Vème REUNION DES INSTITUTS DE RECHERCHES SUR LES VIANDES

PARIS - du 7 au 12 Septembre 1959 -

N° 16

The Effect of Pre-Treatment with Various Drugs on the Time Course of Rigor-Mortis and on Post-Mortem Glycolysis in Rabbits.

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At last year's meeting of the European Meat Workers in Cambridge, Radouco-Thomas reported on the effects of injecting adrenalin and reserpine pre-slaughter on the time-course of post-mortem glycolysis and on the ultimate pH of the muscle. She showed that subcutaneous injection of adrenalin into rabbits four hours before slaughter depleted the glycogen-reserves of the muscles, so that the ultimate pH was raised from the normal value of ~ 5.60 to ~ 6.50, whereas injection of the tranquilliser reserpine tended to lower the ultimate pH. The effect of adrenalin in reducing the muscle glycogen and raising the level of blood lactic acid had already been reported by the Coris (Cori 1925 ; Cori & Cori, 1928). They also showed that the lactic acid formed was reconverted to glycogen in the liver. It is not clear, however, whether the effect is due to a direct action of adrenalin on the glycolytic enzymes or whether it is due to constriction of the capillaries in the musculature, resulting in a lowered oxygen tension.

The present work forms part of a collaborative study with Dr. Lawrie on the effect of stress. It was designed primarily to show whether injection of adrenalin affected the post-mortem rate of glycolysis as well as limiting its extent by reducing the glycogen reserves, and secondly whether such effects could be reversed by injecting the animal with cortisone. At the same time a few experiments were carried out to show the effects of injection of cortisone alone, of nor-adrenalin, of reserpine and of serotonin.

The parameters studied in the psoas muscles were pH, creatine phosphate (C.P.), total adenosine and inosine nucleotides, inosine monophosphate (IMP), adenosine triphosphate (ATP) as acid-labile phosphate, and the extensibility of the muscle. Some values for ultimate pH in the longissimus dorsi muscles are also given. In all cases the animals were killed after immobilising them for ten minutes with myanesin, in order to abolish struggling at death and thereby obtain a high initial pH and C.P. level. The analytical methods are those given by Bendall & Davey (1957).

The following rates were calculated from the time-curves for the individual experiments :

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a) the average rate of fall of pH as pH units per hour during the first 80 mins. of the experiment.

b) the average rate of ATP turnover as μ atoms P/min/g during the first 100 min. of the experiment. This rate represents the sum of the rates of disappearance of C.P. and of ATP itself and of the rate of resynthesis of ATP from the glycolytic cycle. It is given by the sum :

$$-\frac{d\,CP}{dt} - \frac{d\,ATP}{dt} + 97.5\,\frac{d\,pH}{dt}$$

It remains fairly constant during the early stages of rigor mortis at 37° C (c.f. Bate-Smith & Bendall, 1956).

Results

1) The effect of adrenalin and nor-adrenalin

The effect on the various parameters in the psoas muscle of subcutaneous injection of 1.5 mg adrenalin into rabbits four hours before death is summarised in Table 1., with values for fed and fasted controls for comparison.

The main effects of adrenalin injection may be summarised as follows :

1) the initial pH of the muscles is unaffected.
2) the initial level of C.P. is raised significantly.
3) the total nucleotide content and the resting level of ATP are slightly but significantly lowered by the treatment.
4) the ultimate pH is raised to the level characteristic of about three days fasting, although the animals had been fed in the same way as the controls.
5) the rates of pH fall and ATP turnover are significantly raised above those for fed and fasted controls.
6) the times for half-change of extensibility and of ATP are very much reduced below those for the controls, which do not differ significantly from one another in spite of the lowering of the glycogen reserves by fasting.

Compared with these pronounced changes in all the parameters, injection of nor-adrenalin in the same manner has a very different effect. In the two cases where the drug was used it was found that :

a) the initial pH and C.P. values were lower than the controls, but the initial ATP and nucleotide contents were unaffected.
b) the ultimate pH was unaffected and fell to 5.54 in one case and 5.50 in the other.
c) the rate of fall of pH down to pH 6.50 was higher than the control value (0.35 units/hr against 0.24), but the rate from pH 6.50 to pH 5.70 was lower (0.46 units/hr against 0.53).

