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THE MEASUREMENT OF METABOLIC AND VASCULAR RESPONSES IN LIVER AND MUSCLE WITH OBSERVATIONS ON THEIR RESPONSES TO INSULIN AND GLUCOSE

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The interpretation of temperatures recorded from most tissues of the body is a matter of some complexity. Four major factors may usually be considered as of primary importance, namely, the temperature of the blood flowing to the tissue in question, the rate of flow of blood in the tissue, the 'cooling power' of its environment and, lastly, the rate of metabolic heat production within the organ.

Thermocouples heated by means of a current passed through a small heating filament have recently been used by several workers to elucidate blood-flow change in internal organs. It might reasonably be assumed that metabolic heat production would have the same effect on a thermocouple thus artificially heated as on an unheated thermocouple. Acting on this assumption Grayson & Kinnear (1958) devised a method whereby blood flow could be estimated by means of a heated thermocouple technique (internal calorimetry, Grayson, 1952). Steps were taken to allow for the temperature of the blood flowing into the part and to allow for possible variations in heat loss from the part. The result was used to determine the unknown remaining variable, namely, metabolic heat production.

The purpose of the present paper is to report further investigations designed, in particular, to study the validity and scope of this approach to the measurement of metabolic heat production. The physiological aspects of the investigation are, therefore, not to be regarded as complete in themselves, for although interesting comparisons between liver and muscle have been revealed, the primary purpose of the observations was to demonstrate the usefulness of the method.

METHODS

Internal calorimetry

The technique of 'internal calorimetry' for the measurement of blood flow in internal organs has been fully described previously (Grayson, 1952). It depends on the thermoelectric measurement of thermal conductivity. It has been shown elsewhere that conductivity

increment (i.e. the thermal conductivity of living tissue less the thermal conductivity of dead tissue) is an approximately linear function of blood flow (Grayson, 1952).

There are at least three accepted methods for the evaluation of thermal conductivity with heated thermocouples. One is the method described by Grayson (1952) who used a manually operated apparatus and a 'cold junction' outside the organ under investigation. One is the method of Hensel, Ruef & Golenhofen (1954) which uses a needle-mounted heated thermocouple with the cold junction incorporated in the needle shaft. The other is the 'automatic' method of Carlyle & Grayson (1956), which also has a cold junction outside the organ. For reasons which will be apparent later this is the only method applicable to the determination of metabolic heat production.

This technique, in the form used in this laboratory, uses a simplified circuit (Grayson & Mendel, 1957) whereby current is supplied to the heaters of the thermocouple in cyclic fashion as follows: 0·200 A^2 for 24 sec; 0·600 A^2 for 17 sec; 0·315 A^2 for 24 sec; no current for 17 sec. This cycle is continuously repeated.

The heated thermocouple is of the type described by Grayson & Haigh (1954).

Cam-operated switch gear. The source of current is a 6 V car battery. A Muirhead decade resistance (10Ω) on one lead gives a rough control of input current. The other lead is attached to the common pole of three micro-switches operated by cams driven by reduction gear at a rate of one revolution/82 sec from a constant speed motor. There are four cam positions, so that each switch is brought into the circuit in turn, followed by a short period with all switches in the 'off' position.

This is a simplified version of previous circuits. There are no terminals. All connexions are within the box. The output leads pass straight to the heaters of the recording thermocouple. (The manually operated box described in previous papers has now been finally discarded.)

The recording apparatus consists of a Cambridge D'Arsonval galvanometer (internal resistance 50Ω) with a shaft of light recording on a slow moving recording camera (paper width, 120 mm). (In recent experiments a Cambridge four-channel recording camera with two channels converted to temperature recording, by substitution of a D'Arsonval unit for the standard oscillator, has been found satisfactory.)

Insertion of recorder. Wistar strain rats, average weight 200 g, were used in these experiments. Recorders were planted in the liver according to methods described previously (Grayson & Kinnear, 1958). The cold junction was situated intra-abdominally near the portal vein. In the present experiments it was found preferable to bring all leads to the surface of the animal and complete the constantan connexions of the thermo-electric circuit outside the animal.

In the present work all recording of liver blood flow and metabolism was made on the conscious animal. The leads from the apparatus were connected to the thermocouple leads and heater leads from the animal by soldering. The joints were covered with adhesive strapping and the animal was allowed free movement inside its cage.

For intravenous injections a polyethylene cannula was inserted into an external jugular vein at the same time as the insertion of the recorders, and brought out through the back of the neck. During an experiment a connecting length of polyethylene tubing enabled the animal to move freely in its cage, yet to receive intravenous injections without disturbance. Unfortunately it was not possible to withdraw sufficient blood through this type of cannula for blood-sugar estimations.

Muscle recording. In these experiments lightly anaesthetized rats were used. The recording thermocouple was inserted through a stab incision into the substance of the gastrocnemius muscle of one side.

For estimation of heat production it is necessary that the cold junction shall be in a situation representative of the temperature of the afferent blood. In some experiments the cold junction was accordingly introduced through the opposite femoral artery and pushed up to the aorta. In other experiments the cold junction was stitched into the mesentery near its

root (a much simpler and less traumatic procedure). Evidence will be adduced to demonstrate that under the conditions of these experiments mesenteric and aortic temperatures are linearly related. Consequently for most purposes it was thought sufficient to use the mesenteric situation for the cold junction. In all other respects the technique of recording from muscle was identical with that used for the liver.

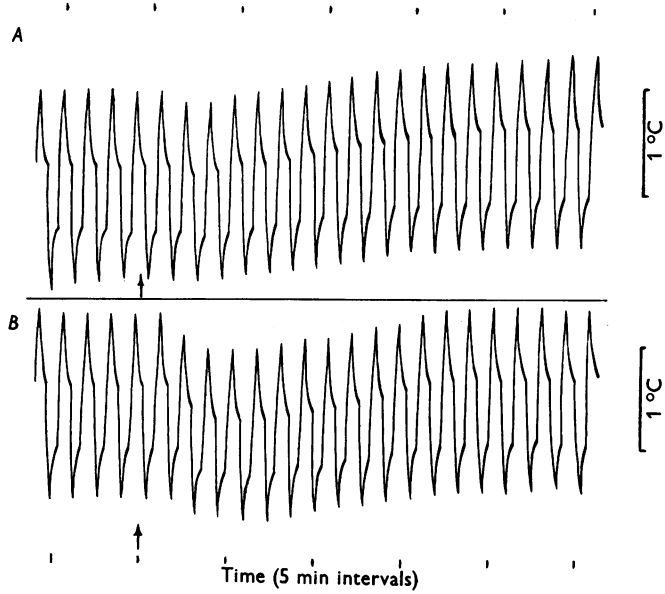

Fig. 1. Photographic records of liver temperature with a heated thermocouple in a conscious rat (tracing from original). *A*, intravenous insulin (2 u.) given at arrow. *B*, intravenous glucose (220 mg) given at arrow. Both records from same rat but on different days.

Determination of metabolic heat production

Figure 1 shows a typical record produced by the automatic recorder. The use of records of this type for the measurement of thermal conductivity and blood flow in living tissues has already been fully described. Nevertheless, the entire concept of metabolic heat production measurement depends on this beginning. Since the investigation of the validity of heat production measurement depends on an understanding of the physical principles involved, some recapitulation is probably necessary at this stage.

It has been shown (Grayson, 1952) that the temperature of a heated thermocouple embedded in a mass of tissue sufficient to be regarded as infinite is governed by the following relation

$$I^2R = 4\pi r\theta k$$

or, more simply, $I^2 = F\theta k$ (where F is an instrumental constant, I is the heating current, R the resistance of the heating elements, θ the temperature rise produced by the current and k the thermal conductivity of the substance or tissue in which the recorder is embedded). This relation applies strictly to a heated thermocouple through the heaters of which a steady current has been passed long enough to establish equilibrium. The present techniques employ a cyclic current in order to speed up temperature equilibration.

