SPECIAL ARTICLES

THE DESTRUCTION OF COENZYME I AND COCARBOXYLASE IN SKELETAL AND CARDIAC MUSCLE AFTER DEATH

IN the course of studies of coenzyme breakdown in heart muscle after experimental coronary ligation in dogs, the idea was entertained that the amount of coenzyme breakdown after one hour of death might be considered the maximum breakdown that could be expected in that length of time following any experimental procedure. In order to test this hypothesis, six dogs (previously on a standard diet of "Pard" dog food) were anesthetized with pentobarbital sodium, the chests were opened and the hearts removed. Immediately on removal of the heart, a sample of left ventricle and a similar sample of deltoid muscle were removed, heated in boiling water for five minutes, homogenized and subsequently analyzed for cocarboxylase and coenzyme I. One hour later a second sample of each tissue was removed and treated in a similar manner. The dog and excised heart were allowed to remain at room temperature during the hour. Coenzyme I was determined by the method of Axelrod and Elvehjem,[1] as modified by Greig.[2] Cocarboxylase was determined manometrically, using split carboxylase prepared as described by Green *et al.*[3]

The results of these analyses are submitted in Tables 1 and 2.

TABLE 1

COENZYME I IN γ/GM OF DRY MUSCLE

Exp. No.	Skeletal muscle control	Skeletal muscle 1 hr. post-mortem	Per cent. change	Heart muscle control	Heart muscle 1 hr. post-mortem	Per cent. change
II 1.	2730	2650	− 2.9	3120	2150	−30.9
II 2.	2430	3380	+28.1	3180	2540	−20.1
II 3.	1649	0	−100.0	881	155	−82.5
II 4.	2640	2235	−15.1	3920	2375	−39.3
II 5.	924	783	−15.3	642	826	+28.7
II 6.	1173	1725	+47.0	2955	2610	−11.7
Average			− 9.7			−25.9

One may be impressed by the surprising lack of destruction under these conditions. Instead of finding nearly complete breakdown of cocarboxylase and coenzyme I, in the case of cocarboxylase in skeletal muscle at least, there is frequently less breakdown than was found previously in shock.[4]

[1] A. E. Axelrod and C. A. Elvehjem, *Jour. Biol. Chem.*, 131: 77, 1939.

[2] Margaret E. Greig, personal communication.

[3] D. E. Green, D. Herbert, V. Subrahmanyan, *Jour. Biol. Chem.*, 138: 327, 1941.

[4] Margaret E. Greig and Wm. M. Govier, *Jour. Pharmacol. and Exp. Therapy*, 79: 169, 1943.

TABLE 2

COCARBOXYLASE IN γ/GM OF DRY MUSCLE

Exp. No.	Skeletal muscle control	Skeletal muscle 1 hr. post-mortem	Per cent. change	Heart muscle control	Heart muscle 1 hr. post-mortem	Per cent. change
II 1.	23.7	22.5	− 3.4	55.0	61.2	+10.1
II 2.	22.8	20.3	−10.9	68.6	53.3	−22.3
II 3.	28.3	18.8	−33.6	63.9	55.0	−13.9
II 4.	21.1	14.2	−32.7	48.6	35.4	−27.1
II 5.	21.3	20.9	− 1.4	71.0	53.0	−25.4
II 6.	18.4	20.4	+ 9.8	65.8	63.3	− 3.8
Average			−12.0			−13.7

By way of explanation, one may suggest that after death the breakdown products of the coenzymes are not removed from the tissue by the circulation, and consequently by their accumulation may serve to inhibit catabolic enzymes. This explanation may be supported by work showing that thiamin inhibits yeast cocarboxylase phosphatase[5] and that nicotinamide inhibits coenzyme I nucleotidase.[6]

WM. M. GOVIER

BOWMAN GRAY SCHOOL OF MEDICINE,
WINSTON-SALEM, N. C.

AN EXPERIMENTAL METHOD FOR EVALUATING BLOOD SUBSTITUTES

REPORT ON SALINE, PLASMA, POLYVINYL ALCOHOL AND ISINGLASS

THE following conditions are desirable in an experiment designed to evaluate substances which may be used for the intravenous treatment of traumatic shock:

(1) The group of animals which is used for the test should be as nearly homogeneous as possible.

(2) The trauma should be of the same degree in each animal and easy to apply. Complicating factors such as anesthesia and hemorrhage into the injured tissues should be reduced to a minimum.

(3) The injury should be highly fatal in the control animals but mild enough to allow a proportion of the treated animals to recover. Percentage survival is considered to be the best single index of the effectiveness of treatment.

(4) The experiments should be carried out with the animals kept at a constant environmental temperature.[1]

(5) If a large number of animals can be handled at one time, there are the obvious advantages of con-

[5] H. G. K. Westenbrink, D. A. vanDorp, M. Gruber, H. Veldman, *Enzymologia*, 9: 73, 1940.

[6] P. J. G. Mann and J. H. Quastel, *Biochem. Jour.*, 35: 502, 1941.