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d) the rate of ATP turnover up to 100' was increased from the control value of 0.59 μatoms/min/g to 0.81 atoms/min/g, that is into the range for adrenalin-treated animals.

e) the combined effect of the above changes is to shorten the time for half-change of ATP from an expected average value of 135' to an average value of 120', and for half-change of extensibility from 160' to 150', allowing for the differences in initial pH between the controls and the treatment. Thus nor-adrenalin seems to modify the rate of post-mortem change more than it does the size of the paramaters.

Comparative values for the ultimate pH of the longissimus dorsi muscles are given in Table 2, and are seen to follow the same trends after adrenalin and nor-adrenalin treatment as the values for the psoas in Table 1. It will also be noted that there is a definite and significant trend under all treatments for the ultimate pH to increase from the lumbar to the cervical ends of the longissimus dorsi.

2) <u>The effect of cortisone in protecting against the effects of adrenalin.</u>

If cortisone (10 mg in 0.5 mls methyl oleate) is injected intramuscularly on the day before death, subsequent injection of adrenalin four hours before death has very much less effect. We then find no significant differences from fed control animals in the following parameters :

initial pH, C.P. and ATP and total nucleotide content. The rates of change are, however, still somewhat higher than the control values, the rate of pH fall being 0.29 $\pm$ 0.03 units/hr against 0.24 for the control (4 cases) and the rate of ATP turnover being 0.64 $\pm$ 0.04 μatoms P/min/g against 0.58 for the control (8 cases). These differences from the controls are not, however, statistically significant, whereas the differences in rate of turnover of ATP from the adrenalin-treated examples given in Table 1 are significant at the level of P = 1.5 %. Thus the treatment has altered the pattern of chemical change back towards the normal, but not completely. This is reflected in the times for half-change of extensibility, which were 127 mins. and 153 mins. in two treated cases, compared with average values for the fed controls of 204 $\pm$ 13 minutes (5) and for the adrenalin-treated animals of 82 $\pm$ 6 (5). Similarly the times for half-change of ATP were 152 $\pm$ 5 (3 cases), 181 $\pm$ 12 (5) and 114 $\pm$ 11 (4), respectively, for adrenalin/cortisone, fed controls and adrenalin treatments.

The most marked protective effect of cortisone is, however, on the glycogen-reserves which is reflected in the values for ultimate pH both in the longissimus dorsi muscles (Table 2) and in the psoas. In the former, the ultimate pH of the cortisone-protected muscles has regained the level of the fed controls, from which it does not differ significantly. In the case of the psoas, the ultimate pH is restored from an average value for adrenalin treatment of 6.48 $\pm$ 0.06 (8) to 6.02 $\pm$ 0.04 (4), compared with 5.70 $\pm$ 0.03 (5) for the controls. This value differs significantly from normal at P = 5 % and from adrenalin treatment at P = 0.1 %

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3) The effect of cortisone alone

The effect of a cortisone injection 24 hours before death is to leave unaffected the following parameters, initial pH, C.P. and ATP, total nucleotide and ultimate pH, none of which differ significantly from the values for fed controls in Table 1. This is well seen in the case of ultimate pH from the values for the longissimus dorsi muscles given in Table 2. There are, however, significant differences from the controls in the rates of change. Thus the overall rate of ATP turnover is raised by the treatment from 0.59 $\pm$ 0.03 μ atom P/min/g (14) for the controls to 0.74 $\pm$ 0.03 (6), a difference which is significant at P = $<$ 0.2 %, but this was offset in one case by a temporary arrest of glycolysis for 37 minutes in the region of pH 6.9, accompanied by a decrease in the rate of C.P. disappearance. This was followed by a phase of increased ATP turnover. The overall effect was to increase the time for half-change of ATP from the control value of 181 mins. to 210 mins., and for half-change of extensibility from 204 to 220 minutes. A similar phenomenon is sometimes encountered in untreated animals and has so far received no satisfactory explanation.

4) The effect of reserpine

Injection of reserpine was carried out in only one case, so that conclusions about its effect must be tentative. In this case, the pH/time curve was close to normal, but with a tendency to be somewhat more protracted, whereas both the initial and ultimate pH values lay in the normal range. The slower fall of pH was reflected in an abnormally long time for half-change of extensibility of 259 mins. The longest time recorded for this parameter in fed controls in the present experiments was 230 mins. and the longest in any experiment was 280 mins., so that this value is certainly exceptional.