From the record shown in Fig. 1 it will be seen that when the current rises from 0 to 0·200 A^2, the temperature also rises to an 'equilibrium' level (lower equilibrium). When the

current rises to 0·600 A^2 (booster), the temperature rises suddenly to a peak; then when the current is lowered to 0·315 A^2 the temperature falls to another 'equilibrium' level (upper equilibrium). When the current is switched off altogether, the temperature falls below lower equilibrium level.

The difference in temperature between upper and lower equilibrium levels forms the basis of measurement for the calculation of thermal conductivity. In the present work the actual level of the lower equilibrium forms the basis for the calculation of metabolic heat production.

Calculation of excess temperature. Starting from an arbitrary point on a record it is easy to measure the actual changes which occur in lower equilibrium temperature. It is also possible, starting from the same point, to determine the changes which should have occurred in lower equilibrium temperature if the only factor concerned were blood-flow change.

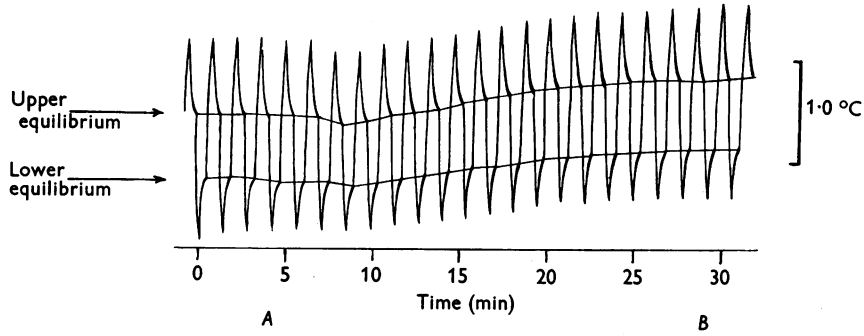

Fig. 2. Treatment of a recording in determination of excess temperature. Same tracing as Fig. 1*A*. Upper equilibria and lower equilibria joined. Vertical distance between upper and lower lines = θ. At *A* (before insulin), θ_1 = 0·64° C; at *B* (after insulin), θ_2 = 0·69° C. At *A*, temperature of lower equilibrium, t_1 measured from bottom of paper = 0·66° C; at *B*, lower equilibrium, t_2 = 0·96° C. Rise in lower equilibrium temperature = 0·3° C. Calculated temperature change due to blood flow alone = $\frac{200}{115}(\theta_2-\theta_1)$ (see text). Excess temperature

$$= (t_2-t_1)-\tfrac{200}{115}(\theta_2-\theta_1) = 0{\cdot}2^\circ \text{ C}.$$

(In practice temperatures are measured in mm, conversions to °C being done by adjusting the ordinate of the derived graph of excess temperature.)

Measurement of actual change. Figure 2 (the same experiment shown in Fig. 1*A*) is a record of the effect of insulin on rat liver. The temperature change between point *A* and point *B* can be determined by measuring from the bottom of the paper at each point and subtracting. In Fig. 2 the lower equilibrium temperature rose by 0·3° C.

Prediction of change due to blood-flow change. The only assumption involved in the following calculation is that, at any one point on the record, I^2 and θ are linearly related.

Let θ be the difference between lower and upper equilibrium temperatures. In Fig. 2 at position *A*, θ = 0·64° C. Under the conditions of thermal conductivity appertaining at that moment, this is the temperature rise produced by a current of 0·115 A^2.

But the lower equilibrium is maintained by a current of 0·200 A^2. By simple proportion, the temperature rise which would be produced by a current of

$$0{\cdot}200 \text{ A}^2 = 0{\cdot}200/0{\cdot}115 \times 0{\cdot}64^\circ \text{ C}.$$

At position *A* this = 1·1° C, which is the amount by which the heating current has raised liver temperature. At position *B*, where the conditions of thermal conductivity were different, the same calculation may be applied. At this position θ = 0·69° C. Whence the amount by which the lower equilibrium heating current has raised liver temperature = 1·2° C. Subtracting the values obtained at *A* from those obtained at *B*, it can thus be estimated

that changes in thermal conductivity occurring as a result of alteration in blood flow should have produced a rise in lower equilibrium temperature of 0·1° C. This is 0·2° C less than what in fact occurred.

In the example shown in Fig. 1*B*, similar calculations show the opposite effect, the actual drop being greater than the calculated effect. Such temperature discrepancies between real and predicted changes will be referred to as 'excess temperature' and evidence will be adduced to demonstrate that 'excess temperature' is, in fact, due to changes in tissue metabolism.

RESULTS

Validity of blood flow and excess temperature calculations

By means of manual recording the validity of the relation, $I^2 = F\theta k$ has been amply demonstrated. For purposes of metabolic heat recording it is, however, necessary to ensure that the same relation holds good with automatic recording. For blood flow determination, I^2 being constant, it will be sufficient to demonstrate linearity between $1/\theta$ and k. For excess temperature determination, however, the relation between $1/\theta$ and k does not enter into the calculation and it will be sufficient to demonstrate linearity between I^2 and $1/\theta$.

Relation between $1/\theta$ *and* k. For the purpose of blood-flow measurement this relation has probably been sufficiently confirmed by previous work. Thus, using a wide series of gelatine/water and gelatine/water/graphite gels, Carlyle & Grayson (1956) showed a linear relation between $1/\theta$ as obtained by the photographic method and thermal conductivity measured by the manually controlled heated thermocouple technique (Grayson, 1952). Further, perfusion experiments in the brain also showed linearity between $1/\theta$ and blood flow.

Later in this paper, however, calculations will be presented which depend on the full validity of the equation, $I^2 = F\theta k$. It was thought desirable, therefore, to repeat these experiments. Figure 3*B* shows the relation between $1/\theta$, recorded photographically, and k obtained by the method of Grayson (1952), using for the purpose a series of gelatine/H_2O and gelatine/graphite/H_2O gels. It is apparent that the photographic method can be used with complete accuracy for the determination of absolute thermal conductivity. Figure 3*A* shows the results of a typical perfusion experiment in which the liver was perfused with dextran solution through the portal vein. The inflow was controlled and measured. It will be seen that the relation between $1/\theta$ and flow was completely linear over the range of perfusion rates selected.

Relation between I^2 *and* θ. Linearity between I^2 and θ has been demonstrated with thermocouples heated by a simple heating current (Grayson, 1952). It has never been confirmed with automatic recording. Since all calculations are limited to upper and lower equilibria it will be sufficient to demonstrate linearity over the range from 0 to 0·315 A^2.

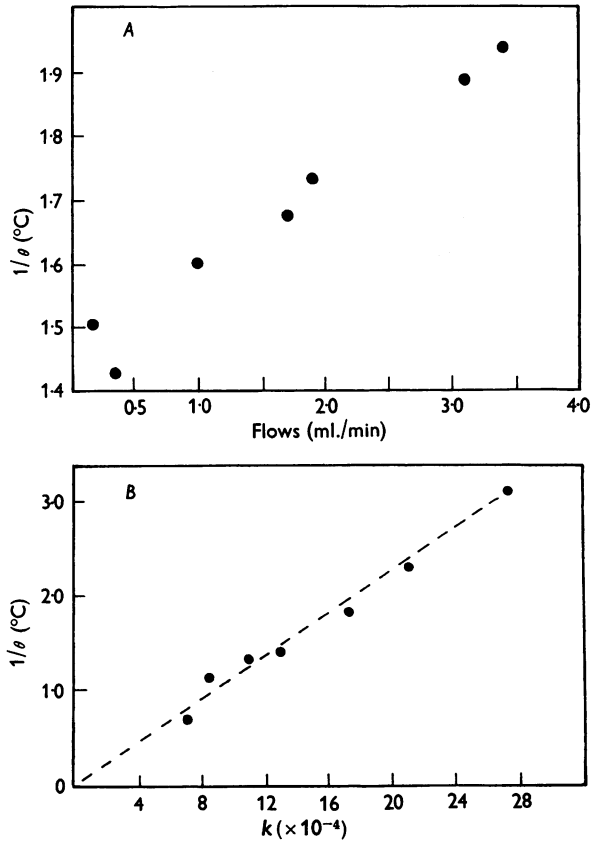

Fig. 3. *A*, Relation between flow and $1/\theta$; liver perfused with dextran through the portal vein. *B*, Relation between $1/\theta$ and thermal conductivity; readings from a series of gelatine/H_2O and gelatine/graphite/H_2O gels.

