In Figure 3 we show data from the 4 experiments with pyruvate as substrate where fluorescence-time integral has been plotted against the total energy production. For each muscle the energy production was varied by allowing the muscle to contract isotonically against a range of afterloads. Use of a *t*-test revealed that none of the intercepts was significantly different from zero. The results are clearly compatible with the assumption of JOBSIS and DUFFIELD[2]. Preliminary experiments also show that this linear proportionality between fluorescence-time integral and energy production is found in other substrates, but that the proportionality factor for a particular muscle depends on the substrate present.

In twitch contractions of skeletal muscle a similar relationship between energy production and time-integral of fluorescence probably exists since in toad sartorius muscle the variation in fluorescence-time integral with load[8] is very similar to the energy variation with load[9]. The relationship apparently breaks down for tetani as shown by the simultaneous heat and fluorescence measurements of GODFRAIND-DE BECKER[7] on amphibian skeletal muscle and from measurements we have made on mammalian skeletal muscle[10]. In metabolically inhibited preparations several authors have demonstrated that a linear relationship exists between phosphocreatine (PC) break-down and energy production (heat + work) in many types of contractions[11–13]. However recent tetanic energy balance studies[14,15] have shown a greater initial energy production in metabolically inhibited amphibian skeletal muscle than would have been predicted on the basis of measured PC and ATP breakdown. The complicated fluorescence data obtained at high stimulus rates or under tetanic conditions may reflect complexities produced by high glycolytic activity as suggested by JOBSIS and DUFFIELD[2] and GODFRAIND-DE BECKER[7], or may reflect the possibility that unknown reactions contribute to total energy production. However, in aerobic cardiac muscle, where total energy production (initial and oxidative recovery heat) is being measured[16–18] the postulate of JOBSIS and DUFFIELD[2] does seem to be confirmed.

The techniques described in this report are now available to study the relationship between pyridine nucleotide fluorescence and total energy production in isolated papillary muscle. This should allow the fluorescence measurements to be calibrated according to the prevailing metabolic conditions and hence provide much needed information as to the nature and extent of the conditions under which the fluorescence-time integral is proportional to total energy turnover.

Zusammenfassung. Nachweis, dass bei isometrischer und bei isotoner Arbeit des Papillarmuskels des Kaninchens die Veränderung der Fluoreszenz direkte Rückschlüsse auf die durch die Arbeit verbrauchte Energie erlaubt.

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Temperature Sensitivity of Bromosulphophthalein Clearance by the Liver

Clearance of bromosulphophthalein (BSP) is used as an index of hepatic function[1] and for the indirect measurement of hepatic blood flow in experimental animals[2,3] and man[4]. In a perfused rat liver, the transport maximum for BSP in the formation of bile is reduced by decrease in temperature[5,6]. Therefore the evaluation of BSP clearance in hypothermic animals may be affected by the temperature dependence of this procedure. This paper reports an investigation of the clearance of BSP by an isolated perfused rat liver at 37 °C and 27 °C in relation to the estimation of hepatic blood flow.

Materials and methods. Liver donors were fed male Wistar albino rats weighing between 250 and 300 g. The liver perfusion system was based on the method used by HEMS et al.[7] and incorporated the modifications suggested by HEMS and WHITTON[8]. Bovine serum albumin used in the perfusate was dialyzed against 3 changes of Kreb's bicarbonate buffer (pH 7.4) over a 48 h period to remove small vasoactive peptides. A water-jacket on the bulb-oxygenator ensured that the temperature of the perfusate was maintained constant. The perfusion pressure (14 cm water) was determined by the height of the perfusate reservoir in the oxygenator above the liver. Perfusate flow rate was measured directly by collecting the outflow in a graduated cylinder. Cannulation of the bile duct allowed direct collection and measurement of bile flow. Perfusate samples were centrifuged at 1000 *g* and the supernatant used. Lactate was assayed using lactate dehydrogenase (Worthington)[9] after precipitation of protein with perchloric acid. Samples for BSP estimation (0.2 ml) were added to 0.5 ml of 0.05 *N* sodium hydroxide and the optical density measured at 565 nm. Results are expressed as mean ± standard error of the mean and are compared by Student's *t*-test.

