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THE ROLE OF THE LIVER IN NON-SHIVERING THERMOGENESIS IN THE RAT

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SUMMARY

1. The temperatures of the liver and afferent aortic and portal blood were measured in 20° C acclimated rats at different environmental temperatures (T_a) between 20 and 37° C. The heated-thermocouple technique was used to measure the metabolic heat production of the liver, its blood flow, and to correct the temperature difference between it and the reference junction for blood flow.

2. The temperature of the liver was higher than that of the afferent blood. On raising T_a from 20 to 30° C the liver temperature fell and the temperature difference between the liver and the aortic blood was reduced despite the decrease in the thermal gradient between the liver and the exterior and a small reduction in hepatic blood flow. A further rise in T_a to 37° C led to an increase in the liver and blood temperatures. The same pattern was seen when the temperature differences were corrected for blood flow.

3. Metabolic heat production in the liver decreased when T_a was raised from 20 to 30° C.

4. In a 20° C environment inhibition of non-shivering thermogenesis with propranolol HCl (10 mg/kg body wt. I.V.) led to a fall in the temperature of the liver and its metabolic heat production towards levels found in untreated rats at T_a = 30° C. Consequently, in treated rats the change in metabolic heat production on raising T_a to 30° C was less than in untreated ones.

5. These results are interpreted as evidence for the participation of the liver in thermoregulatory non-shivering thermogenesis.

INTRODUCTION

The heat produced by the body can be divided into basal and thermoregulatory. Not all the latter is derived from shivering in new-born mammals, in some mammalian species reared at environmental temperatures below their thermoneutral zones or in hibernators. As Donhoffer (1971) has pointed out, although the existence of this thermoregulatory non-shivering thermogenesis (Hsieh, Pun, Li & Ti, 1966) is now accepted, there is still argument about its sites. In particular, although the rat liver is hotter than its surroundings its contribution to this form of heat production is undecided (e.g. Himms-Hagen, 1972). Because of other studies of factors affecting heat production in the rat it became necessary for us to try to answer this question for that species. If non-shivering thermogenesis occurs in the liver it should be possible to show changes in the rate of hepatic heat production as the ambient temperature of the rat acclimated to 20° C (Hart, 1961) is varied between the lower limit of the thermoneutral zone (27·5–30° C) and just above the threshold for the onset of shivering, about 20° C (Stoner, 1971). The effect of this alteration which increases total O_2 consumption 53% (Stoner & Marshall, 1971), has been studied in two ways.

Observations by Grayson & Mendel (1956) and Grayson & Kinnear (1958) indicate that the main pathway for the loss of heat from the liver is through the diaphragm, chest and abdominal walls rather than to the blood. Consequently, when the ambient temperature is raised, to lower the thermal gradient between the liver and the exterior, the liver temperature should also rise if its heat production cannot be controlled.

Grayson, Coulson & Winchester (1971) have developed a heated thermocouple for correcting the temperature difference between the liver and the reference junction for changes in hepatic blood flow and for measuring the metabolic heat production of an organ. Using this it should be possible to measure any changes in hepatic heat production in response to changes in ambient temperature.

The results of these experiments showed that the liver did partake in thermoregulatory non-shivering thermogenesis and a preliminary report of some of the results has already been made (Stoner, 1973).

METHODS

Male albino rats of the Porton strain, body wt 246 ± 4 g (mean $\pm$ S.D.), fed on M.R.C. diet 41B (Bruce & Parkes, 1956) were used. They were kept, from weaning, at an environmental temperature of 18–22° C with controlled lighting giving 12 hr light (7.0 a.m. to 7.0 p.m.) per day. All surgical operations were done under ether anaesthesia 1–7 days (mean $\pm$ S.D., $3{\cdot}6 \pm 1{\cdot}3$) before the experiments. At the end of

the operation each rat was injected I.M. with 10 mg oxytetracycline (Terramycin injectable solution; Pfizer, Sandwich, Kent). The experiments were carried out in the environmental chamber described previously (Stoner, 1971). The temperature of the air (T_a) in the chamber was measured with a thermocouple.