For this purpose the photographic records obtained during normal experiments afford sufficient proof on the basis of excess temperature calculations made at upper and lower equilibrium levels: Calculated on the assumption of linearity between I^2 and θ (see Methods), at lower equilibrium

$$\text{Excess temperature} = (t_2 - t_1) - \tfrac{200}{215}(\theta_2 - \theta_1).$$

Calculated in the same way at upper equilibrium

$$\text{Excess temperature} = (t_2' - t_1') - \tfrac{315}{115}(\theta_2 - \theta_1),$$

where t_2' = upper equilibrium temperature at B,

t_1' = upper equilibrium temperature at A.

But $t_2' = (t_2 + \theta_2)$; $t_1' = (t_1 + \theta_1)$,

$$\begin{aligned}\text{Therefore excess temperature} &= (t_2+\theta_2)-(t_1+\theta_1)-\tfrac{315}{115}\,(\theta_2-\theta_1)\\ &= (t_2-t_1)+(\theta_2-\theta_1)-\tfrac{315}{115}\,(\theta_2-\theta_1)\\ &= t_2-t_1-\tfrac{200}{115}\,(\theta_2-\theta_1).\end{aligned}$$

If the assumptions of linearity between I and θ were not correct the above relations could not hold, and different values of excess temperature would be obtained in upper and lower equilibria. In fact 300 random calculations have been made in twenty experiments. The results of four of the experiments are shown in Fig. 4. It will be seen that excess temperature was always the same whether calculated from upper or from lower equilibrium levels.

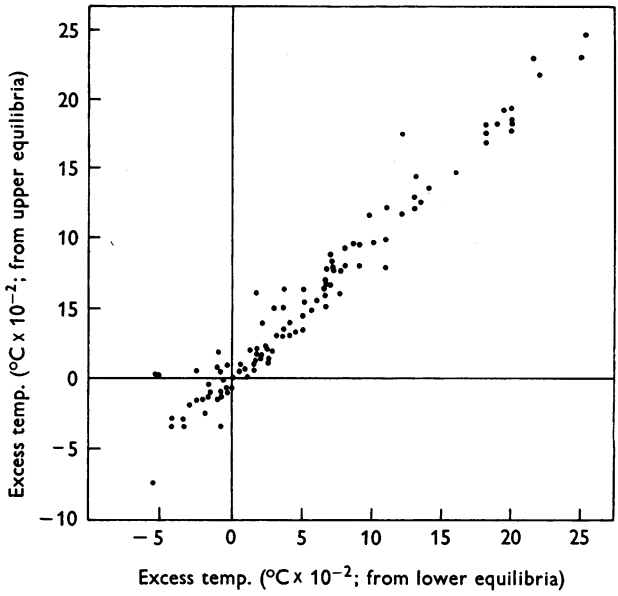

Fig. 4. Relation between I^2 and θ. Excess temperature from four different experiments; ordinate, calculations from upper equilibrium; abscissa, calculations from lower equilibrium.

It must, therefore, be concluded, that under the conditions of the present experiments, I^2 and θ are linearly related.

Experimental assumptions. Reference has already been made to these as regards the liver (Grayson & Kinnear, 1958). There are two. One is that the 'cold junction', fixed to the mesentery near the portal vein, represents closely the mean temperature of the blood flowing to the liver. The other is that heat loss from the liver through the diaphragm and body wall is a fairly constant factor reacting only slowly on over-all liver temperature. The experimental justification of these assumptions has already been fully reported.

With respect to the muscle, for many experiments the cold junction was inserted through a femoral artery into the aorta near its bifurcation. It is reasonable to suppose that this situation represents the temperature of the blood flowing to the limb. In other experiments the cold junction was stitched to the root of the mesentery near the aorta. Figure 5 shows the results of an experiment in which simple temperature was recorded from the aorta and the mesentery during an insulin experiment. It will

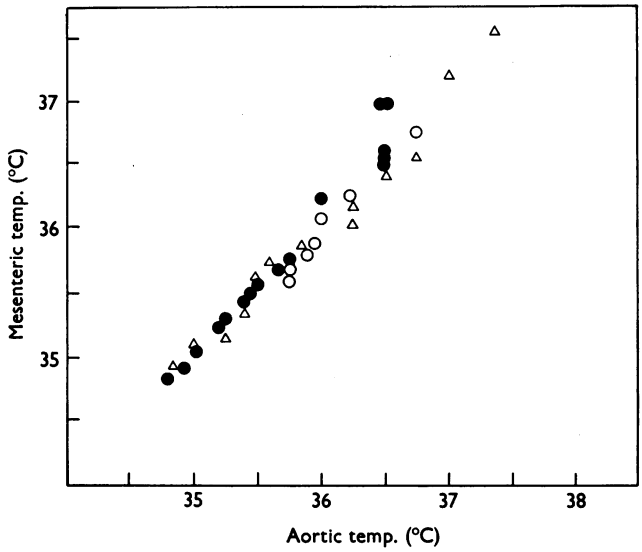

Fig. 5. Relation between the temperature of the mesentery and that of the aorta in three rats. Temperature changes induced by means of pentobarbitone and insulin.

be seen that both temperatures reacted similarly. Similar results were obtained in all such experiments and it was considered justifiable to use the mesenteric situation for the cold junction. Variations in heat loss are not considered to be such a likely source of error as in the liver since the limb was always wrapped in cotton-wool, and the experiments carried out under conditions of constant temperature.

Liver blood flow and metabolism

Conscious rats were used in experiments to determine the effects of insulin, glucose and combined insulin and glucose, on liver blood flow and temperature. After the insertion, under light ether anaesthesia, of a blood-flow recorder in the liver, a cold junction in the mesentery and a polyethylene cannula into a jugular vein, the animals were allowed to recover consciousness before recording was begun. Occasional restless

animals were given 0·02 ml. pentobarbitone sodium through the polyethylene cannula to produce mild sedation.

Insulin. The effect of insulin on liver blood flow and temperature was determined in six experiments. After a preliminary period of recording for 30 min, insulin (Insulin A.B., British Drug Houses, Ltd., 40 u./ml.;

TABLE 1. The effect of insulin on liver blood flow and calculated excess temperature. Values for liver blood flow are expressed as thermal conductivity increments ($\delta k \times 10^{-4}$) above the thermal conductivity of dead liver

	Blood flow		Metabolism, maximum change in excess temperature ($°C \times 10^{-2}$)
Expt. no.	Average initial δk	Average maximal δk after insulin	
1	6·8	9·4	+13
2	5·3	7·5	+12·5
3	1·5	2·3	+19
4	7·8	12·6	+ 6
5	7·4	6·3	+ 6
6	0·6	1·2	+18

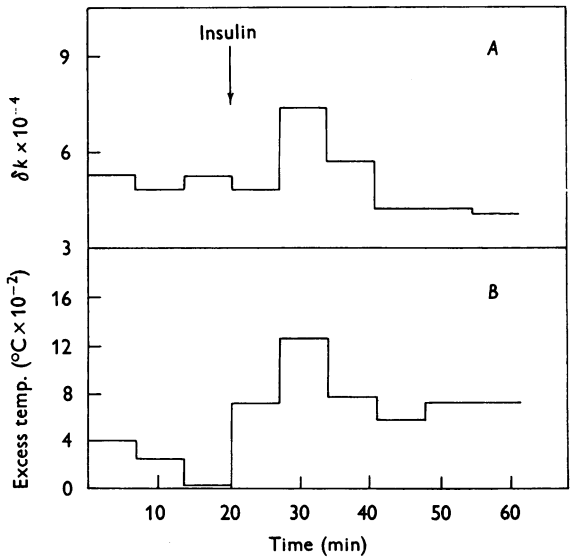

Fig. 6. The typical effect of insulin (I.V.), *A*, on blood flow (δk); *B*, on metabolic heat production (excess temperature) in the liver of a conscious rat.