5) The effect of serotonin

Two experiments were carried out with serotonin, which was injected interperitonally, in one case two hours before death and in the other four hours before death, in the form of serotonin creatinine sulphate (100 mg in 4 mls of 0.45 % NaCl). In the first case, the initial pH and C.P. levels of the psoas were rather low (6.87 and 14.2 μ mols/g) and the ultimate pH was normal (5.78), and in the other the former values were normal (7.06 and 25.5 μ mols/g), whereas the ultimate pH was high (6.30). Similarly the average pH of the longissimus dorsi muscle was higher in the animal injected 4 hours before death (5.87) than in that injected 2 hours before death (5.72). In both cases the rates of ATP turnover were significantly higher than normal (0.71 μ atom P/min/g), and the times for half change of ATP and of extensibility were correspondingly lower than would be expected for controls with the same initial levels of C.P and pH, to the extent of 30 mins.

These results are somewhat similar to those brought about by adrenalin, and suggest that the apparent effect of serotonin may in fact be due to adrenalin release in response to the drug. Further experiments are necessary, however, before reaching such a conclusion, because it is quite apparent that more than 4 hours are necessary for the drug to be fully effective.

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Conclusions

The most striking feature of the results is the very marked effect of subcutaneous injection of adrenalin not only in increasing pre-mortem glycolysis, but also in increasing the rate of ATP-turnover post-mortem, in contrast to lack of effect of nor-adrenalin in the living animal, but its modification of the changes post-mortem. The difference in action between the two drugs on the depletion of glycogen reserves in the living animal has already been noted by Bloom & Russell (1952), who showed that subcutaneous injection of nor-adrenalin into rats is completely without effect on the total carbohydrate of the muscles, and on the blood lactic acid. Nevertheless, the reason for the difference remains obscure, since both drugs have marked vaso-constrictor activity which at first sight might be thought to account for the increased glycolysis in the muscles by reducing the blood flow through them and thus the oxygen tension and, secondly, neither drug appears to have any effect on glycolysis in vitro. It can only be concluded that the specific action of adrenalin must be at the level of the muscle membrane, and that this effect can persist into the post-mortem period, as shown by the marked increase in ATP-turnover. Indeed, we know from the experience of thaw-rigor that damage to the muscle membrane can drastically increase this latter parameter in the excised muscle. Even so, it is still difficult to explain why such increases in the living animal cannot easily be overcome by oxidative phosphorylation, thus sparing the glycogen reserves, unless the oxygen supply to the muscle is decreased also by the action of the drug. In this connection, it is interesting that serotonin which is known to act on the adrenal medulla has somewhat similar effects to adrenalin itself, particularly about four hours after injection.

Protection against the effects of adrenalin is afforded by cortisone, which probably acts by increasing the rate of formation of carbohydrate from protein (gluco-neo-genesis) as soon as the glycogen-reserves have been reduced by the action of adrenalin. Nevertheless, cortisone by itself tends to increase the post-mortem rate both of glycolysis and of ATP turnover, and to reduce the times for half-change of ATP and extensibility. It is not clear how this comes about, unless this hormone can evoke the release of adrenalin, or some other substance with similar effects.

Besides their bearing on the normal post-mortem changes in muscle, the present results are of some interest in relation to the muscle-degeneration (M.D) disease found in Landrace pigs on the one hand, and to dark-cutting beef, on the other. As Ludvigsen has shown, the M.D disease is characterised by very rapid post-mortem glycolysis, but, unlike the adrenalin syndrome, the glycogen-reserves are unaffected and the ultimate pH is either normal or lower than normal. Similar high rates of glycolysis in apparently normal pigs were reported last year at this meeting by Wismer-Pedersen. It was hoped in the present studies to be able to simulate these effects in rabbits, but so far no drug, with the possible exception of nor-adrenalin, has been found which will accelerate post-mortem glycolysis, without also reducing the glycogen reserves before death. It is, nevertheless, a possibility that the high rates encountered in the normal pig could be due to abnormal release of adrenalin at death, which would be expected to have an effect of this sort, particularly when it is remembered that the range over which the pH is changing in such cases, 6.4 to 5.4, is characterised in most animals by a rate of glycolysis nearly double that in the high range, 7.1 to 6.6., discussed in the present paper. Similarly, one would

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expect adrenalin to be somehow involved in the "dark-cutting" syndrome of beef, which, at the other extreme, is characterised by high ultimate pH.