Copper–constantan thermocouples (36 S.W.G. copper, 38 S.W.G. 'Eureka' except for the aortic thermocouples which were 42 S.W.G.) were made from enamelled wire and the junctions were insulated with Formite Damarda lacquer (Bakelite Ltd). The aortic temperature (T_{aorta}) was measured with a thermocouple sealed into the end of a polyethylene cannula (PP 25, Portex Ltd, Hythe, Kent; Popovic & Popovic, 1960) with Kleber Tixo K 20 adhesive (Tenant Trading Co., London) and inserted into the lower third of the thoracic aorta via the left carotid artery. The temperature of the portal vein (T_{pv}) was measured by attaching a thermocouple to the wall of the vein in the base of the mesentery with isobutyl 2-cyanoacrylate tissue adhesive (Ethicon Ltd, Edinburgh). The wires were brought out through the skin of the dorsum. Colon temperature (T_c) was measured with a thermocouple inserted 6–8 cm from the anus and the subcutaneous temperature (T_{sc}) with a thermocouple inserted under the skin of the dorsum. The liver temperature (T_L) was measured with either a heated or simple thermocouple inserted in the right lobe of the liver and the wires brought out through the skin of the dorsum as before (Stoner, 1956). The temperatures were recorded on a multichannel hot-wire recorder (Devices Sales Ltd, Welwyn Garden City).

The heated thermocouples were of the improved type described by Grayson *et al.* (1971). The heater coil was made from 42 S.W.G. enamelled copper wire and was 3·5 mm long. It was fitted over the copper wire of a standard thermocouple and the probe treated with Silastic cement (Dow-Corning medical adhesive 891) as shown by Grayson *et al.* (1971). Current was applied to the heater coil with a modified version (available on request) of the circuit of Carlyle & Grayson (1956) to give a cycle of 0, 0·387, 0·591 and 0·500 A. The duration of the 0·591 A current was 17 sec but Omron Subminy timers type STP-NH (Omron Tateisi Electronics Co., Japan) were introduced into the circuit so that the duration of the other parts of the cycle could be altered individually. It was thus possible to ensure at the beginning of each experiment that the duration of the 0, 0·387 and 0·500 A currents was sufficient for temperature equilibrium to be reached. Either the aortic or portal vein thermocouple was used as the reference junction, the two constantan leads being soldered together outside the rat. At the end of the experiment the rat was killed with sodium pentobarbitone (Veterinary Nembutal, Abbott) and recordings made on the dead liver (Grayson *et al.* 1971). This form of heated thermocouple gave a linear relationship between I^2 and the change in liver temperature. From the recordings the temperature difference between the liver and the reference thermocouple corrected for blood flow, the I^2 equivalent of the metabolic heat of the liver and the increase in the thermal conductivity of the liver (Δk) due to blood flow were calculated (Grayson *et al.* 1971). The thermal conductivity of dead rat liver was assumed to be $11{\cdot}8 \times 10^{-4}$ c.g.s. units as found previously (Stoner, 1956).

Where possible the results have been expressed as the mean $\pm$ S.D. and the means have been compared by Student's *t* test as modified by Fisher (1934) for small samples.

RESULTS

Effect of environmental changes on liver and blood temperatures

The variations in the temperature of the liver and its afferent blood supply with changes in the environmental temperature were observed in twenty-one unanaesthetized 20° C acclimated rats. It was not possible to determine all the temperature combinations in each rat. The temperatures of the liver, aortic blood and portal vein under the different conditions are shown in Table 1.

TABLE 1. Temperature of the liver, aortic blood and portal vein of the 20° C acclimated rat at different environmental temperatures

	Temperature, °C (mean ± S.D.)		
Environmental	Liver	Aorta	Portal vein
20	38·4 ± 0·53* (20)	37·6 ± 0·32 (14)	38·4 ± 0·44 (12)
30	37·9 ± 0·60† (17)	37·4 ± 0·32 (11)	38·1 ± 0·18 (10)
37	39·0 ± 0·44 (9)	38·8 ± 0·05 (4)	39·1 ± 0·34 (8)

All temperatures at 37° C significantly ($P < 0·01$) higher than the corresponding temperatures at the lower environmental temperatures.

No. of rats shown in parentheses.

* Significantly greater than T_{aorta} at $T_a = 20°$ C; $P < 0·001$.

† Significantly ($P < 0·02$) greater than T_{aorta} at either $T_a = 20$ or 30° C and less than T_L at $T_a = 20°$ C.

When $T_a = 20°$ C T_L was always greater than T_{aorta} (Table 1) and in individual rats the temperature difference was $+0·99 \pm 0·21°$ C ($n = 13$). The temperature differences between the liver and the portal vein varied between $+0·4$ and $-0·7°$ C, T_L being the same or greater in eight out of eleven rats.

