Protein Turnover in Skeletal Muscle and Liver of Rats Bearing the Walker 256 Carcinoma

In an investigation of the effect of tumour growth on protein degradation in host tissues, a study has been made of the effect of the Walker 256 carcinoma on labelling patterns in muscle and liver of rats injected with ^{3}H-L-lysine 7 days before tumour implantation.

Methods. Female Wistar rats of mean body weight 192 g were injected i.p. under ether anaesthesia with 20 μCi L-lysine-(4,5)-^{3}H·HCl (250 mCi/mmole) per 100 g body weight. Some of the animals were killed after 7 days (control group) and the remainder were anaesthetized with ether and injected s.c. either with a suspension of Walker 256 carcinoma cells or with 1 ml 0.9% (w/v) saline and killed after a further 10 days, when the mean tumour weight was 24.9 g. Each animal was fed daily 12 g finely ground rat cake (North Eastern Agricultural Co-operative, Ltd.) supplemented with L-^{12}C-lysine to reduce the reutilisation of labelled amino acid[1–3]. Total daily available lysine intake was 135.5 mg, estimated according to CARPENTER[4]. Total protein of liver, soleus and gastrocnemius was isolated, estimated and counted as previously described[5]. Statistical evaluation of results was carried out using Student's *t*-test.

Results and discussion. The data in the Table indicate that there was no loss of protein from the soleus due to tumour growth and the fall in protein specific activity and loss of total labelled protein were the same in both the tumour-bearing and saline-injected rats. The percentage loss of total labelled protein from the 7th to 17th day following isotope injection was 21.1% in both groups which was not significantly greater than the percentage loss from the gastrocnemius (15.1%) in the saline-injected rats and significantly less ($P < 0.001$) than the percentage loss from the gastrocnemius of the tumour-bearing rats (31.7%). It would appear that tumour implantation and growth had no effect on protein turnover in the soleus during this period.

The increased loss of total labelled protein from the gastrocnemius of the tumour-bearing rat was accompanied by a net loss of protein and a greater fall in protein specific activity compared to the saline-injected rat. A fall in total labelled protein reflects the extent of protein catabolism in a tissue and thus these results would suggest that during the period of tumour implantation and growth there is an increase in protein catabolism in the gastrocnemius. The increased fall in specific activity of gastrocnemius protein in the tumour-bearing rat probably results from the greater relative loss of labelled protein in this group. On the other hand, in the liver of the tumour-bearing rat in which there was an increase in protein content and a retention of more labelled protein than in the liver of the saline-injected rat, the decrease in protein specific activity is most readily interpreted as being due to an increased rate of protein synthesis. This would be in accord with the finding of an increase in the in vivo incorporation of ^{14}C-glycine into liver protein of tumour-bearing rats during short-term labelling[6]. The greater amount of radioactive protein remaining in the liver of the tumour-bearing rat would suggest that there has been a reduction in the rate of protein catabolism.

Thus the difference in the response of the soleus and gastrocnemius to the Walker 256 carcinoma is due not only to a differential effect of the tumour on protein

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Specific activity and total radioactivity of proteins of soleus, gastrocnemius and liver of control, saline-injected and tumour-bearing rats

	Control	Saline injected	Tumour-bearing	*P*
mg protein per soleus per 100 g initial body wt.	15.8 ± 0.7	16.1 ± 0.6	16.2 ± 0.8	n.s.
dpm per mg soleus protein	1262 ± 79	969 ± 36	962 ± 25	n.s.
dpm × 10^{-3} per total soleus protein per 100 g initial body wt.	19.7 ± 1.1	15.7 ± 0.8	15.6 ± 1.0	n.s.
Mean percentage loss total labelled protein	—	20.8 ± 4.1	21.3 ± 4.9	n.s.
mg protein per gastrocnemius per 100 g initial body wt.	116.1 ± 2.9	114.6 ± 1.4	103.9 ± 1.9	<0.001
dpm per mg gastrocnemius protein	1119 ± 40	958 ± 25	850 ± 30	<0.002
dpm × 10^{-3} per total gastrocnemius protein per 100 g initial body wt.	129.3 ± 2.9	109.8 ± 3.5	88.3 ± 3.4	<0.001
Mean percentage loss total labelled protein	—	15.1 ± 2.7	31.7 ± 2.6	<0.001
mg protein per liver per 100 g initial body wt.	561 ± 13	601 ± 19	763 ± 21	<0.001
dpm per mg liver protein	2451 ± 78	844 ± 15	789 ± 21	<0.05
dpm × 10^{-3} per total liver protein per 100 g initial body wt.	1377 ± 65	507 ± 18	600 ± 14	<0.001
Mean percentage loss total labelled protein	—	63.2 ± 1.3	56.5 ± 1.0	<0.001

Results are expressed as the mean ± S.E. of 8 experiments. Paired soleus and right gastrocnemius muscles were analysed. *P* value given for saline-injected versus tumour-bearing group.

biosynthesis in the two tissues as shown previously[5,7] but also to its specific effect on protein catabolism in the latter.