[1] F. M. Allen, *Arch. Surg.*, 41: 155, 1939.

venience and of improving the conditions for regulating the comparison between the substances under test.

Using rats in which shock of the tourniquet type was produced by a slight adaptation of a technique described by Haist and Hamilton[2] we have found that most of these conditions can be met. The present report deals with rat plasma, 4 per cent. solutions in 0.85 per cent. saline of isinglass[3] and of polyvinyl alcohol (Type RH623 E. I. du Pont de Nemours and Company) and of 0.85 per cent. saline alone.

Ten 224–250 gm rats were used each day. Two served as controls while the remaining eight rats were divided into groups of two, each group receiving one of the solutions to be tested. A metal tourniquet which could be tightened to exert a constant pressure, was applied high on the thighs of both hind legs for five hours. Transfusion through a cannula in the right jugular vein was started forty-five minutes after release of the tourniquets. Ten cc of solution were injected into each rat at a rate of 2-2.1 cc per hour. Pentothal sodium (0.3 cc, 1 per cent. solution per 100 gm of rat given intraperitoneally) was given to permit cannulation. The anesthesia was light enough so that the effects wore off a few minutes after the cannulation was completed. Apart from this no anesthetic was used or was considered desirable for any reason throughout the experiment. Except for the 10 to 15 minutes used for the cannulation the animals were kept in thermostatically controlled warming cabinets at a temperature of 27–28° C.

The first 27 rats received unsterilized solutions. Thereafter the saline and isinglass solutions were autoclaved and the plasma and polyvinyl alcohol solution were passed through a Seitz filter. The plasma was prepared the same day as it was used from blood obtained by exsanguinating etherized rats through the carotid artery. About 10 units of heparin per cc of blood were used as an anticoagulant. The plasma was allowed to stand for several hours in the ice box and then centrifuged to remove any precipitate which had formed. The filtration was performed about two hours before the injection was started.

The results are set forth in Table 1.

TABLE 1.

SURVIVAL RATES IN RATS TREATED FOR SHOCK

Substances tested	No. of animals	No. survived	Per cent. survived	Average survival time of the remainder in hours
Plasma	20	5	25	12 (range 2–38)
Saline	20	8	40	14 (range 3–33)
Isinglass	20	5	25	14 (range 2–31)
Polyvinyl alcohol	20	13	65	10 (range 4–20)
Control	25	2	8	10 (range 2–23)

Rats living more than 48 hours after the shock was initiated are considered to have "survived."

The effectiveness of polyvinyl alcohol is striking. The preparation used in this work (RH 623) has an approximate viscosity in 4 per cent. aqueous solution of 5 centipoises at 20° C. The molecular size has not been accurately worked out. Using preparation RH 391, a polyvinyl alcohol with a viscosity of 55 centipoises under the same conditions and therefore with a different molecular size, Heuper *et al.*[4] report many undesirable side effects following its parenteral administration in experimental animals. Although the toxic effects of RH 623 may be similar to those of RH 391 this should not be assumed on the basis of the work with RH 391 alone. For this reason we have undertaken to determine the toxicity of RH 623 separately. At any rate the fact that RH 623 is so effective for the immediate treatment of shock, is most interesting.

The relatively low survival rate in the group receiving plasma is worthy of comment. In accord with the findings reported here Allen[1] and Rosenthal[5] have found saline more effective than homologous plasma or serum in treating tourniquet shock in rats and mice. On the other hand, Mylon, Winternitz and de Süto-Nagy[6] working with dogs in tourniquet shock, report a 10 per cent. recovery with saline and a 76 per cent. recovery with citrated plasma. In a recent paper Green[7] states that homologous plasma did not decrease significantly the mortality in rats in tourniquet shock. It is apparent that the rating of plasma in the treatment of tourniquet shock is not clearly established.

The author wishes to thank Warrant Officer (S.B.) J. Scattergood, R.C.N.V.R., for his technical assistance, and Lt. (S.B.) C. R. Cowan, R.C.N.V.R., for technical advice.

WILLIAM LOCKE,
Surgeon Lieutenant, R.C.N.V.R.
R.C.N. MEDICAL RESEARCH UNIT,
BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH,
UNIVERSITY OF TORONTO

ASSOCIATIVE DYNAMIC EFFECTS OF PROTEIN, CARBOHYDRATE AND FAT

IN spite of published evidence warranting a different understanding, a general belief still prevails

[2] R. E. Haist and J. Hamilton. In press (personal communication).

[3] Kindly supplied by Professor N. B. Taylor, department of physiology, University of Toronto.

[4] W. C. Heuper, J. W. Landsberg and L. C. Eksridge, *Jour. Pharm. and Exper. Therap.*, 70: 201, 1940.

[5] S. M. Rosenthal, *Pub. Health Rep.*, 58: 1429, 1943.

[6] E. Mylon, M. C. Winternitz and G. J. de Süto-Nagy, *Amer. Jour. Physiol.*, 139: 313, 1943.

[7] H. N. Green, *Lancet*, ii: 148, 1943.