conditions, appreciably inhibited, presumably as a result of the block in the essential energy-yielding mechanism of respiration.

The effect of other cocaine-simulating drugs on citrate synthesis is being investigated.

SUMMARY

1. The effects of cocaine on the respiration of yeast and of mammalian tissues with various substrates have been investigated.

2. The effects of cocaine on citrate synthesis, pyruvate degradation and acetylating systems have been investigated. Evidence is presented that cocaine inhibits cellular respiration by blocking the entry of active acetate into the tricarboxylic acid cycle.

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The Effect of Nucleotide and Ischaemic Shock on the Level of Energy-Rich Phosphates in the Tissues

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Shock is best defined, simply, as the general reaction of the body to injury and its most characteristic features in small mammals, such as the rat, are depression of oxygen consumption and body temperature (Tabor & Rosenthal, 1947; Green & Stoner, 1950, 1954). These changes which occur during excessive loss from the carbohydrate stores (Stoner, Threlfall & Green, 1952*a*; Threlfall & Stoner, 1954), imply decreased energy production by the shocked animal and the idea that this is the essential metabolic change after severe injury has been discussed (Green & Stoner, 1954). The possible explanations of the decreased energy production which have been considered may be summarized as follows: (1) Exhaustion of the stored energy of the body—the 'energy depletion' hypothesis of McShan, Potter, Goldman, Shipley & Meyer (1945). (2) 'Uncoupling' of phosphorylation from the oxidative production of energy. (3) Incomplete metabolism of energy-yielding substrate.

Attempts have been made to analyse these possibilities (Stoner *et al.* 1952*a*, *b*; Threlfall & Stoner, 1954) and in the experiments now described the view that the depressed metabolism of the animal in shock is due to exhaustion of its stores of energy-rich compounds has been examined. In the light of current biochemical thought (Lipmann, 1941; Krebs, 1953) this would be represented by depletion of the phosphocreatine (PC) and adenosine triphosphate (ATP) of the tissues which satisfy their immediate energy requirements at the expense of the 'energy-rich bonds' ($\sim$P) of these compounds.

The 'energy-depletion' hypothesis was first put forward by Potter and his co-workers (McShan *et al.* 1945; LePage, 1946*a*, *b*) and was based on the appearance of the breakdown products of carbohydrate and nucleotide metabolism in excessive amounts in the blood of the shocked animal and a fall in the ~P content of the tissues. The most favourable evidence was found when shock was produced by haemorrhage or by the revolving drum described by Noble & Collip (1941), i.e. in animals with generalized tissue damage, but as pointed out by Wilhelmi (1948), the tissue changes were not gross. In shock following more limited tissue injury (hind-limb ischaemia), Bollman & Flock (1944) and Goranson, Hamilton & Haist (1948) did not find gross changes in the tissue nucleotides outside the damaged area although Kovách *et al.* (1952) have recently reported a generalized fall in the tissue ATP content. PC and ATP are, of course, broken down in the ischaemic tissue (Bollman & Flock, 1944; Macfarlane & Spooner, 1946; Bielschowsky, unpublished observations; Goranson *et al.* 1948; Kovách *et al.* 1952).

To investigate the 'energy-depletion' hypothesis more fully, ~P levels in the muscle, brain and liver of rats in both nucleotide and ischaemic shock have been studied. Although nucleotide shock is, in one sense, artificial it closely resembles traumatic shock (Green & Stoner, 1950). The processes of shock are accelerated by a raised environmental temperature (Tabor & Rosenthal, 1947; Green & Stoner, 1950) and advantage was taken of this in order to emphasize any changes in ~P distribution. Nucleotide shock was studied in adrenalectomized rats for the same reason (Stoner & Green, 1950).

METHODS

For experiments on muscle, albino rats (average weight 180 g.) were obtained from the same source as before (Stoner *et al.* 1952*a*, *b*) and fed on Edinburgh rat cube no. 86 (North-Eastern Agricultural Cooperative Society, Aberdeen). For experiments on liver and brain, albino rats of the Porton strain were used, fed on M.R.C. diet 41 (Bruce & Parkes, 1949). In all experiments the animals were fasted overnight before use. The response of the two strains to the shock-inducing procedures was identical. No difference was found in the P distribution in the muscle of the two strains.