The pattern of the temperature changes on raising T_a from 20 to 30° C is shown in Fig. 1. There was little change in T_{aorta} but T_{sc} rose and there was a fall in T_{pv} and T_L, particularly in the latter. The change in the liver began with the change in T_a and T_L soon fell to a new steady-state which was usually maintained while T_a remained at 30° C. In these experiments the rats were kept at 30° C for between 28 and 102 min (44·8 ± 18·3 min). At this T_a the mean T_L was significantly lower than in a 20° C environment (Table 1). In the seventeen experiments in which the effect on T_L of raising T_a from 20 to 30° C was measured T_L fell in fifteen, the mean fall

being $0{\cdot}57 \pm 0{\cdot}23°$ C. The difference between T_L and T_{aorta}, $+0{\cdot}67 \pm 0{\cdot}24°$ C ($n = 10$) was also significantly ($P < 0{\cdot}01$) less than at $T_a = 20°$ C.

Although when $T_a = 30°$ C the mean aortic and portal vein temperatures did not differ significantly from those when $T_a = 20°$ C (Table 1), in nine out of twelve of these tests T_{aorta} fell $0{\cdot}31 \pm 0{\cdot}26°$ C and in seven out of ten T_{pv} fell $0{\cdot}51 \pm 0{\cdot}21°$ C.

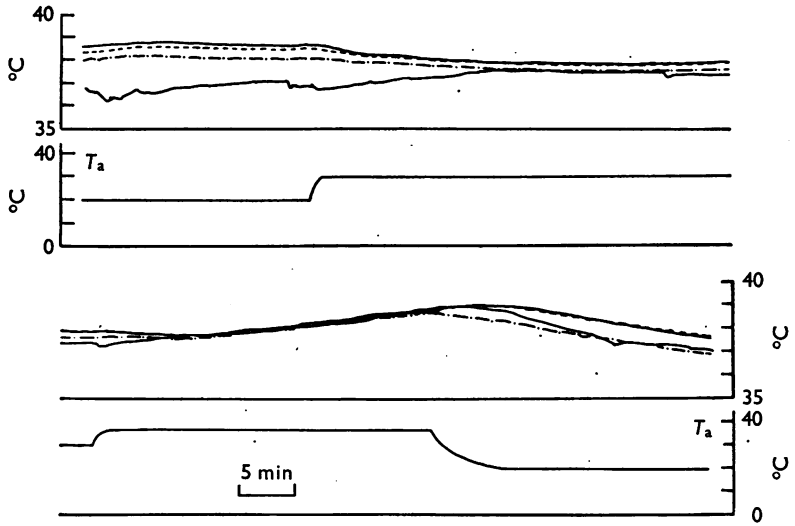

Fig. 1. Superimposed tracings of the recordings of the temperatures of the liver, portal vein, aortic blood and dorsal subcutaneous tissue (order from above downwards at the start of the tracing) of a rat at different environmental temperatures (T_a). The upper and lower sections are parts of a continuous recording which should be read from left to right starting in the upper part.

This picture was completely altered when T_a was further increased to 37° C. Then, after a lag of about 5 min, all the temperatures rose and after 21–52 min were significantly higher ($P < 0{\cdot}01$) than the corresponding temperatures when $T_a = 20$ or 30° C (Table 1). Although the temperatures were not significantly different from one another, in individual rats T_L was still higher than T_{aorta}, $+0{\cdot}5 \pm 0{\cdot}29°$ C ($n = 4$) but the difference between T_L and T_{pv} varied between $+0{\cdot}6$ and $-0{\cdot}6°$ C with T_L being lower in five out of eight rats. When T_a was restored to 20° C normal temperature relationships were not restored immediately and the temperature continued to rise for a short time, particularly in the liver and portal vein (Fig. 1).

Effect of β-blockade

After the I.V. injection of 10 mg propranolol HCl/kg body wt. in a 20° C environment the temperature at the various sites fell to new equilibrium values after about 30 min. In six rats T_L was then 0·70 ± 0·22° C and T_{aorta} 0·68 ± 0·19° C below the pre-injection levels. These rats responded differently to an increase in the ambient temperature from 20 to 30° C (Fig. 2). There was usually no further fall in liver temperature but after a short interval T_L and T_{aorta} rose. The response in these treated rats resembled that of untreated rats in a 37° C environment.

