and fed on experimental diets which were pelleted. The diet contained (g/kg): wheat starch 452, sunflower oil 126, sucrose 200, potato starch 50, cod-liver oil (as a source of retinol and cholecalciferol) 20, mineral mixture (Phillips & Hart, 1935) 40, vitamin mixture (Miller & Bender, 1955) 10; the protein source was casein with free amino-N contents varying from 175 to 935·8 mg/g. The variation in free amino-N content was achieved by enzymic hydrolysis of casein using pepsin (for 1·5 and 3·0 h), pancreatin (for 24 h), pepsin (for 3 h) and pancreatin (for 6, 12 and 24 h). Hydrolysates were neutralized with sodium hydroxide, dried under vacuum and ground. Total N was determined in the hydrolysates using the Kjeldahl method, and followed by determination of N non-precipitable in 150 g trichloroacetic acid/l (free amino-N).

In all experimental diets, the total N content was approximately 11·5 g/kg, and the ME (determined according to Miller & Payne, 1959) 16·953 MJ/kg.

On each occasion, after completion of respiration measurements, the amount of diet consumed by each rat was estimated.

Measurement of the metabolic rate

Measurement of the metabolic rate was carried out between 09.00 and 12.00 hours, and started immediately after completion of food intake by the rat. Metabolic rate was determined using a closed-circuit respiration technique. The oxygen and carbon dioxide contents in the air in the metabolism cage were determined continuously for 60 min using a gas analyser (Mijnhardt, Odijk, Holland), connected to a recorder and Orsat apparatus (Gawęcki, Urbanowicz, Lis & Buchowski, 1976). After 60 min the air in the cage was completely exchanged for a period of 3 min and the measurements were continued for another 60 min. Changes in the concentration of respiratory gases in the cage, after corrections for pressure and temperature, served as the basis for calculating the O_2 consumption and the volume of CO_2 produced within subsequent periods of 10 min. Respiratory quotient (RQ; O_2 intake : CO_2 produced) was calculated and the amount of energy produced (metabolic rate) was calculated as kJ/kg metabolic body-weight ($kg^{0.75}$).

Fasting metabolism (basal metabolic rate; BMR) was determined in the same way.

Statistical analysis

Statistical analysis was carried out using analysis of variance and Student's *t* test. The coefficient of variation for the results was 5·4.

RESULTS

N consumption and the total amount of heat produced by the rat during 120 min after intake of diet containing 200 g of non-hydrolysed and enzymically hydrolysed casein/kg are shown in Table 1. N consumption by the animals fed on diets with casein of high extent of hydrolysis (above 70% free amino N/g N) appeared approximately twice as high as in groups of rats given diets with casein non-hydrolysed or hydrolysed with pepsin for 1·5 h (below 10% free amino N/g N). Despite considerable differences in N consumption between groups of animals given casein of different extent of hydrolysis, the total amount of heat ($kJ/kg^{0.75}$) produced by the rat during 120 min has changed negligibly. Correlation coefficients between N consumption and total heat production calculated for particular groups of rats were high and varied from +0·78 to +0·96.

The total amount of heat produced by the rat within 120 min of completion of diets containing 200 g non-hydrolysed and enzymically hydrolysed casein/kg is presented in

Table 1. *Total and additional (specific dynamic action; SDA) heat production in the rat within 120 min after intake of diets containing non-hydrolysed casein and casein enzymically hydrolysed to different extents*

(Mean values with their standard errors for six rats/group)

Group no. / Hydrolysis procedure	1 No hydrolysis	2 Pepsin for 1·5 h	3 Pepsin for 3 h	4 Pepsin for 3 h + pancreatin for 6 h	5 Pepsin for 3 h + pancreatin for 12 h	6 Pepsin for 3 h + pancreatin for 24 h	7 Pancreatin for 24 h
Extent of hydrolysis (%) (free amino-nitrogen: total N)	1·73	8·56	25·00	52·78	72·97	93·58	89·02
Nitrogen intake (g)							
Mean	0·0872	0·0858	0·1104	0·1235	0·1767	0·1710	0·1960
SE	0·0068	0·0075	0·0096	0·0087	0·0140	0·0139	0·0259
Total heat production (kJ/kg$^{0·75}$)							
Mean	43·02	39·61	39·00	38·10	43·79	38·37	45·61
SE	1·54	1·13	1·65	1·28	2·39	0·86	1·56
Correlation coefficient between total heat production (kJ/kg$^{0·75}$) and N intake in each group of rats	+0·95	+0·96	+0·92	+0·88	+0·78	+0·96	+0·88
Heat production (metabolic rate) (kJ/kg$^{0·75}$ per g N)‡							
Mean	493·4	461·6	353·3	308·5	247·8	224·4	232·7
SE	17·6	13·2	14·8	10·4	13·5	5·0	17·9
SDA† (kJ/kg$^{0·75}$ per g N)‡							
Mean	224·5	183·7	137·7	117·8	102·4	85·6	95·1
SE	15·2	24·1	15·6	14·6	8·7	12·7	11·8