Production of shock. Four-hour periods of bilateral hind-limb ischaemia were produced with rubber tourniquets (Rosenthal, 1943). Nucleotide shock was produced by the injection of MgATP (the magnesium salt of adenosine triphosphate), intraperitoneally except in the adrenalectomized rats when it was given intramuscularly. The dose, which varied with the nature of the experiment, is given in the tables. MgATP was prepared from the Ba salt (Boots Pure Drug Co. Ltd.) either as previously described (Green & Stoner, 1950) or by shaking the Ba salt, suspended in water, with Amberlite IR-120 to convert it into the free acid (Osborn, 1953) and then neutralizing it with $Mg(OH)_2$. In a few experiments ATP was obtained as the free acid (L. Light and Co. Ltd.) and neutralized with $Mg(OH)_2$. The purity of the samples was determined chromatographically (Eggleston & Hems, 1952) and only those samples in which the ratio of ATP to ADP was not less than approximately 70/30 were used. The volume of solution used for injection was based on the amount of P liberated by 7 min. hydrolysis in N-HCl at 100°, expressed as ATP. The material did not appear to contain inorganic pyrophosphate.

Adrenalectomy. Adrenalectomy was carried out using the dorsal approach; the rats were under ether anaesthesia and were given 1% (w/v) NaCl to drink after the operation. They were used at the end of 3 days.

Preparation of tissue samples for analysis. Muscle samples were obtained from the hind limb in nucleotide shock and from the fore limb and pectoral muscles in ischaemic shock. Pentobarbitone sodium (Nembutal—Abbott Laboratories Ltd.) and $\alpha\beta$-dihydroxy-γ-(2-methoxyphenoxy)propane (Myanesin—British Drug Houses Ltd.) were used in the controls and experimental animals sampled before death and the extraction of the muscle with 10% (w/v) trichloroacetic acid (TCA) was carried out as described before (Stoner *et al.* 1952*b*).

Samples of liver and brain were obtained from rats frozen in liquid O_2. The controls and the experimental animals sampled before death were anaesthetized with Nembutal (5 mg./100 g. body wt. intraperitoneally) before freezing. When the rat was frozen the head was removed, divided along the sagittal plane with a chisel and the brain removed as completely as possible. The abdomen was broken open and samples of liver removed, avoiding the large blood vessels as far as possible. Brain was ground up in an ice-cold mortar with 6 vol. ice-cold 10% (w/v) TCA and liver was extracted with 6 vol. TCA in a small 'homogenizer' (Folley & Watson, 1948). The protein precipitate was removed by centrifuging in a refrigerated centrifuge as for the muscle extract.

Analytical methods. The muscle extracts were analysed as before (Stoner *et al.* 1952*b*). In this scheme PC-P is the difference between the inorganic P precipitated, as $MgNH_4PO_4$, from the extract before and after standing for 16 hr. at 21°. The further P liberated by 7 min. acid hydrolysis in N-HCl at 100° (Δ 7 min. P) has been taken to represent the two labile phosphate groups of ATP. The contribution of other acid-labile phosphates to this fraction is negligible in muscle (Bendall, 1951). This was confirmed (Table 1) by finding that the Δ 7 min. P was almost exactly twice the P of the terminal phosphate group of ATP estimated by the potato pyrophosphatase method (*vide infra*).

Table 1. *Comparison of methods for the estimation of ATP in muscle*

Δ 7 min. P, determined by hydrolysis with N-HCl and terminal P of ATP, determined by the potato pyrophosphatase method, in rat muscle. Theoretically the Δ 7 min. P should be twice the terminal P of ATP level.

Rat no.	Δ 7 min. P (mg. P/g. wet wt.)	Terminal P of ATP (mg. P/g. wet wt.)
498	0·36	0·19
499	0·41	0·20
550	0·38	0·20
15	0·38	0·18

The total acid-soluble P, inorganic P and PC-P of the brain extracts were determined as for the muscle extracts. The Δ 7 min. P was also estimated, but the contribution from other labile phosphates is too great for this to be equated with the labile groups of ATP (Kerr, 1942; LePage, 1948). The terminal P of ATP was determined, after removing the TCA with ether, by the potato pyrophosphatase method of Lee & Eiler (1951). The enzyme was prepared from mature potatoes and the optimum conditions (temperature, ratio of enzyme to substrate) for the liberation of the terminal P only were determined for each batch of enzyme. The more cumbersome method of Kalckar (1947) for the determination of nucleotides was tried but found unsatisfactory for the reasons given by Munch-Petersen (1953).