Besides the effects of the various drugs, considerable interest attaches to the variations in rate of pH fall encountered in normal animals in both high and low ranges of pH. We have mentioned the case of the cortisone treated animal, where the pH of the psoas fell to 6.90 and then remained constant for 40 minutes, and similar cases are found amongst control animals. That they are not artefacts, d e to errors in pH measurement, is shown by the fact that the time for half-change of ATP and of extensibility is always increased in such cases. Moreover, such delays in pH fall are more the rule than the exception in whale muscles with high initial pH. No satisfactory explanation to account for these differences can be advanced from the factors usually considered to govern the post-mortem changes, and it remains to be seen whether they can be explained in terms of changes in the muscle membrane.

Références

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Summary

1) It is shown that the well-known effect of subcutaneous injection of adrenalin of depleting the glycogen content of the muscles, is accompanied in immobilised rabbits by an increase in the resting level of creatine phosphate, (C.P), slight decreases in the content of adenine and inosine nucleotides and of ATP, an increase in ultimate pH of 0.80 units, and increases in rate of glycolysis and of ATP turnover. This results in very marked acceleration of the onset of rigor mortis and of the decay of ATP.

2) Nor-adrenalin injection does not deplete the glycogen reserves, nor alter the resting level of ATP or nucleotides, but lowers the initial pH and C.P levels. The ultimate pH does not differ from that of normally fed controls, but the rates of glycolysis and of ATP turnover are significantly increased, and the time for onset of rigor is decreased.

3) Cortisone protects against the effects of adrenalin, and restores all the parameters to nearly their normal levels.

4) Cortisone alone has no effect on the resting levels of the parameters, nor on the ultimate pH, but tends to increase the rate of glycolysis.

5) Serotonin, four hours after interperitoneal injection, has left unaffected the initial pH, ATP and nucleotide levels, but accelerates glycolysis and raises the ultimate pH. It is suggested that the effect is mediated through adrenalin release.

6) Reserpine does not affect any of the parameters, except the rate of glycolysis

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which is slightly lower than normal.

7) The results are discussed in relation to findings in other animals, such as the pig, beef and whale.

Résumé

1) Il est démontré que l'effet bien connu de l'injection sous-cutanée d'adrénaline de diminuer la teneur en glycogène des muscles est accompagné chez les lapins immobilisés d'une augmentation du niveau initial du phosphate de créatine, d'une diminution légère de la teneur en nucléotides, en adénine, inosine et en A.T.P., et d'une grande augmentation du pH final, de la vitesse de la glycolyse et du "turnover" de l'ATP. Ceci se traduit par une accélération très marquée du commencement de la rigidité et du décroissement de l'ATP.

2) L'injection de nor-adrénaline ne diminue ni les réserves de glycogène ni la teneur initiale en ATP ou en nucléotides, mais abaisse légèrement le niveau initial du pH et du C.P. Le pH final ne diffère pas du pH des témoins, mais la vitesse de la glycolyse et du "turnover" de l'ATP est sensiblement augmentée, et la rigidité se déroule plus tôt.

3) La cortisone protège l'animal contre les effets de l'adrénaline et restitue tous les paramètres à peu près à leurs niveaux normaux.

4) La cortisone par elle-même n'agit pas sur les niveaux initiaux des paramètres, ni sur le pH final, mais tend à augmenter la vitesse de la glycolyse.

5) La sérètonine, 4 heures après injection inter-péritonéale, n'a pas eu d'effet sur les niveaux initiaux des paramètres, mais accélère la glycolyse et élève le pH final. On croit que l'effet est dû à la libération de l'adrénaline provoquée par la drogue.

6) La réserpine n'affecte aucun des paramètres, seule la vitesse de la glycolyse est sensiblement plus basse que la normale.

7) On compare ces résultats avec ceux faits sur d'autres animaux, tel que le porc, le boeuf et la baleine.

Zusammenfassung

1) Der wohl bekannte Effekt von subcutane Spritzen von Adrenalin der zu die Erniederung der Glykogen Gehalt von Muskeln leitet wird im immobilisieren Kaninchen durch Erhöhung des Ruhe-Niveau des Kreatin Phosphate, kleine Verminderung von den Gehalt von Adenin und Inosine Nukleotiden und A.T.P., ein grosse Erhöhung des Schluss-pH und Vergrösserung der Geschwindigkeit von der Glykolyse und den Umsatz von A.T.P. begleitet. Dadurch wird der Anfang des Erstarrung und der Abbau von A.T.P. erheblich beschleunigt.