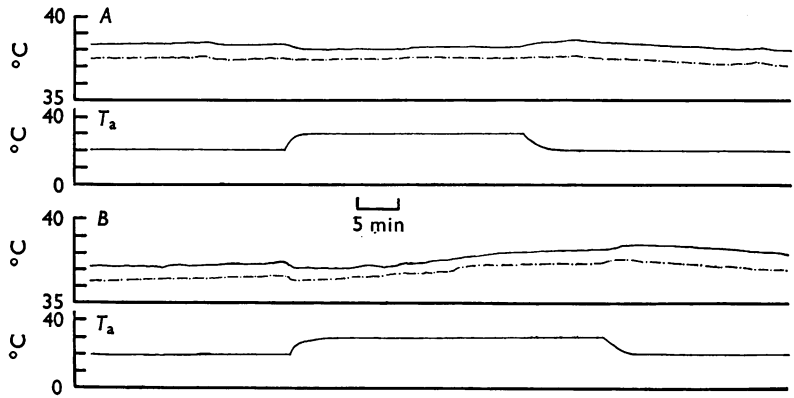

Fig. 2. The effect of a change in environmental temperature (T_a) on the liver (——) and aortic blood (—·—) temperatures before (*A*) and 30 min after (*B*) the intravenous injection of 10 mg propranolol HCl/kg body wt.

Effect of environmental temperature on hepatic heat production

A typical recording of the changes in T_L produced by the cycle of heating currents is shown in Fig. 3. Once equilibrium had been reached at any particular T_a a relatively constant pattern was observed. The transition from one equilibrium to another on increasing T_a from 20 to 30° C is shown in Fig. 3. In view of the constancy of the temperature pattern calculations were not made on every cycle but, as far as possible, at 10 min intervals, avoiding the first 10 min after a change in T_a. For each rat the mean values for each of the three parameters calculated was based on 4–16 cycles at each T_a. The group mean values in Table 2 were derived from these individual means. The groups were separated according to whether the reference junction was in the aorta or on the portal vein. Different rats were used for each group.

The corrected liver temperature was always higher than that in the aorta in both 20 and 30° C environments. These differences (Table 2) were

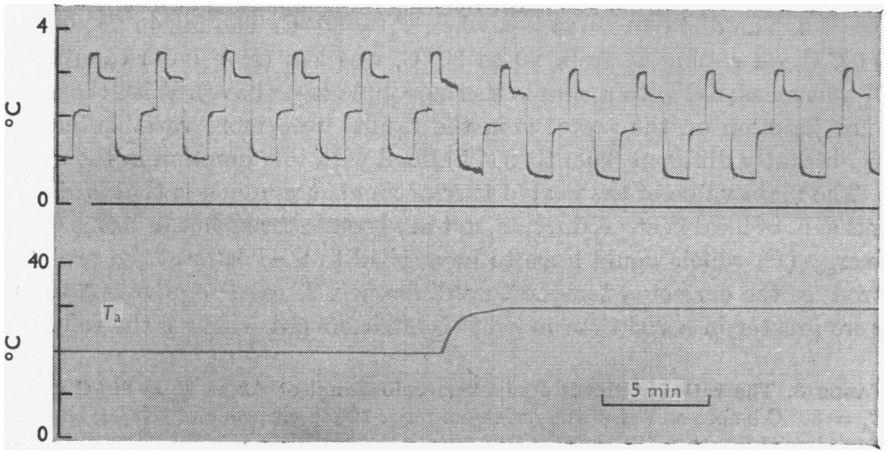

Fig. 3. The effect of a change in environmental temperature (T_a) on the recording from a heated thermocouple in the liver of a rat. Reference junction in aorta.

TABLE 2. The effect of environmental temperature on the corrected temperature difference between liver and reference junction, the I^2 equivalent of hepatic heat production and the change in thermal conductivity due to blood flow

		Site of reference junction	
	T_a(°C)	Aorta	Portal vein
		Mean ± S.D.	
Temp. difference between liver and reference junction (° C)	20	+0·87 ± 0·21 (23)	+0·76 ± 0·36 (8)
	30	+0·50 ± 0·04* (23)	+0·51 ± 0·23 (8)
I^2 equivalent (A^2)	20	0·102 ± 0·035 (23)	0·108 ± 0·061 (8)
	30	0·056 ± 0·024* (23)	0·069 ± 0·037 (8)
Ratio		1·86 ± 0·34 (23)	1·51 ± 0·18 (8)
Thermal conductivity change (10^4 Δk c.g.s. units)	20	6·5 ± 2·8 (21)	7·0 ± 3·3 (8)
	30	6·0 ± 2·7 (21)	6·4 ± 2·8 (8)
Ratio		1·10 ± 0·10 (21)	1·08 ± 0·12 (8)