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Results and discussion. Lactate metabolism. Two series of 6 livers were used to monitor the viability of the perfusion system, one series at 37 °C and one at 27 °C. In each case an initial sample (2.5 ml) of the original perfusate was retained for analysis. All subsequent samples were replaced with perfusate and a correction made for the small dilution effect when calculating the total substrate content of the perfusate. Samples (2.5 ml) were collected during an equilibration period at 15 and 30 min after establishing the perfusion. After 35 min 1500 µmoles of lactate (0.12 *M* solution of Li-DL-lactate) was added to the collecting vessel following the removal of an equivalent volume of perfusate. This gave a lactate concentration in the perfusion medium of 20 m*M*. The rate of removal of lactate by the liver was followed in serial samples taken after 5, 25, 55 and 85 min.

At 37 °C, lactate was taken up from the perfusate at a mean rate of 0.84 µmoles/min/g wet weight of liver (Figure 1). This is consistent with the data obtained by HEMS et al.[7] and WILLIAMSON et al.[10] using 24 h starved rats. In the livers perfused at 27 °C the rate of lactate consumption was reduced by 45% to 0.46 µmoles/min/g.

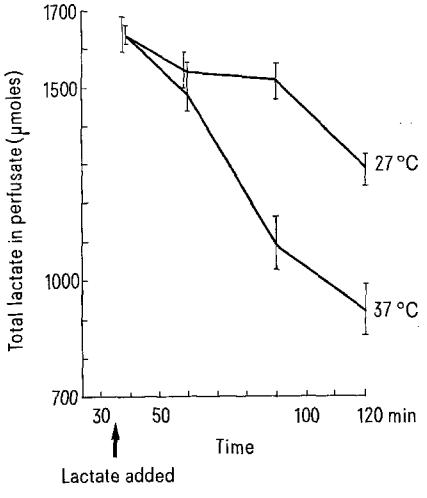

Fig. 1. Mean lactate contents of perfusates at 37 °C and 27 °C. Results are shown as the mean of 6 experiments standard error of the mean.

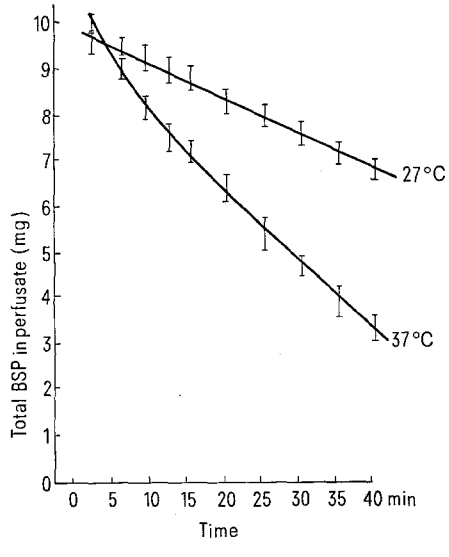

Fig. 2. Mean BSP contents of perfusates of livers at 37 °C and 27 °C.

Bile secretion rate. The mean rate of bile secretion at 37 °C was 0.82 ± 0.27 µl/min/g. At 27 °C the bile flow rate was reduced by 47% to 0.43 ± 0.4 µl/min/g ($p < 0.01$). Similar results have previously been reported[6,11].

E.M. histology. Electronmicrographs (×31,000) of sections from livers perfused at 37 °C and at 27 °C and from normal (non-perfused) livers were compared. Perfusion at either temperature did not result in any marked changes in the structure of the liver cells.

BSP clearance by the liver. The effect of temperature on the ability of the liver to clear BSP (sulphobromophthalein; Koch-Light) from the perfusate was investigated in 2 further series of 6 liver perfusions, 1 group at 37 °C and 1 at 27 °C. BSP (10 mg as a 0.2% solution, pH 7.0) was added to the collecting vessel after an equilibration period of 30 min. Samples for BSP assay were taken from the hepatic portal vein cannula and from the venous outflow cannula every 3 min for the first 15 min, and then every 5 min until 40 min after addition of the dye. The initial mean rate of removal of BSP at 37 °C was 21.7 µg/min/g wet weight of liver and at 27 °C was reduced to 7.4 µg/min/g (Figure 2). These results are in close agreement with the values calculated by other workers[6] using rat livers perfused at 40 °C and 25–30 °C. The reduction in hepatic clearance of BSP could not be explained by changes in the flow rate of perfusate through the 2 groups of livers. Mean perfusate flow rate measured by collection of the venous outflow was 23.2 ± 1.6 ml/min at 37 °C and 21.0±1.1 ml/min at 27°C and the difference was not significant.