1 i.u./100 g rat) was given intravenously and recording continued for a further 60 min. The changes in the thermal conductivity of the liver (expressed as thermal conductivity above that of the dead liver (δk) and the calculated 'excess' temperature are shown in Table 1. The pattern of a typical response is shown in Fig. 6.

It can be seen that in three experiments there was a rise in δk values amounting to 38–62 % of the initial values. In one experiment there was a slight fall in δk values, and in two experiments, where the initial δk values were low, being $1{\cdot}5 \times 10^{-4}$ and $0{\cdot}6 \times 10^{-4}$, there was probably no change. In the experiments in which a rise in δk values occurred the change started within 10 min after giving insulin, and was maintained for a further 10–30 min. The response shown in Fig. 6*A* represents Expt. 2 (Table 1), and the blocks are the average δk values for five successive cycles.

The results for the calculated maximum excess temperature were more constant than the blood-flow response, a rise occurring in every case (Table 1). The maximum calculated excess temperature varied from 6×10^{-2} to 19×10^{-2} ° C. The response shown in Fig. 6*B* has also been plotted from the average calculated values in five successive cycles. The rise in excess temperature started within 10 min of insulin injection, reached a maximum in from 10 to 20 min, and then fell. In many experiments, however, it remained above resting values for the remainder of the experiment.

TABLE 2. The effect of glucose on liver blood flow and calculated excess temperature

	Blood flow		
Expt. no.	Average initial δk	Average maximal δk after glucose	Metabolism, maximum change in excess temperature (°C × 10^{-2})
1	6·0	8·4	−29
2	3·3	7·1	−17
3	3·3	4·3	−20
4	6·3	8·3	+20
5	4·7	6·8	+13
6	3·7	5·0	+30
7	8·9	10·4	−12
8	1·0	2·1	−48
9	11·8	12·2	−29
10	5·9	7·4	−65

Glucose. The effect of glucose on liver blood flow and metabolism was determined in ten experiments. Glucose was given through the polyethylene cannula in the dose 100 mg/100 g rat, 100 mg glucose being dissolved in 0·3 ml. saline. The change in δk values and calculated excess temperature are shown in Table 2, and a typical response (Expt. 2) is shown in Fig. 7.

It can be seen from Table 2 that there was an increase in δk values in eight experiments amounting to from 17 to 100 % of the initial values. In one experiment (Expt. 9) there was no significant change in flow, and in Expt. no. 8, where the initial δk value was $1{\cdot}0 \times 10^{-4}$, the change was

probably also not significant. Changes in δk values started within 10 min of giving glucose, and in five experiments reached a maximum within 20 min, and then declined to resting values after a further 10 min. In three experiments, however, the rise in δk values occurred more slowly and remained high for the rest of the experiment (Fig. 7*A*).

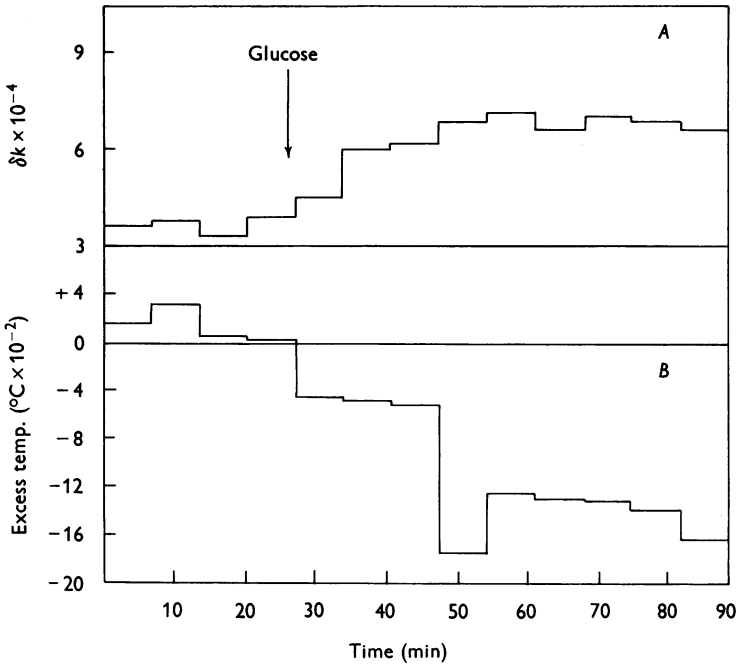

Fig. 7. The typical effect of glucose (100 mg/100 g rat, I.V.) *A*, on blood flow; *B*, on metabolic heat production in the liver.

In seven of these experiments there was a fall in the calculated average excess temperature (Table 2) varying from -12 to -65×10^{-2} °C. In three experiments there was a rise in excess temperature. The fall in excess temperature occurred within 5 min of the injection of glucose, the temperature remaining depressed for over 60 min in four experiments (Fig. 7*B*), and for 30 min in three experiments.

Glucose and insulin. The effect of combined glucose (100 mg/100 g rat) and insulin (1 i.u./100 g rat) on liver blood flow and temperature was determined in ten experiments. The results for δk values and excess temperature are shown in Table 3, and the varying pattern of response in Fig. 8. From Table 3 it can be seen that there was an increase in δk values in six experiments, a fall in two and no change in two experiments. In the experiments in which there was a rise in δk values the response, which was usually slight, occurred within 5 min and lasted from 10 to

20 min (Fig. 8*B*). Where a fall in δk values occurred, the response tended to persist for some 50 min before recovery to initial δk levels.

The changes in calculated excess temperature shown in Table 3 were very variable. In five experiments there was a rise in calculated excess temperature, in two of which the changes were only 1 and 4×10^{-2} ° C.

Figure 8 shows the pattern of response in three experiments. In the experiment shown in Fig. 8*A* there was no change in flow, and a small rise in excess temperature occurred 30 min after injection of glucose and insulin. In the experiment shown in Fig. 8*B* there was a rise in blood flow

TABLE 3. The effect of glucose and insulin on liver blood flow and calculated excess temperature

	Blood flow		Metabolism,
Expt. no.	Average initial δk	Average maximum δk after glucose and insulin	maximum change in excess temperature ($°C \times 10^{-2}$)
1	6·0	7·2	− 4
2	3·2	3·8	+ 4
3	5·6	4·4	+ 5
4	3·0	1·7	−19
5	1·0	3·0	+ 1
6	1·3	2·9	+12
7	4·6	6·6	−23
8	16·7	16·5	−50
9	8·4	11·4	+14
10	3·2	4·3	− 3

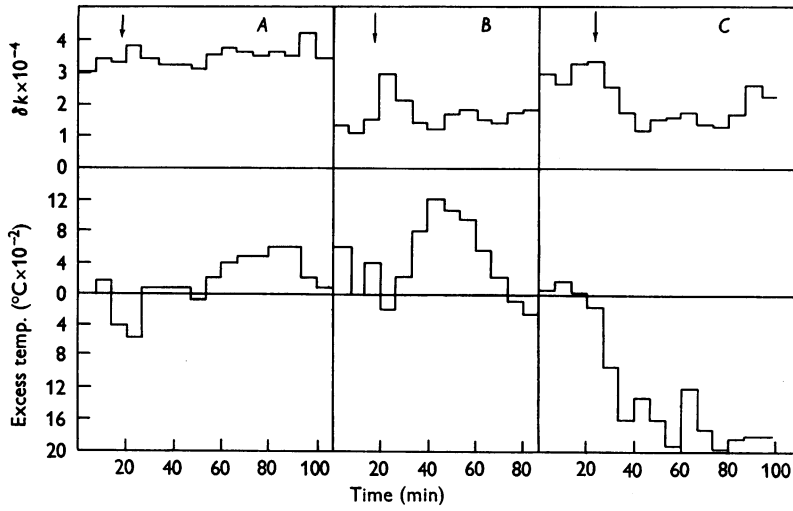

Fig. 8. Liver blood flow and metabolism; typical responses to combinations of insulin (1 i.u./100 g rat) and glucose (100 mg/100 g rat; three experiments *A*, *B* and *C*). I.V. injections given at arrows.

of short duration and marked rise in calculated excess temperature, and in the experiment shown in Fig. 8*C* there was a fall in blood flow which persisted for 60 min, and a marked fall in calculated excess temperature.