No. of rats shown in parentheses.
* Significantly different from value at T_a = 20° C, $P < 0·001$.

not significantly different from those measured with simple thermocouples. The difference was less when T_a = 30° C. The fall in T_L, 0·37 ± 0·03° C, on raising T_a from 20 to 30° C, was less ($P < 0·01$) than when T_L was measured with a simple thermocouple (see above). With the reference junction on the portal vein the results were more variable but not significantly different from those obtained with this junction in the aorta.

The main value of the heated thermocouple technique is that it gives a measure of local heat production, not in absolute terms but in terms of the energy (I^2) which would have to be applied to the heater of the probe to produce the corrected temperature difference. These I^2 equivalent values were greater in a 20° than in a 30° C environment whether the reference

TABLE 3. The ratios of the I^2 equivalent values and of Δk at T_a = 20° C and at T_a = 30° C before and after the I.V. injection of 10 mg propranolol HCl/kg body wt. Each line refers to a different rat

$I^2_{20°}/I^2_{30°}$		$\Delta k_{20°}/\Delta k_{30°}$	
Before propranolol	After propranolol	Before propranolol	After propranolol
1·90	1·52	1·18	1·26
2·10	1·00	1·20	1·01
2·08	1·68	1·07	1·06
2·02	1·79	1·03	1·05
1·66	2·39	0·95	1·21
1·75	2·81	1·04	1·10
1·77	1·30	1·07	1·04

junction was in the aorta or on the portal vein (Table 2). The variability was high and the difference between the mean values at 20 and 30° C was only statistically significant when the reference junction was in the aorta. However, as each rat contributed values at both these environmental temperatures the variability could be overcome by considering the ratio of the I^2 equivalent value at 20° C to that at 30° C for each rat. In all thirty-one cases this ratio exceeded 1·0 and the mean values are shown in Table 2. When the reference junction was in the aorta the mean value was significantly ($P < 0·001$) greater than when it was on the portal vein.

The effects of raising T_a from 30 to 37° C were studied in five rats. In four the I^2 equivalent values at 37° C did not differ significantly from those at 30° C; in the fifth rat the value was lower ($P < 0·05$).

After the I.V. injection of 10 mg propranolol HCl/kg body wt. in a 20° C environment the I^2 equivalent values fell in six out of seven rats although the difference between the mean values was not statistically significant. When T_a was raised to 30° C the I^2 equivalent values fell further ($P < 0·05$)

to the same level as at 30° C before propranolol treatment. Because of this difference in the initial values, with two exceptions, the change was not as great as in the untreated rats (Table 3). The reasons for the aberrant behaviour of two of the rats were not apparent.

Effect of environmental temperature on liver blood flow

The value of $10^4 \Delta k$ (the increase in thermal conductivity due to blood flow) was lower than the previous value of $10{\cdot}1 \pm 3{\cdot}4$ c.g.s. units obtained by the less accurate manual method (Stoner, 1956). The mean values did not differ significantly whether the reference junction was in the aorta or on the portal vein or whether the rat was in a 20 or 30° C environment (Table 2). This confirmed previous findings (Stoner, 1958). However, using each rat as its own control, the ratio of the mean Δk at T_a = 20° C to that when T_a = 30° C was greater than 1·0 in seventeen of the twenty-one rats in which the reference junction was in the aorta and in seven of the eight rats in which it was on the portal vein. Hence, Δk was significantly greater at the 1 % level (Quenouille, 1959) in the 20° C environment. The fall in hepatic blood flow on raising T_a from 20 to 30° C was not great (Table 2) and never exceeded 26 %.

When T_a was raised from 30° to 37° C the hepatic blood flow decreased further in three out of five rats, the ratios $\Delta k_{30°}/\Delta k_{37°}$ being 1·34, 1·21, 0·91, 1·21 and 0·91. The treatment with propranolol had no effect on Δk or on the response to raising T_a from 20 to 30° C (Table 3)

DISCUSSION

These results strongly support the view that the liver contributes to thermoregulatory non-shivering thermogenesis in the rat and can be added to the other evidence for that view (see review by Donhoffer, 1971).