The percentage extraction of BSP was calculated as follows:

$$\% \text{ extraction of BSP} = \frac{(\text{Hepatic portal vein conc.}) - (\text{Hepatic vein conc.}) \times 100}{(\text{Hepatic portal vein conc.})}$$

The percentage extraction of BSP was 10.0% ± 0.55 at 37 °C but was reduced to 5.3% ± 0.34 at 27 °C ($p < 0.001$).

The decay curves shown in Figure 2 indicate that the characteristics of BSP clearance are different at the 2 temperatures. At 27 °C there is a linear disappearance of BSP from the perfusate. However, at 37 °C there is a much faster rate of removal at the initial high BSP concentrations that at the subsequent lower concentration. The uptake of BSP is reported to be at least a two-stage process involving an active transport into the parenchymal cells followed by a ratelimiting transfer into the bile[12]. The difference in the shape of the BSP decay curves at the 2 temperatures studied may indicate some alteration in the relative rate of the two processes.

Using the figures quoted earlier for the percentage extraction of BSP at 27 °C and 37 °C, the correlation between the flow rate through the liver as measured by application of the Fick principle to BSP clearance, and direct collection of the venous outflow was investigated. Mean perfusate flow rate at 37 °C was 23.2 ± 1.6 ml/min by direct measurement. The corresponding BSP clearance measurements gave a value of 23.7 ± 1.2 ml/min, which was not significantly different. At 27 °C, mean perfusate flow rate from direct measurements was 21.0 ± 1.1 ml/min and from BSP clearance, 19.1 ± 1.8 ml/min, which

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again was not significantly different. The close agreement between the two methods demonstrates that under the conditions described, provided the values presented for percentage extraction are used, mean hepatic blood flow may be calculated from BSP clearance at reduced temperature.

Résumé. L'épuration de la bromosulphophtaléine (BSP) par le foie de rat perfusé a été étudiée à 37 °C et à 27 °C. Les courbes de disparition de la BSP indiquent un changement dans le mécanisme d'épuration à la température inférieure. L'épuration de la BSP est utilisée comme mesure de la vitesse d'écoulement aux 2 températures; moyennant une correction appropriée, un bon accord est obtenu avec les mesures directes.

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Rhythmische Änderungen der Reaktion auf verschiedene olfaktorische Reize beim Krallenfrosch (*Xenopus laevis*)

Nachdem Trincker[1,2] die bei Anpassungsvorgängen durch Lernen (Dressur) auftretenden Änderungen des motorischen Verhaltens von Amphibien (*Ambystoma*) und Fischen (*Carassius*) als Schwingungsvorgänge im mathematischen Sinne auffasste, wies Ewert[3,4] nach, dass bei optischer Dauerreizung von Erdkröten (*Bufo bufo*) der exponentiell verlaufende Abfall der Handlungsbereitschaft von einem zentralen, periodisch abklingenden Vorgang überlagert wird. Entsprechende rhythmische Änderungen der ethometrisch als Reaktionsgrösse einer Verhaltensweise erfassbaren Handlungsbereitschaft konnten nun auf olfaktorische Reize beim Krallenfrosch (*Xenopus laevis*) nachgewiesen werden.

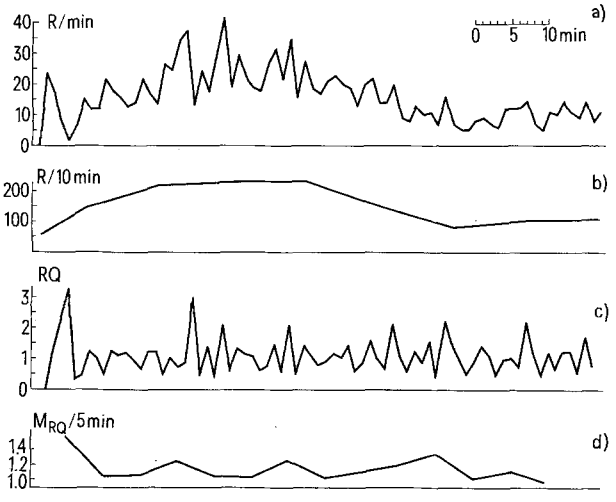

Fig. 1. a) und b). Reaktionskurven bei Registrierung über 75 min; Ordinate: Reaktionen pro min bzw. pro 10 min c) Reaktionsquotientenkurve. d) Kurve der Mittelwerte aller Reaktionsquotienten für jeweils 5 min zur Erfassung der Schwebung. Abszisse jeweils Zeitverlauf; Mittelwerte aus 6 Messungen.