The following fractions were determined on liver extracts: total acid-soluble P, inorganic P after 16 hr. at 21° and Δ 7 min. P. No attempt was made to determine any PC present in the liver (Ennor & Rosenberg, 1952). As in brain, phosphate esters other than ATP contribute to the Δ 7 min. P (LePage, 1948) so that the terminal P of ATP was again determined with potato pyrophosphatase.

To obtain confirmation of the origin of the inorganic P liberated from these extracts by potato pyrophosphatase additional tests were carried out on the extracts from six livers and four brains. Good agreement was found between the values for the terminal P of ATP determined with the pyrophosphatase and the values obtained when the adenine nucleotides were absorbed from the extract on to a column of activated charcoal (British Drug Houses Ltd.) followed by elution with 5% (v/v) aqueous pyridine (Crane & Lipmann, 1953) and chromatographic separation of the nucleotides (Eggleston & Hems, 1952).

Plasma inorganic P was determined after precipitation as $MgNH_4PO_4$ (Stoner *et al.* 1952*b*).

In all cases P was determined by the method of Fiske & Subbarow (1925), using either a 'Spekker' absorptiometer (Hilger and Watts, London) or a Coleman 'Junior' spectrophotometer (Coleman Instruments Inc., Maywood, Ill.) for the colour comparison. The results of the tissue determinations are expressed as mg. P/g. wet wt. The water content of the muscle, brain and liver has been taken as 75, 80 and 73% respectively, being the means of several determinations. These values were also used for the shocked animals as it was found that any differences which occurred were too slight to warrant a separate determination in each case. The values shown in the tables are means ± standard error except for the results obtained on the liver and brain in rats dying in nucleotide shock at 30° where the range is shown because of the small number of animals and the high degree of variability. The statistical comparison of the means has been done by Student's '*t*' test.

The P distribution found in normal tissues was in good agreement with that described for the rat by other workers. Because of the significantly lower Δ 7 min. P level in the fore-limb muscle and its lower total acid-soluble and inorganic P content at 20°, muscle from the experimental animals was always compared with muscle removed under similar environmental conditions from the same site in the controls. This principle was also applied to liver and brain, although P distribution there was not significantly affected by environmental temperature. In brain the inorganic P levels were rather higher and the PC-P levels rather lower than those reported by others (LePage, 1946*a*; Richter,

Table 2. *Phosphate distribution in the hind-limb muscles of normal rats at death in nucleotide shock*

Adenosine triphosphate administered intraperitoneally to normal rats and intramuscularly to adrenalectomized rats. Number of observations in each case shown in parentheses.

Treatment	Environmental temperature (°)	Time after injection (average)	Total acid-soluble P	Inorganic P	Phosphocreatine P	Δ 7 min. P
			(mg. P/g. wet wt.)			
Controls	20	—	1·81 ± 0·02 (45)	0·40 ± 0·02 (45)	0·55 ± 0·02 (45)	0·45 ± 0·02 (44)
Controls	30	—	1·81 ± 0·02 (6)	0·41 ± 0·04 (6)	0·63 ± 0·03 (6)	0·48 ± 0·02 (6)
ATP (125–200 mg. Mg ATP/100 g. body wt.)	20	5 hr. 7 min.	1·98 ± 0·02* (23)	0·51 ± 0·03* (23)	0·51 ± 0·03 (23)	0·44 ± 0·01 (22)
ATP (150–220 mg. Mg ATP/100 g. body wt.)	30	2 hr. 56 min.	1·93 ± 0·03* (4)	0·51 ± 0·07 (4)	0·59 ± 0·03 (4)	0·49 ± 0·03 (4)
Adrenalectomized controls	20	—	1·77 ± 0·03 (5)	0·37 ± 0·02 (5)	0·52 ± 0·03 (5)	0·48 ± 0·01 (5)
Adrenalectomized + ATP (50–60 mg. Mg ATP/100 g. body wt.)	20	4 hr. 50 min.	1·77 ± 0·05 (5)	0·54 ± 0·05† (5)	0·46 ± 0·06 (5)	0·47 ± 0·01 (5)

* Significantly different from corresponding control mean at $P<0.01$.
† Significantly different from corresponding control mean at $P<0.05$.