The temperature of the liver was about the same as that found previously in rats with a similar environmental history (Birnie & Grayson, 1952; Stoner, 1956, 1958). It was always greater than that of the aortic blood and nearly always greater than that of the blood in the portal vein. Raising T_a from 20 to 30° C so as to eliminate thermoregulatory non-shivering thermogenesis, led to a fall in liver temperature both absolutely and relative to the afferent blood which showed less change in temperature. This occurred despite a fall in liver blood flow, presumably due to a shift of blood from core to shell at the higher T_a. The change in blood flow was small and was not observed previously (Grayson & Mendel, 1956; Stoner, 1958). If hepatic heat production was not controlled and remained constant during changes in T_a, raising T_a from 20 to 30° C to lower the temperature gradient between the core and the shell should have led to

a rise in liver temperature since the main heat loss from the liver is through the body wall. The liver temperature did rise when T_a was raised above the upper limit of the thermoneutral zone to 37° C, presumably because hepatic heat production could not be reduced further nor hepatic heat loss increased.

Further evidence came from the results obtained by the heated thermocouple technique. The difference between the afferent blood and liver temperatures could then be corrected for hepatic blood flow. This corrected difference was still reduced when T_a was raised from 20 to 30° C. There was also a prompt reduction in the energy which had to be applied to the heater of the probe to reproduce the corrected temperature difference (Table 2, Fig. 3). This is the most convincing single piece of evidence in favour of a role for the liver in thermoregulatory non-shivering thermogenesis. The rapidity of the response to a change in T_a has also been seen in indirect calorimetry measurements (Depocas, Hart & Héroux, 1957).

Since thermoregulatory non-shivering thermogenesis is controlled by the sympathetic nervous system (Hsieh *et al.* 1966) it can be inhibited by β-blockade (Brück & Wünnenberg, 1965; Heim & Hull, 1966). In the present experiments β-blockade with propranolol had the expected effects on the hepatic responses. When propranolol was given at $T_a = 20°$ C, T_c and T_L fell and the I^2 equivalent value was reduced towards the level previously found in a 30° C environment. Consequently, when T_a was raised to 30° C the fall in the I^2 equivalent value was usually less than in the untreated rats (Table 3). After propranolol the temperature of the liver ($37{\cdot}2 \pm 0{\cdot}2°$ C) was significantly ($P < 0{\cdot}001$) reduced so that T_L was lower than usual at the time T_a was raised to 30° C. Since at this T_a basal heat production can maintain body temperature a rise in T_L was to be expected.

These results have been obtained on temperate zone rats living, after weaning, 10° C below their thermoneutral zone and therefore endowed with some cold acclimation. This probably accounts for the differences between their responses and those of the tropically adapted ($T_a = 28°$ C) rats used by Grayson & Mendel (1956) in which T_L fell when T_a was reduced below the thermoneutral zone. In our rats T_c and T_L always rose when T_a was lowered from 30° C towards 0° C (Stoner, 1958, 1968, 1971).

While the liver can now be said to partake in thermoregulatory non-shivering thermogenesis in the rat a number of problems remain.

The liver is not the only site of non-shivering heat production. Although the results have been expressed as quantitatively as possible, they do not indicate what fraction of thermoregulatory non-shivering thermogenesis was contributed by the liver in these 20° C acclimated rats at 20° C, whether this non-shivering thermogenesis was maximal or whether it

would have increased had T_a been lowered into the zone of shivering thermogenesis.

Control is exercised through the sympathetic nervous system but it is not known if this is done through the innervation of the liver (Shimazu & Fukuda, 1965; Shimazu, Fukuda & Ban, 1966; Shimazu & Amakawu, 1968; Edwards, 1972) or indirectly. Similarly it is not known whether the substrates for this heat production are derived from the hepatic stores or from the blood-stream or whether their source and nature vary with the duration of the exposure to the environmental stimulus.

The increase in the temperature of the blood during its passage from the thoracic aorta to the portal vein (Table 1) is in keeping with the view that the intestines are also a source of heat (Federov & Shur, 1942; Grayson & Mendel, 1956) although the fraction of this heat obtained by conduction from the liver and kidneys is not known. The difference between the temperatures in the portal vein and aorta decreased when T_a was raised from 20 to 30° C and Jansky (1971) considers that the intestines, and possibly also the kidneys, make small contributions to non-shivering thermogenesis.

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