56° C. and in others by adding disinfectants, and in each case allowed to incubate at 37° C. for 6 days. Sterilized thus by heat, by phenol and by gentian violet there is no evidence of toxin production. Experiments to test more thoroughly these various hypotheses are being planned. Details will be published in full elsewhere.

15 (1762)

The change in reaction of dying tissue.

By WITHROW MORSE and H. C. VAN DER HEYDE.

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In the studies of tissue enzyme action which the senior writer has been making since 1910, there has always been the question of the stages immediately following the death of the tissue and also of the conditions of reaction of medium, which have been shown to regulate the character of the process, that is, the rate and equilibrium. An attempt was made by Strauss and Morse[1] to determine the reaction of medium in the kidney during hematogenous infarction brought on by ligation of the blood vessels and at the same time to determine whether autolysis proceeded or not. The former collaborator (D. C. S.) being called for service rendered it impossible to complete this series of studies. Earlier still[2] the Sørensen colorimetric method was employed in similar work, but the obvious difficulty of the time element involved in the dialysis inhibited very critical conclusions. Recently, Dernby[3] applied the Sørensen solutions with the Clark-Lubs indicators to the study of the problem, but the critical point regarding the inception of autolysis and the state of reaction of medium in the earliest stages was not investigated. In his third paper in the "Studies of Autolysis" [4] Bradley and collaborator found "soon

[1] Strauss, D. C., and Morse, M. W., 1917, PROC. SOC. EXP. BIOL. AND MED. 1917, xiv, 171.

[2] Morse, M., *J. Biol. Chem.*, 1916, xxiv, 163.

[3] Dernby, K. G., *J. Biol. Chem.*, 1918, xxxv, 179.

[4] Bradley, H. C., and Taylor, J., *J. Biol. Chem.*, 1916, xxv, 261.

after death" a reaction of P_H = 7.00 in normal liver, but inasmuch as beef and pig livers were used, it is probable that the source of supply was slaughter-house material as in previous work in the series, while there is nothing to indicate that the experiment with horse liver involved the incipient stages, so that no data seem to have been given which would permit one to judge how soon *post mortem* the experiments were conducted. Here, as in the studies of Dernby, the colorimetric method involving dialysis was employed (p. 263, l.c.). The writers are unable to find in biochemical literature any other studies of this nature and the following results of their work are presented with the view of interesting investigators in the problem where facilities are available for furthur work.

Method.—Guinea pigs were used, the pig being struck on the head with an iron mallet, laparotomy rapidly performed, the liver exposed and frozen *in situ* by means of an ethyl chloride spray. Then the liver was excised while the heart still beat, transferred to a cold mortar in an ice-bath at − 5° C., wherein it was ground to a snow. The temperature of the liver mass, however, varied but little from zero Centigrade; in this connection it is well to recall the findings of Foster and Moyle[1] in studies on muscle, where exposure to temperatures of from − 5° C. to − 8° C. led to relatively great development of acidity (lactic), the low temperature acting similarly to mechanical injury. The snow obtained in this way was transferred to the electrode vessel of the gas chain apparatus[2] and the temperature of the mass within the vessel was brought rapidly to about 20° C. by means of the warm hand. Potentiometer readings were made at frequent intervals and the readings followed for thirty-six hours. The contents of the vessel were agitated, moderately, by means of a stirrer, operated by a small motor. In order to check the apparatus, controls were run on Sørensen NaOH − KH_2PO_4 buffer mixtures, the variation from the expected being but slight in any one case. By this means, likewise, the time for reaching equilibrium was established as far as the phosphate-alkali mixtures were concerned, twelve minutes

[1] Foster, D. L., and Moyle, D. M., *Biochem. J.*, 1921, xv, 334.

[2] The writers were permitted to use the apparatus belonging to the Department of Soils, West Virginia University.

PROTOCOL.