The effect of adrenalectomy. In nine experiments the adrenal glands were removed at the time of the insertion of the liver blood-flow recorder and the cold junction, and the experiments with insulin, glucose and glucose and insulin were repeated 2 hr after operation.

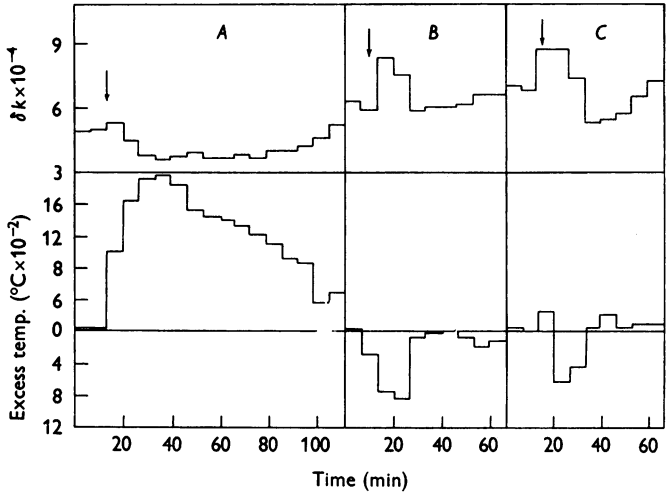

Fig. 9. The influence of acute adrenalectomy on blood flow and metabolic responses of rat liver, *A*, to insulin alone; *B*, to glucose alone; *C*, to a combination of glucose and insulin. All doses as before; I.V. injections given at arrows.

The results are shown in Fig. 9. Figure 9*A* shows a response with insulin alone. In this experiment there was a fall in δk values and a rise in calculated excess temperature. A fall in δk values occurred in two of the three experiments and in the other there was no change. A rise in calculated excess temperature occurred in all these experiments. Figure 9*B* shows the response with glucose. It can be seen that there was a rise in δk values of short duration, and a moderate fall in calculated excess temperature. A similar response was obtained with glucose and insulin (Fig. 9*C*). These changes were obtained with the other two experiments in each group.

Muscle blood flow and metabolism

Records were obtained from the belly of the left gastrocnemius muscle of the lightly anaesthetized rat (0·15 ml./100 g pentobarbitone sodium, reinforced in some cases by one further dose of 0·05 ml.). Intravenous injections were made through a cannula in the external jugular vein. In

each case an initial period of 1 hr was allowed with the heater circuits running for equilibration before recording was begun.

The effect of insulin on muscle blood flow and metabolism. The results of a typical experiment are shown in Fig. 10. Insulin (2 u.) was injected after recording the base line for 20 min. No significant change occurred in blood flow. There was a marked increase in excess temperature, which

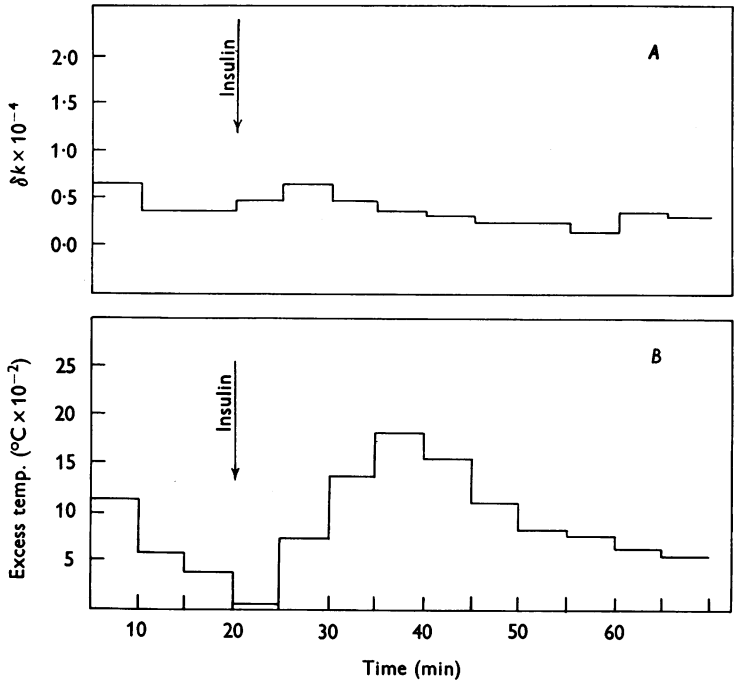

Fig. 10. Effect of insulin *A*, on blood flow (δk), and *B*, on metabolic heat production (excess temperature) in rat gastrocnemius muscle.

reached a maximum 20 min after giving insulin. Thereafter excess temperature subsided slowly to resting levels. Table 4 gives the results of eleven similar experiments. It will be seen that significant changes in blood flow were recorded in only three experiments, but that excess temperature rose in nine, the peak effect occurring 20–40 min after the injection.

The effect of glucose on muscle blood flow and metabolism. Figure 11 shows the results of a typical experiment in which 220 mg glucose was injected intravenously after 20 min of base-line recording. There was no change in blood flow. Excess temperature, however, fell markedly, reaching its lowest point 40 min after the injection. Thereafter it began to recover slowly.

Table 5 gives the results of eleven similar experiments. The effects on blood flow were usually slight. In three instances a slight rise was recorded,

in one case there was a fall, in the others no significant blood-flow change could be detected. The effects on excess temperature were more pronounced. In nine experiments there occurred pronounced depressions in excess temperature and metabolic heat production. In two experiments small

TABLE 4. The effect of I.V. insulin on rat skeletal muscle blood flow and excess temperature

Expt. no.	Change in δk	Maximum change in excess temperature ($°C \times 10^{-2}$)	Time to maximum effect (min)
1	nil	nil	—
2	nil	nil	—
3	nil	+2·5	35
4	nil	+1·1	20
5	0·6 rising to 1·2	+4·4	20
6	nil	+0·2	40
7	nil	+4·1	40
8	1·9 rising to 5·9	+4·9	40
9	4·7 rising to 5·2	+4·4	40
10	nil	+4·3	20
11	nil	+4·5	30

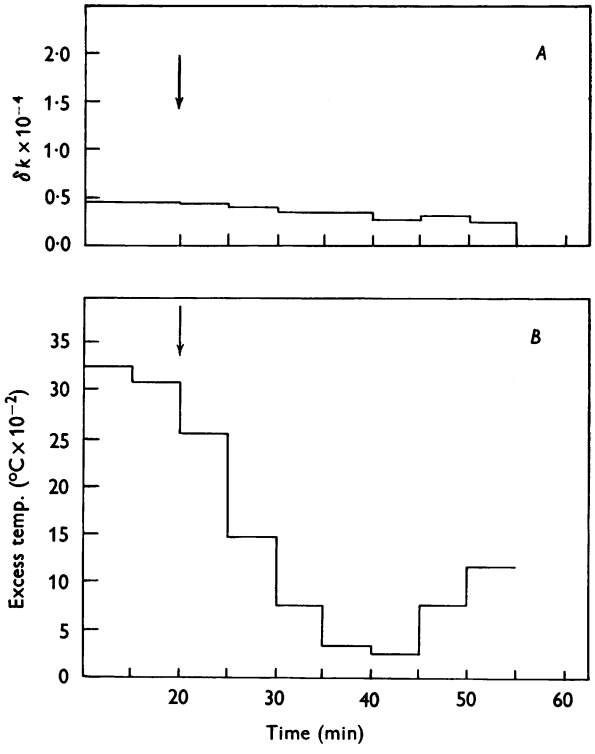

Fig. 11. Effect of I.V. glucose (100 mg/100 g rat), *A*, on blood flow; *B*, on metabolic heat production in rat gastrocnemius muscle.