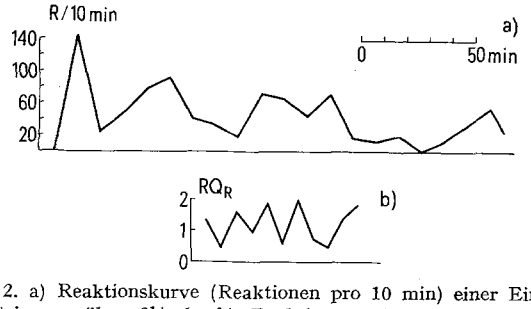

Fig. 2. a) Reaktionskurve (Reaktionen pro 10 min) einer Einzelregistrierung über $3^1/_4$ h. b) Reaktionsquotientenkurve für die Reaktionen pro Raffbewegungsserie (Mittel aus 6 Messungen in der 1. Reaktionsminute mit je 12 Raffbewegungsserien).

Als Versuchstiere dienten 7 adulte Krallenfrösche, die zwischen den Tests in einem grossen Gemeinschaftsbecken gehalten wurden. Durch regelmässiges, individuelles Füttern mit jeweils der gleichen, nicht ad libitum verabreichten Nahrungsmenge wurde versucht, einen vergleichbaren Sättigungs- bzw. Hungerzustand aller Tiere zu erzielen. Zur Versuchsdurchführung wurde jeweils ein einzelner Krallenfrosch in ein Aquarium gebracht, in dessen Wasser als stark wirksamer olfaktorischer Reiz ein durch Zerreiben von 3 Mehlkäferlarven gewonnenes Konzentrat bzw. verschiedene reine Prüfsubstanzen gleichmässig in 2 l Wasser verteilt waren. Zur quantitativen Erfassung der Reaktion wurde die Zahl der für diese Art beim Beuteerwerb typischen Raffbewegungen mit den Vorderbeinen pro Minute bestimmt. Aus den erhaltenen Reaktionszahlen (R) zweier jeweils aufeinander folgender Minuten wurde entsprechend der von Ewert[3,4] benutzten Methode ein Reaktionsquotient (RQ) errechnet ($RQ = R_i/R_{i+1}$).

Bei über 75 min durchgeführten Registrierungen zeigt sich ein Reaktionsverlauf (Figur 1a), der bei einer auswertenden Zusammenziehung der Reaktionszahlen für einen Zeitraum von jeweils 10 min zum Zwecke einer möglichst guten Kurvenglättung dem Bild einer normalen Handlungsbereitschaftskurve (z.B.[5]) auf Dauerreizung mit Anlaufphase, Phase maximaler Reaktion und Gewöhnungsphase entspricht (Figur 1b). Wie der zeitliche Verlauf des RQ belegt, ist dieser Kurve eine Reaktionsoszillation mit einer mittleren Schwingungsdauer von 2 min 52 sec (s = ± 10 sec) überlagert (Figur 1c; Berechnung der Schwingungsdauer über die Zahl der Kurvengipfel in den ersten 50 min, während denen noch bei keinem Individuum Pausen auftraten). Nachdem diese Oszillation des RQ einer als Schwebung auffassbaren periodischen Amplitudenschwankung unterliegt, die am klarsten bei einem Zusammenziehen der Werte für jeweils 5 min als 15-min-Rhythmik zur Darstellung gebracht werden kann (Figur 1d), ist auf die tatsächliche Überlagerung zweier Grundschwingungen zu schliessen (Dauer der $Schwingung_1$ 2 min 52 sec, der $Schwingung_2$ nach $f_{Schwebung} = f_1 - f_2$ ca. $3^1/_2$ min). Einer $3^1/_4$ h langen

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