	Time.	Temperature.	Millivolts.	Calculated P_H
I.	3:45 (Pig killed)		(Saturated electrode)	
	3:51 (Transferred to electrode vessel)			
	3:54.....	13° C.	420	
	3:59.....	20	510	4.50
	4:02.....	20	530	4.84
	4:05.....	20	535	4.96
	4:07.....	20	540	5.01
	4:08.....	21	542	5.05
	4:11.....	21	550	5.18
	4:19.....	20	615	6.29
	4:22.....	20	635	6.63
	4:23.....	20	645	6.81
	4:28.....	20	660	7.08
	4:37.....	20	664	7.15
	4:45.....	21	665	7.15
	5:01.....	21	660	7.06
	5:15.....	21	657	6.99
	5:28.....	21	652	6.93
	5:37.....	21	648	6.86
	5:45.....	21	646	6.82
	7:39 (Toluene added)	21	630	6.55
	9:05 (A.M.) ...	19	640	6.40
	11:15.....	22	590	5.90
	1:45 P.M.....	21	587	
	10:10 A.M.....	21	537	4.95
	9:45 A.M.....	22	485	
	Discontinued.			
II.	10:14 Killed.....			
	10:20 Transferred to electrode vessel; heart in cadaver still beating.			
	10:23.....	20	370	2.09
	10:25.....	26 (Vessel warmed)	495	4.21
	10:31.....	20 (Vessel cooled)	490	4.15
	10:35.....	21	497	4.27
	10:39.....	21	500	4.32
	10:44.....	21	500	4.32
	10:55.....	22	502	4.35
	11:11.....	22	497	4.26
	11:29.....	22	496	4.25
	11:40.....	22	495	4.23
	1:15.....	23	622	6.39
	1:40.....	22	641	6.73
	1:47.....	22	643	6.76
	3:38.....	23	626	6.46
	4:05.....	23	620	6.80
	4:45.....	22	605	6.11
	5:15.....	23	590	6.20
	8:45 A.M.....	20	520	4.67
	8:50.....	20	527	4.79
	9:05.....	20	528	4.81
	Discontinued.			

being necessary. This figure is taken as a basis for the tissue work. The writers are unable to determine any factor in the tissue which may prolong the period of reaching equilibrium and while it is possible to explain the results obtained, as having to do with inequilibrium, the burden of proof is rather upon this aspect of the question, for one must show why liver tissue should demand more time for reaching equilibrium than the buffers.

The protocols following are those of two experiments. A third was conducted with practically identical results:

The results are striking, the reaction of the tissue being decidedly acid at the first reading, taken within five minutes after the time of excision of the liver. Then there is a slow fall to neutrality, which is reached within about 45 minutes. A rise ensues, which continues for a considerable length of time, over 24 hours at least.

The meaning of these findings is not clear, but they may be due to the fact that acid is produced at first in an explosive way, a conclusion which is justified by the studies of Fletcher,[1] who found that one fifth of the CO_2 produced by an excised muscle arose in the earliest stages; by the studies of Fletcher and Hopkins,[2] who found, always, in dying tissues lactic acid; and by the investigations of Foster and Moyle,[3] who found 0.218 per cent. lactic acid developed in injured muscle (minced) as compared to uninjured muscle 12 days at 0° C., 0.017 per cent. Secondly, the buffer action of the proteins, etc., in the tissue may exert its effect, causing a "fixing" of the free acid, but finally this effect is nullified by a saturation of the buffers and a rise in free acid begins.

If these results are free from criticism, a more substantial basis for the conception of how autolysis proceeds is available. Bradley showed in his first series of studies that the proteins of the substrate in autolysis became altered in some way whereby they became more digestible in tissue hydrolysis under the influence of the tissue protease and Dernby virtually substantiates these findings. The older work of Dochez, of Hedin and of Rowland point to this conclusion and the interpretation of relation of reaction to substrate is in keeping with the recent studies of Northrup,

[1] Fletcher, W. M., *J. Physiol.*, 1902, xxviii, 354.

[2] Fletcher, W. M., and Hopkins, F. G., *J. Physiol.*, 1907, xxxv, 247.

[3] *loc. cit.*

Falk and others upon different material. If we assume that the alkalinity of the tissue is slowly changed to acid reaction, it is difficult to see how low hydrogen ion concentration can operate to render the tissues more digestible, whereas a high degree of acid, such as we have found developed in the liver in the present study, may well be imagined to exert a profound influence upon the character of the proteins of the liver, for this concentration resembles that of gastric juice, especially that of the young subject,[1] where proteins are digested rapidly.

Since the above statements were written, the electrometric method has been checked by the Sørensen colorimetric method supplemented by the indicators of Clark and Lubs. Practically identical results have been obtained with both liver and kidney. The details of the method, with results and discussion, will be given in another place under the following title: "Further Studies on the Reaction of Dying Tissues," by Withrow Morse and R. Goldberg. The question will be raised therein, whether the suggestion made by Paul Erlich ("Die Aenaemie") that the reaction of the nucleus is acid, is applicable here.

16 (1763)

The cure of infantile rickets by sunlight as demonstrated by a chemical alteration of the blood.

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It has been shown by one of us (A. F. H.) that the rickets of infants can be cured merely by frequent exposures to the sun's rays.[2] Animal experiments carried out in this laboratory confirmed these clinical observations. They clearly demonstrated that rickets could be either prevented or brought about in rats fed a standard diet, according to whether they were subjected for

[1] McClendon, J. F., *Amer. J. Physiol.*, 1915, xxxviii, 191.

[2] Hess, A. F., and Unger, L. J., PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 298.

Hess, A. F., and Unger, L. J., *J. A. M. A.*, 1921, lxxvii, 39.