TABLE 5. The effect of I.V. glucose on muscle blood flow and excess temperature

Expt. no.	Change in δk	Maximum change in excess temperature (° C × 10^{-2})	Time to maximum effect (min)
1	nil	−2·5	17
2	nil	−16·4	50
3	1·8 rising to 3·1	−3·7	10
4	nil	−1·8	50
5	3·7 rising to 4·8	−1·4	50
6	8·2 rising to 10·8	−0·5	50
7	nil	−0·6	45
8	nil	−0·4	10
9	4·2 falling to 1·3	+0·7	15
10	nil	+1·2	30
11	nil	−3·0	40

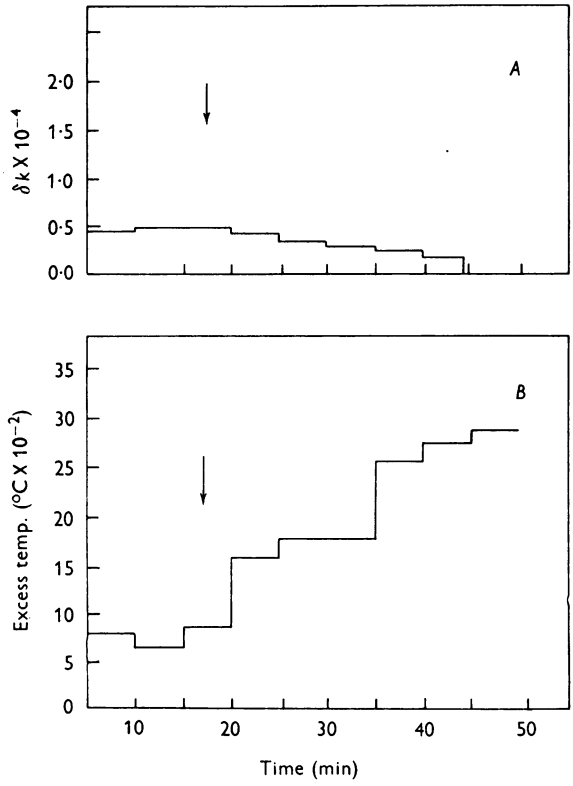

Fig. 12. Effect of insulin (1 i.u./100 g rat) and glucose (100 mg/100 g rat), *A*, on blood flow and *B*, on metabolism in rat gastrocnemius muscle.

increases were recorded. The timing of the maximum effects varied from 10 to 50 min after the injection.

The effect of glucose plus insulin on muscle blood flow and metabolism. Figure 12 shows the results of a typical experiment in which 2 u. insulin and 220 mg glucose were given together. No significant change occurred in muscle blood flow. There was, however, a marked effect on excess temperature which began to rise almost immediately reaching a plateau of effect after 20 min. The results for calculated excess temperature in six similar experiments are given in Table 6. In every case there was a pronounced rise in excess temperature beginning early and reaching a maximum in 20–45 min. There was no change in muscle blood flow in any of these experiments.

TABLE 6. The effect of I.V. glucose + insulin on rat skeletal muscle excess temperature

Expt. no.	Maximum change in excess temperature ($°C \times 10^{-2}$)	Time to max. effect (min)
1	+ 5·4	25
2	+13·9	45
3	+11·0	40
4	+ 8·4	30
5	+33·9	40
6	+21·2	20

Quantitative calculations of heat production

Excess temperature as used hitherto in this paper is merely a qualitative interpretation of changes in heat production beginning from an arbitrarily selected point. The following approach is an attempt to evaluate liver heat production in more absolute terms.

Heat changes between heated thermocouple and the surrounding tissues are governed by the equation $I^2 = F\theta k$. The fact that the liver itself is also a producer of heat is normally only indirectly allowed for. Consider two points A and B on the record (Fig. 2). Let the heat production of the liver at A be H_1 and at B, H_2. Let the temperature of the lower equilibrium, in excess of the afferent blood, at A be t_1 and at B, t_2. The square of the heating current at lower equilibrium = 0·200 A^2. Whence

(i) at A; $H_1 + 0{\cdot}200 = Ft_1k_1$ (t_1 and t_2 are herein substituted for θ in the formula $I^2 = F\theta k$);

(ii) and at B; $H_2 + 0{\cdot}200 = Ft_2k_2$.

But at position B, heat production in the liver has changed by an amount giving rise to an excess temperature e, which can be measured by methods already clarified. Let the difference between upper and lower equilibrium temperature be θ_1 at A and θ_2 at B.

At B, θ_2 is the temperature rise produced by 0·115 A^2; whence a temperature of e would be produced by a current of $(0{\cdot}115e/\theta_2)$ A^2

(iii) whence $$H_2 = H_1 + \frac{0{\cdot}115e}{\theta_2}$$

from (ii) $$H_1 + 0{\cdot}200 + \frac{0{\cdot}115e}{\theta_2} = Ft_2k_2,$$

but from (i) $$F = (H_1 + 0{\cdot}200)/t_1k_1;$$

(iv) whence $$H_1 + 0{\cdot}200 + \frac{0{\cdot}115e}{\theta_2} = \frac{t_2k_2}{t_1k_1}(H_1 + 0{\cdot}200).$$

In any experiment e, θ_2, k_1 and k_2 are known or can be calculated from the record; t_1 and t_2, however, give rise to difficulty. The value t_1 represents a temperature increment over and above the temperature of the inflowing blood produced partly by a heating current of 0·200 A^2, partly by the metabolic heat production of the tissue. In the case of liver there is considerable heat loss through the diaphragm and abdominal wall, which cannot be evaluated; t_1 cannot, therefore be measured directly. If the metabolic work done by the tissue were small in comparison to the work done by the heating element it might be sufficiently accurate to consider only the temperature increments produced by the heating current. In the case of liver these assumptions might not be justified.

The following calculation was made on a record from an experiment in which, although the blood flow changed, the recorded temperature difference between point A and point B did not change. The value t_2/t_1 was therefore unity and the calculation could be carried out without making any further assumptions. Figure 13 is the graph of excess temperature.

At position B, $e = 0{\cdot}23°$ C., $\theta_2 = 0{\cdot}71°$ C, $k_2 = 18{\cdot}8 \times 10^{-4}$, $k_1 = 16{\cdot}9 \times 10^{-4}$.

Applying the formula:

$$H_1 + 0{\cdot}200 + 0{\cdot}0374 = 1{\cdot}11H_1 + 0{\cdot}222,$$

whence $$H_1 = 0{\cdot}138\ A^2,$$

and $$H_2 = 0{\cdot}175\ A^2.$$

In two other experiments only were similar conditions found, i.e. no change in the level of t_1 and t_2, with, however, changes in k. In these, the corresponding values of H were 0·070 and 0·120 A^2, respectively. It is clear, then, that the heat production of the liver substance is very considerable and cannot be ignored in the calculation. At present, therefore, knowledge of heat production in the liver in quantitative terms is derived from these three rats. As will be shown, however, they do give some further information which may be of value.

