Similarity of the Rigor Mortis Process in Normal and Germ-free Rats

Bate-Smith and Bendall[1] reported that rigor mortis in muscle was caused by the irreversible association of actin and myosin as adenosine triphosphate (ATP) became depleted. Conflicting reports have appeared concerning the process of rigor mortis in germ-free animals. Harmon[2] has observed that germ-free rats do not become rigid after death. These observations were only visual, but classical theories of the process would require critical re-evaluation if muscle from germ-free animals showed no rigor mortis. We have designed an experiment to investigate the time course of rigor mortis development and associated parameters in normal and germ-free rats (Table 1).

Table 1. COMPARISON OF GERM-FREE AND NORMAL RATS WITH RESPECT TO RIGOR MORTIS AND ASSOCIATED PARAMETERS

Parameter	Muscle	Animal group: Control $\bar{x}$	Control $s_{\bar{x}}$	Germ-free $\bar{x}$	Germ-free $s_{\bar{x}}$	
Rigor mortis† (min)	B.F.	437·7	40·01	366·5	36·01	*N.S.*
Duration of contractility‡ (min)	L.D.	351·0	19·48	275·5	17·06	*
Post-rigor sarcomere length (μ)	L.D.	2·20	0·01	2·14	0·04	*N.S.*
Phosphocreatine (moles/g of muscle)						
15 min§	G.M.	3·98	0·23	2·99	0·43	*
4 h§	G.M.	1·17	0·13	1·77	0·39	**
15 min	Quad.	4·38	0·68	3·25	0·34	*N.S.*
4 h	Quad.	0·96	0·03	1·87	0·31	*
Adenosine triphosphate (moles/g of muscle)						
15 min	G.M.	4·14	0·31	4·15	0·29	*N.S.*
4 h	G.M.	0·89	0·09	0·79	0·04	**
15 min	Quad.	3·59	0·32	2·85	0·32	*N.S.*
4 h	Quad.	0·85	0·09	0·80	0·03	*N.S.*
Lactic acid (moles/g of muscle)						
15 min	G.M.	7·00	0·95	12·12	2·08	**
4 h	G.M.	33·11	3·16	30·07	3·83	*N.S.*
15 min	Quad.	9·40	1·73	9·10	1·43	*N.S.*
4 h	Quad.	28·10	3·90	22·67	2·07	*N.S.*

B.F., biceps femoris; L.D., longissimus dorsi; G.M., gluteus medius; quad., quadriceps.
* ($P < 0·05$).
** ($P < 0·01$).
† Time (min) required for complete loss of muscle extensibility[3].
‡ Time (sec) during which the muscle has the ability to sustain a contractile force of 10 g with a 50 V electrical stimulation at a frequency of 2/c/s and a stimulus duration of 0·1 m.sec[5].
§ Time post-mortem.

Ten germ-free and ten normal disease-free control rats (weighing 400 g) were killed by stunning and decapitation. Because the muscles were small several of them had to be used. The biceps femoris muscles were immediately excised and placed in a rigorometer apparatus[3] to determine the time (min) required for complete loss of extensibility, at 37° C, which has been associated with ATP depletion and actomyosin formation (Bendall[4]). There was no significant ($P > 0·05$) difference in the time course of rigor mortis between germ-free and normal disease-free control rats (Table 1). Because striated muscle, which goes into rigor mortis slowly (long delay phase), is more responsive to electrical stimulation than muscle with a short delay phase of rigor mortis[5] muscle response to electrical stimulation was also investigated. The mean differences (Table 1) in the stimulatory response were very small, but the germ-free rats showed a significantly ($P < 0·05$) shorter duration of contractility on repeated stimulation. Statistically these data suggest that, if there were any real difference, the germ-free rats would develop rigor mortis more quickly than disease-free controls.

Certain post-mortem chemical changes are shown in Table 1. Creatine phosphate[6] appeared to be initially lower in muscles of germ-free rats. The concentration of ATP[6] decreased in both groups of rats to about 30 per cent of initial values by 4 h post-mortem. Lactic acid values increased in both groups at a rate similar to that reported for rabbits (personal communication from Bendall).

Post-rigor sarcomere lengths[7] were also determined because the sarcomere length is associated with the duration of the delay phase of rigor mortis[8]. No significant differences in post-rigor sarcomere lengths were found between the two groups of rats (Table 1).

In addition to the physical and chemical parameters, all of which indicated a normal rigor mortis process, visual observations revealed obvious post-mortem stiffening in both groups. In order to ensure absolute germ-free conditions, an additional group of four germ-free rats were killed by cervical fracture and were left undisturbed in the isolator for 4 h. These four rats stiffened and this supports the observations of the post-mortem stiffening of germ-free guinea-pigs[9] and germ-free mice[10].

The results of the present investigation, supported with objective chemical, physical and histological techniques, clearly show that the normal rigor mortis process occurs in germ-free rats.

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Urea Formation by the Lactating Goat Mammary Gland

NEARLY thirty years ago Graham *et al.*[1] suggested, on the basis of arteriovenous difference measurements, that urea was produced by the lactating goat mammary gland. This view gained credence with the discovery of arginase in lactating mammary tissue and in a review article in 1949 Folley[2] considered it probable that the deamination of amino-acids (with related urea production) was an important source of the carbohydrate required for milk secretion. Later results of arteriovenous difference measurements and perfusion experiments in the cow, together with the demonstration that amino-acids absorbed by the gland are used for milk protein formation, however, appeared to invalidate this view (see Barry[3]).

In a study of arteriovenous differences of amino-acids across lactating goat mammary glands, in which simultaneous measurement of mammary blood flow and milk protein yield allowed a quantitative comparison of the amino-acids absorbed by the glands with the corresponding amino-acid residues secreted in milk protein, we reported a consistently excessive absorption of arginine. On average, about three times as much arginine was