2) Einspritzung von Nor-Adrenalin vermindert der Glykogen - Gehalt der Muskeln durchaus nicht, und beeinflussert weder das Ruhe-Niveau des A.T.P. noch der Nukleotiden. Dagegen vermindert sie das Anfangs-Niveau von C.P und pH etwas. Der Schluss-pH ist derselbe wie in normalle Tieren, aber die Geschwindigkeit von den Glykolyse und von den ATP-Umsatz gehoben wurden und die Zeit bis zum Anfang

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des Erstarrung verkürzen.

3) Cortisone schütz gegen die Effekte des Adrenalin und alle Parameteren beinahe zu ihren normal Niveau zurück bringt.

4) Cortisone allein hat keiner Einfluss auf das Ruhe-Niveau des verscheidener Parameteren oder auf der Schluss-pH, aber die Glykolyse-Geschwindigkeit etwas vergrössert.

5) Vier Stunden nach inter-peritonealer Einspritzung hat Serotonin den ursprunglich pH -, ATP-und Nukleotiden - Niveaux nicht beeinflusst aber die Glykolyse-Geschwindigkeit vergrössert und der Schluss-pH gesteigert. Es wird erwägt das diese Effekte das Resultat einer Adrenalin-Befreiung sind.

6) Reserpine keinen der gemessenen Parameter en beeinflussert ausser die Glykolyse-Geschwindigkeit die etwas niedriger als normal wird.

7) Die Resultate werden mit dem Befund in anderen Tieren wie das Schwein, das Rind und der Wallfisch besprochen und verglichen.

Table 1

Effect of injection of adrenalin on post-mortem events in the psoas muscle at 37° C. S.E.'s of means are given, with numbers of animals in brackets.

Parameter	Controls		Adrenalin-Treated	Significance of differences	
	Fed	Fasted (3 days)		Cols 1 & 3 P %	Cols 2 & 3 P %
Initial pH	7.11 ± 0.03 (5)	7.10 ± 0.04 (4)	7.12 ± 0.04 (5)	$\gg$10	$\gg$10
Initial CP (μmols/g)	21.6 ± 0.95 (5)	21.0 ± 0.8 (4)	26.4 ± 0.9 (4)	$<$ 1	$<$1
Initial ATP (μmols/g)	8.9 ± 0.3 (5)	8.8 ± 0.3 (4)	7.5 ± 0.5 (4)	$<$1	$<$1
Total nucleotide (μmols/g)	12.4 ± 0.2 (5)	-	10.9 ± 0.6 (4)	$<$1	-
Rate of pH fall (pH Units/hr)	0.24 ± 0.01 (5)	0.24 ± 0.02 (4)	0.32 ± 0.02 (5)	$<$0.1	$<$0.1
Rate of ATP turnover (μatoms P/min/g)	0.58 ± 0.03 (14)	0.59 ± 0.02 (10)	0.79 ± 0.04 (11)	$<$0.1	$<$0.1
Time for ½ change of extensibility (min)	204 ± 13(5)	174 ± 12 (4)	82 ± 6 (5)	$<$0.1	$<$0.1
Time for ½ change of ATP (Min)	181 ± 12 (5)	185 ± 10 (5)	114 ± 11 (4)	$<$0.1	$<$0.1
Ultimate pH	5.70 ± 0.03 (5)	6.48 ± 0.03 (4)	6.48 ± 0.06 (8)	$<$0.1	$\gg$10.0

Table 2

Ultimate pH values of longissimus dorsi muscles of rabbits after various treatments (Cut 1 is made at the thick end (tail) and cut 4 at the thin end (head), for which S.E.'s of means are given.)

Treatment	Ultimate pH				Significance of differences from control. P %	
	Cut 1	2	3	4	Cut 1	Cut 4
Control (fed) (10)	5.69 ± .03	5.72	5.78	5.84 ± 0.04	-	-
Adrenalin-injected (10)	6.09 ±0.13	6.26	6.43	6.51 ± 0.06	$<$0.1	$<$0.1
Nor-Adrenalin (4)	5.72 ±0.40	5.75	5.75	5.82 ± .02	$>$5.0	$>$5.0
Adrenalin-Cortisone (8)	5.80 ±0.03	5.88	5.99	6.06 ± 0.07	$\sim$5.0	$\sim$5.0
Cortisone (4)	5.68 ± .03	5.75	5.75	5.83 ± .02	$>$5.0	$>$5.0
Serotonin (4)	5.74 ± .05	5.76	5.83	5.85 ± .04	$>$5.0	$>$5.0