The situation with regard to calculations in muscle is rather different. From three cases where the lower equilibrium temperature did not change, values for H were found to be 0·003; 0·005; 0·0004 A^2. These are exceedingly small and indicate a low resting metabolism, small in comparison with the heat produced by the heaters of the recorders. It is considered that in muscle, therefore, the tissue contribution to t_1 may be ignored and the

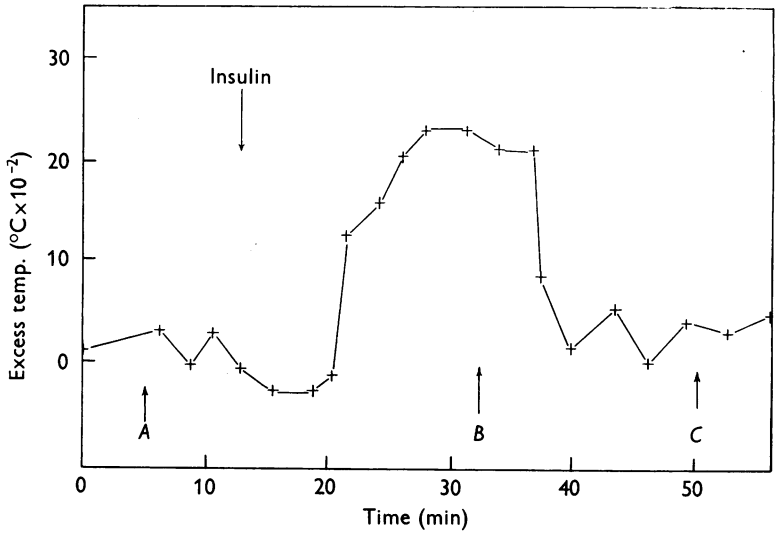

Fig. 13. Rat liver excess temperature changes following insulin; experiment to illustrate further calculations regarding heat production. In this experiment, although the blood flow changed, there was no change in lower equilibrium temperature between A and B, thus enabling heat production at A to be calculated in quantitative terms (see text). Values for position C were then determined from excess temperature and blood-flow data.

calculation applied more widely. The figures thus obtained give a comparison between heat production of the liver and the heat production of an electrically heated thermocouple operating under the same conditions in muscle.

It is, perhaps, difficult to visualize the meaning of this in terms of liver function. A further extension of the calculation, may, perhaps, clarify the issue. From the experimental values, at point A (Fig. 13), $\theta = 0{\cdot}77°$ C, i.e. a current of 0·115 A^2 produced a temperature rise of 0·77° C, whence a current of 0·138 A^2 would have produced a temperature rise of 0·92° C.

Now the flow of blood through liver is so high that the bulk of liver tissue available to be heated may be ignored. It may reasonably be assumed that most of the heat goes to raise the temperature of the blood flowing through the liver. Therefore 0·92° C is the temperature by which metabolic heat has raised that of the blood flowing into the liver. Now,

if the blood flow through the liver were known in absolute terms it would clearly be simple to calculate heat production in terms of calories. But although in the present experiments total flow has not been recorded, δk, which is a linear function of blood flow, has. Multiplying the metabolic temperature rise by δk will, therefore, give a figure in arbitrary units which is a simple function of heat production in calories.

Referring to the experiment above and applying this calculation to Fig. 13 we get:

at point A, $\delta k = 6{\cdot}2 \times 10^{-4}$; $\theta = 0{\cdot}77°$ C: metabolic temperature increment = $0{\cdot}92°$ C,

at point B, $\delta k = 8{\cdot}1 \times 10^{-4}$; $\theta = 0{\cdot}71°$ C: metabolic temperature increment = $1{\cdot}09°$ C,

at point C, $\delta k = 4{\cdot}6 \times 10^{-4}$; $\theta = 0{\cdot}83°$ C: metabolic temperature increment = $1{\cdot}09°$ C.

Whence, in arbitrary units, heat production is as follows:

$$\begin{aligned} \text{at point } A \quad & -0{\cdot}92 \times 6{\cdot}2 = 5{\cdot}7, \\ \text{at point } B \quad & -1{\cdot}09 \times 8{\cdot}1 = 8{\cdot}8, \\ \text{at point } C \quad & -1{\cdot}09 \times 4{\cdot}6 = 5{\cdot}6. \end{aligned}$$

For most of our present purposes this is as far as the calculations may be taken with any confidence. However, for the purposes of comparison with other data concerning liver metabolism the following approximation may have its interest.

In an earlier paper, Grayson (1952) suggested that a mean δk level of 12×10^{-4} usually approximated to an over-all liver blood flow of about 100 ml./100 g tissue/min. At point A, δk was $6{\cdot}1 \times 10^{-4}$. The average weight of the livers of the rats used in the present experiments was 8·0 g. It is reasonable, therefore, to assume that the total liver blood flow in this experiment was in the region of 4 ml./min. A further assumption which, in fact, is confirmed by unpublished observations on human liver (Grayson & Kinnear) is that no great temperature gradients occur within the liver. Now the temperature increment at point A (Fig. 13) produced by metabolic heat is $0{\cdot}77(0{\cdot}138/0{\cdot}115) = 0{\cdot}92°$ C. It is assumed that this temperature increment is evenly distributed. It follows, then, that in this rat the heat production of liver (this was a conscious rat) at rest was $0{\cdot}92 \times 4$ cal/min = 3·68 cal/min.

The calculation of heat production in terms of I^2 equivalents may, obviously, be equally applied to muscle. The further treatment of the figures may probably be applied with equal validity, since we are dealing only with fully equilibrated temperatures. At equilibrium the initial

task of heating the bulk of muscle tissue has already been accomplished. The further heat production is spent in heating the admittedly small flow of blood and in maintaining the temperature of the muscle bulk. In these experiments heat loss from the muscle was restricted by enclosing the limb in cotton-wool and may probably be ignored.

In calculations on muscle, however, consistency was difficult to obtain since the differences in total thermal conductivity before and after giving insulin were usually small and minor faults in recording made the value $t_2 k_2/t_1 k_1$ hard to evaluate reliably. The figures shown in Table 7 were from experiments which gave consistent results. A comparison of the figures from liver and muscle shows a very marked difference in resting heat production per unit mass of tissue, heat production in liver being almost 95 times that of resting muscle. On the other hand the effect of insulin is to raise the heat production of liver by 44%, that of muscle by 670%. Nevertheless, the gross rise produced by insulin remains far greater in liver than in muscle.

DISCUSSION

Measurement of metabolic heat production

The present paper presents a technique for the evaluation of metabolic heat production in liver and muscle. Most of the findings have been presented in terms of 'excess temperature', a simple concept indicating the difference between what actually occurs in liver or muscle and what would have occurred if, during the experiment, the only factor serving to alter tissue temperature were blood flow.

One assumption is of the highest importance, namely, that the cold junction is in a region representative of the temperature of the blood flowing to the organ. In the present work, this assumption presents little difficulty for reasons which have been fully described earlier in this paper, but it is apparent that instruments in which the cold junction is incorporated in the shaft of the needle housing the heated thermocouple (Hensel *et al.* 1954) would not be suitable for metabolic heat determinations.

The only other experimental factor which could easily affect the validity of the conclusions would be if large changes were to occur in the direct heat loss from liver or muscle. In the case of the liver it has been shown that, although the liver does, in fact, lose a considerable amount of heat by direct transfer through the diaphragm and through the abdominal wall, this factor cannot account for the phenomenon of 'excess temperature' (Grayson & Kinnear, 1958). In the case of muscle this factor is even less important since the experiments were carried out in constant-temperature rooms and the limb was, moreover, encased in cotton-wool so that the

ambient temperature of the limb approximated to that of the deep structures of the leg.

The only other major assumption is that I^2 and θ are linearly related. This point has been fully dealt with and there can be no doubt of the relation.

There can be little reasonable doubt that 'excess temperature' gives a qualitative picture of changes in metabolic heat production during the course of an acute experiment. The further mathematical treatment of the results, however, requires considerably more care.

In the first place the calculation cannot be applied to experiments where changes are too rapid. It is essential that reasonable time has been allowed for full equilibration of temperatures in relation to changed blood flow and heat production. In many experiments changes were too rapid for these conditions to be fulfilled. Accordingly, most of the results have been presented in the form of excess temperature and are intended to give merely a qualitative picture.