Statistically significant differences in heat production (kJ/kg$^{0·75}$ per g N) between experimental groups

Group no.							
1	—	NS	***	***	***	***	***
2	NS	—	***	***	***	***	***
3	***	***	—	*	***	***	***
4	***	***	*	—	**	***	**
5	***	***	***	**	—	NS	NS
6	***	***	***	***	NS	—	NS
7	***	***	***	**	NS	NS	—

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Basal metabolic rate 21·27–23·80 kJ/kg$^{0·75}$.
‡ N consumed in the diet.

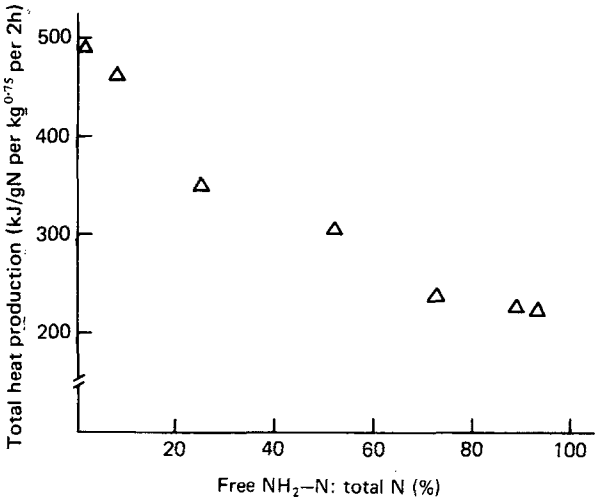

Fig. 1. Relationship between total heat production (kJ/g nitrogen per kg$^{0.75}$ per 2 h in the rat within 2 h after intake of a diet containing enzymically hydrolysed casein and the extent of hydrolysis (%) (free amino-N : total N).

Table 1. The highest heat production (/g total N consumed and /kg$^{0.75}$) was found in the rats fed on the diet containing non-hydrolysed casein. In the experimental diet in which casein subjected to pepsin action for 1·5 h was the only source of protein a negligible, statistically non-significant decrease in the total heat production was observed, as compared with the control group of animals fed on the diet containing non-hydrolysed casein. However, increasing the period of casein digestion with pepsin from 1·5 to 3 h resulted in a significant decrease in total heat production within 120 min after food intake, as compared with the control group. In vitro hydrolysis of casein with pepsin, followed by hydrolysis with pancreatin for 6, 12 or 24 h, resulted in a further significant decrease in heat production in the rat; the value for the 24 h pancreatin hydrolysate was 54·5% of that for the non-hydrolysed casein. When casein was subjected to pancreatin action only for 24 h, there were no significant differences in heat production when compared with that of casein previously hydrolysed with pepsin.

Similar results were obtained with regard to changes in the additional heat production (SDA) above the level observed in fasted rat (BMR); maximum decrease in the heat-producing effect of casein (61·8%) of the basal value was obtained with enzymically hydrolysed casein with the highest extent of hydrolysis (93·58%).

The experimental results indicated a high correlation between the free amino-N content of the dietary protein and the amount of heat produced by rat within 120 min after food intake (Fig. 1) ($r - 0{\cdot}92$).

In Figs 2 and 3 the dynamics of changes in heat production in rat are presented for 10 min periods, within the first 60 min after intake of diets containing non-hydrolysed and enzymically hydrolysed casein. The highest stimulation effect of the metabolic rate resulting from food intake was found within the first 10 min for all diets. Heat production gradually decreased with the period after completion of food intake by the rat, and within the last 10 min of the second hour of measurements, approached the level of heat production by fasted animals (BMR) (mean 2·0 kJ/kg$^{0.75}$ per 10 min). In the final 10 min period, the metabolic rate in the rats given the hydrolysed-casein diets, varied from 2·61 to 3·26 kJ/kg$^{0.75}$ per 10 min.