Résumé. Les protéines des tissus de rats ont été marquées au moyen de L-Lysine-4, 5-H3. Une inoculation de Carcinome Walker 256 a été effectuée 7 jours après. Des observations de perte de protéines radioactives indiquent que chez les rats avec tumeur la dégradation des protéines du gastrocnémius augmente celle des protéines du foie, diminue et que celle du soléus n'est pas modifiée.

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Platelet Aggregation by Thrombofax®. Studies on the Mechanism of Action[1]

We have reported in a previous communication[2] the platelet-aggregating activity of Thrombofax (Ortho), a commercial cephalin prepared as an ether extract of acetone-dried brain[3]. This reagent is ready-to-use, stable and gives highly reproducible results. It has been shown to aggregate platelets from humans[2], dogs, guinea-pigs and rats[4].

The addition of 0.2 ml Thrombofax to 0.8 ml stirred normal citrated platelet-rich plasma (PRP, 300,000 platelets/µl) in a Born aggregometer provokes a strong platelet aggregation after a latent period; the duration of the latent period is inversely, and the initial rate of aggregation is directly, related to the amount of Thrombofax added (Figure 1). A continuous stirring of the system is required to obtain platelet aggregation. Previous incubation of PRP with Thrombofax at 37 °C, before stirring is started, shortens or abolishes the latent period before aggregation (Figure 2). In the presence of non-aggregating amounts of Thrombofax, a strong second wave of aggregation appears following addition of either adenosine-5′-diphosphate (ADP) (Figure 3) or adrenaline (Figure 4) at concentrations unable to provoke by themselves a second wave of aggregation, indicating synergism of Thrombofax with these aggregating agents. Recently it has been shown[5] that Thrombofax, like other aggregating agents, induces retraction in PRP clotted by reptilase.

The possible role of thrombin in mediating the aggregating activity of Thrombofax could be excluded by the observation that concentrations of heparin, which inhibit the aggregating activity of at least 130 N.I.H. units/ml thrombin, are ineffective on aggregation by Thrombofax; in addition, formation of plasma clots during or after aggregation by Thrombofax was never observed; finally, platelets from patients with congenital deficiency of factor V, VII or VIII reacted normally to Thrombofax.

Thrombofax preincubated at 37 °C with 8 mg/ml human albumin (Hyland) progressively loses its aggregating activity which usually disappears within 30–45 min. Preincubation of Thrombofax with normal human platelet-poor plasma does not modify its aggregating properties. Since it has been demonstrated by WARNER et al.[6] that albumin inhibits the aggregating property of free fatty acids (FFA), it is likely that FFA, which indeed constitute about $^1/_3$ of the total lipid content of Thrombofax[7], are responsible for its aggregating activity. The aggregating activity of Thrombofax was not linked to the thermolabile fraction in this reagent, which precipitates at 56 °C. Platelet aggregation by phospholipids and FFA has been reported by several authors[6,8–10]. HASLAM[9] demonstrated that the aggregating effect of FFA was mediated by the release of endogenous platelet ADP.

That Thrombofax induces platelet aggregation by a similar mechanism is supported by the following observa-

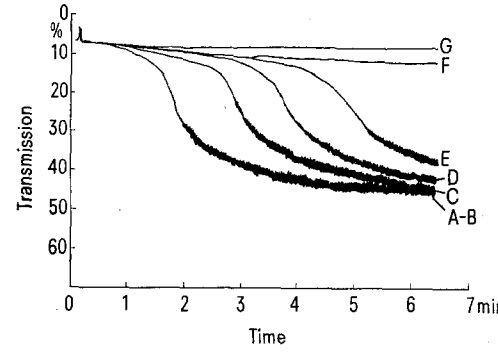

Fig. 1. Human platelet aggregation by different concentrations of Thrombofax. To 0.8 ml PRP were added 0.2 ml of Thrombofax at the following dilutions (in isotonic saline): A, undiluted; B, dilution × 2; C, dilution × 4; D, dilution × 8; E, dilution × 16; F, dilution × 32; G, dilution × 64.

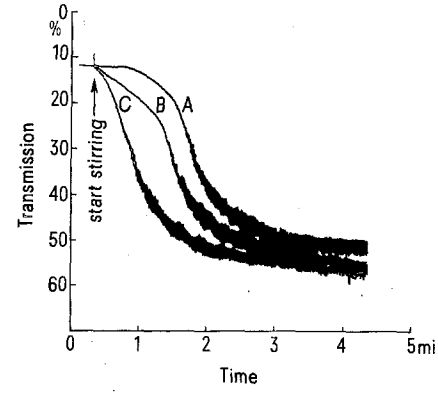

Fig. 2. Effect of previous incubation of human PRP with Thrombofax at 37 °C, without stirring, on subsequent platelet aggregation. A, no preincubation; B, 30 sec preincubation; C, 60 sec preincubation.

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