In a number of experiments (Table 7), however, consistent results were obtained. In these experiments it seems reasonable to suppose that the full calculation gives some indication of the quantitative changes, since the only assumption additional to those involved in 'excess temperature' is that blood flow and δk are linearly related. This has now been confirmed so many times on the basis of perfusion experiments that it can no longer be held in reasonable doubt (Grayson, 1952; Hensel *et al.* 1954).

Heat production in liver. Table 7 gives the resting heat production of liver in three rats. The figures are, of course, arbitrary units, the mean value being 3·4. On the basis of the approximations as to weight and blood flow dealt with earlier in this paper this is consistent with a mean heat production for the whole rat liver of 2·2 cal/min or 132 cal/hr. According to Behnke (1958) the total oxygen consumption of rat liver is 197 ml./hr, approximately 12 % of the rat's total consumption. This figure suggests a total heat output per hour for rat liver of approximately 98·5 cal.

Insulin and the liver

Blood flow responses. The present experiments add little to the findings of Grayson & Kinnear (1958) in this respect. The over-all response is confirmed, namely, that usually the hypoglycaemic phase is accompanied by a rise in liver blood flow.

The results of combining insulin with glucose were much the same as those previously reported. In most experiments a combination of these two substances produced no significant change in liver blood flow.

One must probably accept the conclusions of Grayson & Kinnear (1958) that insulin in doses sufficient to produce hypoglycaemia produces vasodilatation probably in the hepatic arterial radicles, that this vasodilatation is

not nervously mediated, nor can it be accounted for solely on the basis of adrenaline release.

Nevertheless, one additional fact has been elicited in the present work which further complicates the picture, namely, that glucose alone usually causes some significant increase in hepatic blood flow. It may be that this glucose vasodilatation has no connexion with the effects of insulin. The original suggestion of Grayson & Kinnear (1958), that insulin does not directly cause vasodilatation but produces its effect on blood flow only as a result of hypoglycaemia, may still be correct. Nevertheless, it is clear that the hepatic blood-flow responses in relation to blood-sugar levels are more complex than had been thought and that the present evidence is insufficient to warrant further speculation at this stage.

TABLE 7. Liver and muscle heat production per unit mass of tissue (arbitrary units).

	Liver		Muscle	
	Pre-insulin	Post-insulin	Pre-insulin	Post-insulin
	5·64	8·82	0·011	0·15
	2·42	2·90	0·020	0·18
			0·115	0·85
			0·030	0·15
	2·10	3·10	0·005	0·06
Means	3·4	4·9	0·036	0·28

Heat production responses. The heat production responses in the liver were more consistent in many ways than the blood-flow changes. In the first place insulin always produced a rise in excess temperature. The extent of the rise was variable, but from Table 7 it is seen that the mean changes were from a resting heat production of 3·4 arbitrary units per unit mass of tissue to 4·9 units per unit mass, i.e. a mean rise in heat production of 44 %.

The effect of glucose alone was not so constant but in seven out of ten experiments there was a definite fall in excess temperature.

The effect of combining insulin and glucose was to produce a variable picture. In five experiments there was a fall; in five there was a rise. But in most cases the changes were small and the general picture of reaction was of a response intermediate between that produced by insulin alone and that produced by glucose alone. Clearly, measurements of blood glucose levels would have helped greatly in assessing the results; unfortunately technical considerations made it impossible in these experiments.

The evidence at present does not permit a definitive conclusion as to the origin of the increased heat production that follows administration of insulin. The fact that glucose alone usually depresses heat production and

that a combination of insulin and glucose radically modifies the insulin effect suggests that it may be the level of blood glucose rather than the direct effect of insulin itself which determines the reaction. If this be so it is hard to avoid the further conclusion that it is the break-down of liver glycogen (in response to the low blood sugar) that is the main exothermic reaction involved.

This argument may be taken somewhat further. One of the effects of imposing on the animal a raised blood sugar by means of intravenous injection of glucose might be expected to be the inhibition of glycogen break down. This procedure usually also inhibited heat production, a finding which adds further weight to the possible importance of glycogen break-down as the exothermic reaction concerned.

Muscle and insulin

Blood-flow responses. It has been reported elsewhere that insulin usually produces an increase in blood flow in human muscle (Allwood, Ginsburg & Paton, 1957). In the present experiments this result was not so clearly established, only three out of eleven experiments showing clear-cut increments. One reason for this may be found in the nature of the technique. Internal calorimetry is not a highly sensitive method. It is hard to detect changes in very low blood flows. Conductivity increments recorded from rat muscle are usually of the order of $0{\cdot}1$–$0{\cdot}5 \times 10^{-4}$ c.g.s. units. Occasionally, however, the recorder is in a situation which yields higher values—possibly this may be due to the proximity of large vessels or to some other unknown cause. The three examples which yielded positive blood-flow responses were all cases with relatively high resting conductivity increments. It is felt, therefore, that the present results are consistent with the view that in the rat, as in man, insulin may produce vasodilation in muscle. Combining the insulin with glucose uniformly produced no detectable effect on blood flow. Glucose alone had no effect that could be detected in seven experiments. In three out of the other four experiments it caused a rise in flow. In the remaining experiment it produced a fall. There is a superficial similarity between these and the findings obtained in liver but clearly further investigation—probably by other techniques—will be necessary before the mechanisms involved can be elucidated.

Heat production in muscle. The heat-production responses were much more consistent than the blood-flow results. Thus in nine out of eleven experiments there was a clear-cut rise in excess temperature, no change in the other two.

It is seen from Table 7 that the resting levels of heat production in muscle were very much lower than those in liver. Insulin produced rises

of from 0·036 to 0·28 arbitrary units per unit mass. In terms of percentage change this is a considerable increase. In absolute terms, however, compared with the liver values, they still remain slight.

The effect of glucose alone was to depress the resting metabolism still further in nine out of eleven experiments.

The effect of combining insulin with glucose, however, was to produce increments in heat output in every case of a very high order. Indeed, the results obtained suggest that glucose might even potentiate the insulin response in muscle. This is in striking contrast to the findings in liver.

Presumably, following insulin administration there is both increased glycogen break-down within the muscle and increased glucose utilization by the cells. The simultaneous administration of glucose will help to maintain glycogen stores that might otherwise become depleted—and possibly, thereby, potentiate the insulin effect. Glucose could be regarded as acting within the cell—whither, from experience with tissue space measurement, it is clear it will diffuse rapidly. It could be pictured as supplying the needs of the cell directly without the necessity for breaking down muscle glycogen. The present work shows that glucose inhibits muscle heat production. It might well be that in muscle, probably, as in liver, glycogen break-down is also a potent source of heat.

SUMMARY

1. 'Internal calorimetry' has been used for the determination of metabolic heat production as well as blood flow.

2. The calculation of 'excess temperature', i.e. the contribution of metabolism to recorded temperature changes, is described, together with experiments to justify the assumptions involved.

3. The method has been applied to a preliminary study of the effects of insulin and glucose on blood flow and metabolism in liver and muscle.

4. Resting heat production in rat liver may be expressed in arbitrary units which enable quantitative comparisons to be made. Resting heat production in liver appears to be about 95 times that in resting muscle.

5. Insulin (I.V.) produces a 44% increase in liver heat production and a 670% increase in muscle heat production.

6. Glucose (I.V.) depresses both liver and muscle heat production.

7. Combinations of insulin and glucose (I.V.) produce variable but usually slight effects in liver. In contrast, combinations of insulin and glucose (I.V.) result in a greatly increased heat production in muscle.

8. The possible relation of these effects to glycogen break-down is discussed.

9. In the liver it is confirmed that insulin usually produces vasodilatation which, however, is prevented by the simultaneous administration of glucose. Glucose alone, however, usually also causes vasodilatation.

10. Subject to the sensitivity limitations of 'internal calorimetry' in the measurement of muscle blood-flow change it has also been confirmed that insulin may produce vasodilatation in muscle, preventable by the simultaneous administration of glucose, but that glucose alone may sometimes also produce vasodilatation.

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