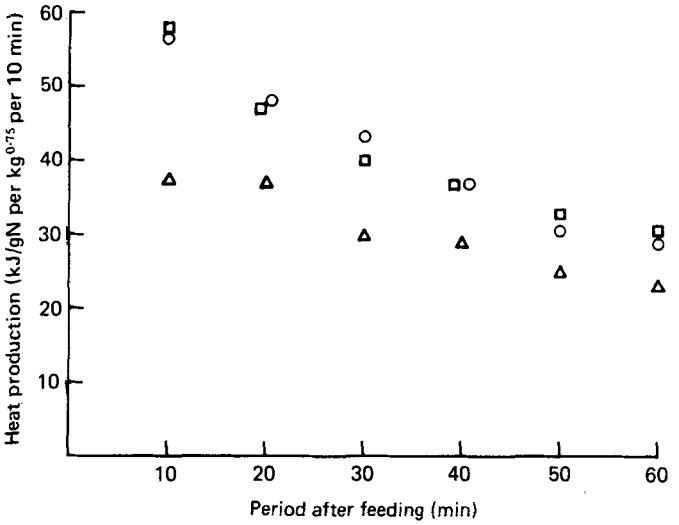

Fig. 2. Dynamics of changes in heat production (metabolic rate; kJ/g nitrogen per kg$^{0.75}$ per 10 min) in the rat within the first hour after intake of a diet containing non-hydrolysed casein (□) and casein hydrolysed with pepsin for 1·5 h (○) or 3 h (△).

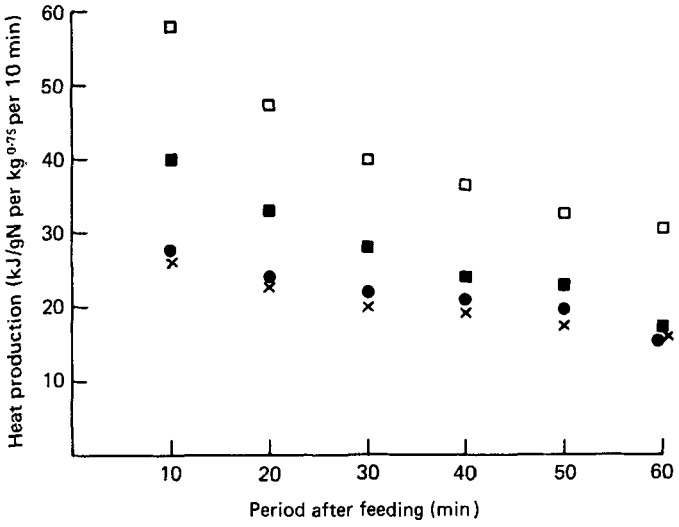

Fig. 3. Dynamics of changes in heat production (metabolic rate; kJ/g nitrogen per kg$^{0.75}$ per 10 min) in the rat within the first hour after intake of diet containing non-hydrolysed casein (□), and casein hydrolysed with pepsin for 3 h and pancreatin for 6 h (■), 12 h (●), or 24 h (×).

DISCUSSION

The experimental findings indicated a visible increase in heat production in the rat after intake of a semi-purified diet, containing 200 g casein and 17 MJ ME/kg diet. Replacement in the diet of casein by its enzymic hydrolysates of various content of free amino-N (from 8·56 to 93·58%) resulted in a considerable increase in N consumption associated simultaneously with negligible changes in the total amount of heat produced. This might indicate that with increasing food intake the heat-producing effect of the diet decreased. However,

correlation coefficients between the N intake and the total heat production (kJ/kg$^{0.75}$) calculated within particular experimental groups were found high (mean +0·91), and demonstrated that the observed differences in N consumption had no significant effect on the SDA when expressed as kJ/g of N consumed.

It was found that diets containing enzymic hydrolysates of casein exhibited a significantly lower SDA, expressed in kJ/g N consumed. The metabolic rate (kJ/kg$^{0.75}$ per g N) in the rat fed on diets containing hydrolysed casein was inversely proportional to the extent of casein hydrolysis, measured by the ratio of free amino-N content to total N content in the hydrolysate (r −0·92).

In the light of the studies of Passmore and Ritchie (1957) and Brobeck (1948) it could be assumed that the observed decrease in heat production in the rat fed on a diet in which casein had been replaced by its hydrolysates was the main reason for an increased consumption of food and of nitrogen. In this case the more rapid absorption of free amino acids and their effect on satiety mechanism (Mellinkoff, Frankland, Boyle & Greipel, 1956), as well as a higher palatability of the diets could be of some importance, too.

The observed decrease in the heat-producing effect of the diet associated with the increase in the extent of protein hydrolysis seemed to indicate a close relationship between the digestion process and SDA of protein; such a relationship was suggested by Bidder & Schmidt (1877). However, in other studies (Benedict & Emmes, 1912; Borsook, 1936) an increase in the metabolic rate was observed after intravenous injection of an amino acid mixture. Recent studies of Russian physiologists (Golinko & Sysojew, 1975) carried out on dogs with stomach fistulas indicated that parenteral administration of a casein hydrolysate and a mixture of amino acids resulted in an intensive secretion of gastric juice as early as 10–15 min after injection. Thus the increase in heat production observed after injection of an amino acid mixture does not rule out the contribution of digestion processes to the occurrence of SDA. However, it seems to indicate that the stimulation of metabolic rate after protein intake is primarily affected by enzyme synthesis and secretion of gastrointestinal juices rather than by the peristaltic movements or absorption process.

The absence of an increase of heat production in dogs fed on an extract of bone and meat (Rubner, 1902) has been regarded as a further argument against the involvement of digestion processes in the occurrence of SDA. However, such extracts usually contain a high glutamic acid content, and when administered intravenously do not result in the secretion of gastric juice, but strongly inhibit the excretion activity in the stomach resulting from injection of other amino acids (Golinko & Szłygin, 1976). This finding was confirmed by Chambers & Lusk (1930), who demonstrated that glutamic acid exhibited no SDA. Besides, Wilhelmj, Bollman & Mann (1928) reported that intravenous administration of amino acids induced SDA in animals but not after hepatectomy, thus suggesting the liver as the source of SDA. Tanaka & Yano (1975) found that the respiration quotient (RQ) reached 0·9–1·0 in the first 3 h after protein intake, and they also observed that a similar increase of the metabolic rate and of the RQ was achieved after administration of glucagone with insulin. They also believed that their findings indicated the glycogen of the liver mobilized by hormonal and nervous mechanisms as the source of energy in the initial phase of SDA of protein. Since both mechanisms stimulating glycogenolysis react after the intake of food and its presence in the stomach (Konturek, 1976), it can be assumed that the mobilized glycogen of the liver is producing energy for the normal action of the digestion process (synthesis and secretion of enzymes, gastric movements). However, some other functions of this organ in the development of SDA cannot be excluded.

In our experiments, the greatest relative decrease in the heat-producing effect of casein was observed after its in vitro hydrolysis with pepsin for 3 h, thus indicating that gastric processes have a primary role in the SDA of casein. Also, the highest increase in the meta-

bolic rate was observed in the rat 10–20 min after protein intake (Tanaka & Yano, 1975; Gawęcki *et al.* 1978), however, this finding may be associated with the amino acid composition of the protein.

The hypothesis that the synthesis and secretion of digestive enzymes are the main reasons for SDA of food can be indirectly justified by differences between the increase in metabolic rate resulting from the intake of proteins, fats and carbohydrates. The number and quantity of enzymes participating in proteolysis, particularly with regard to the stomach, is markedly higher than is necessary for the digestion of the remaining nutrients (Konturek, 1976). It seems that the effect of sensory stimuli on SDA of protein, observed by Tanaka & Yano (1975), as well as the demonstrated relationship between SDA and the satiety mechanism (Brobeck, 1948; Passmore & Ritchie, 1957) could be attributed primarily to the secretory activity in the stomach. The nervous system plays a main part in the first phase of gastric juice secretion (Pawlow & Schumowa-Simanowskaja, 1895).

The experimental diets containing casein which had been almost completely hydrolysed by enzymes (pepsin for 3 h, pancreatin for 24 h) produced a visible heat-producing effect, reaching 39·2% of that obtained with the diet containing non-hydrolysed casein. Taking into consideration the stimulatory effect of fats and carbohydrates in the experimental diets on the metabolic rate, this finding seems to indicate, however, that the processes associated with protein hydrolysis in the digestive tract are the main but not the only reason for the increase in heat production observed after ingestion of this nutrient by the animals.

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