Table 3. *Phosphate distribution in the brain of normal rats, rats in nucleotide shock and rats after a 4 hr. period of bilateral hind-limb ischaemia*

Adenosine triphosphate administered intraperitoneally. Number of observations in each case shown in parentheses.

Treatment	(Environmental) temperature (°)	Clinical state	Time after release of tourniquets or injection (average)	Total acid-soluble P (mg. P/g. wet wt.)	Inorganic P (mg. P/g. wet wt.)	Phosphocreatine P (mg. P/g. wet wt.)	Δ 7 min. P (mg. P/g. wet wt.)	Terminal P of ATP (mg. P/g. wet wt.)
Controls	20	—	—	0·84 ± 0·02 (16)	0·23 ± 0·02 (16)	0·06 ± 0·005 (15)	0·15 ± 0·005 (16)	0·05 ± 0·003 (16)
Controls	30	—	—	0·81 ± 0·02 (10)	0·25 ± 0·02 (10)	0·07 ± 0·01 (12)	0·18 ± 0·02 (11)	0·04 ± 0·003 (11)
ATP (200 mg. Mg ATP/100 g. body wt.)	20	Death	5 hr. 7 min.	0·88 ± 0·08 (6)	0·37 ± 0·08 (6)	0·07 ± 0·02 (6)	0·13 ± 0·02 (6)	0·03 ± 0·004* (6)
ATP (280 mg. Mg ATP/100 g. body wt.)	30	Death	3 hr. 8 min.	0·79–1·09† (3)	0·33–0·61† (3)	0·02–0·09† (3)	0·20–0·59† (3)	0·02–0·03† (3)
Ischaemia	20	Ill	5 hr. 8 min.	0·83 ± 0·03 (9)	0·28 ± 0·04 (9)	0·06 ± 0·01 (9)	0·14 ± 0·01 (9)	0·05 ± 0·01 (9)
Ischaemia	20	Death	9 hr. 47 min.	0·87 ± 0·04 (9)	0·36 ± 0·04 (9)	0·06 ± 0·01 (9)	0·12 ± 0·03 (8)	0·04 ± 0·08 (6)
Ischaemia	30	Ill	34 min.	0·81 ± 0·03 (4)	0·25 ± 0·03 (4)	0·06 ± 0·01 (4)	0·16 ± 0·01 (4)	0·03 ± 0·005 (4)
Ischaemia	30	Death	1 hr. 23 min.	0·89 ± 0·03* (9)	0·46 ± 0·04‡ (9)	N.D. (11)	0·11 ± 0·02‡ (11)	N.D. (7)

* Significantly different from corresponding control mean at $P<0·05$.
‡ Significantly different from corresponding control mean at $P<0·01$.
† Range.
N.D. Not detectable (see text).

Table 4. *Phosphate distribution in the liver of normal rats, rats in nucleotide shock and rats after a 4 hr. period of bilateral hind-limb ischaemia*

Adenosine triphosphate administered intraperitoneally. Number of observations in each case shown in parentheses.

Treatment	Environmental temperature (°)	Clinical state	Time after release of tourniquets or injection (average)	Total acid-soluble P (mg. P/g. wet wt.)	Inorganic P (mg. P/g. wet wt.)	Δ 7 min. P (mg. P/g. wet wt.)	Terminal P of ATP (mg. P/g. wet wt.)
Controls	20	—	—	1·01 ± 0·04 (14)	0·30 ± 0·02 (12)	0·18 ± 0·01 (12)	0·04 ± 0·01 (12)
Controls	30	—	—	0·94 ± 0·05 (8)	0·22 ± 0·01 (8)	0·19 ± 0·01 (8)	0·04 ± 0·004 (7)
ATP (200 mg. Mg ATP/100 g. body wt.)	20	Death	5 hr. 7 min.	1·13 ± 0·08 (6)	0·60 ± 0·03* (6)	0·16 ± 0·03 (6)	0·03 ± 0·004 (6)
ATP (280 mg. Mg ATP/100 g. body wt.)	30	Death	3 hr. 8 min.	1·07–1·26† (3)	0·51–0·58† (3)	0·05–0·08† (3)	N.D.–0·02† (3)
Ischaemia	20	Ill	5 hr. 8 min.	1·11 ± 0·06 (10)	0·44 ± 0·03 (8)	0·12 ± 0·02 (8)	0·04 ± 0·004 (8)
Ischaemia	20	Death	9 hr. 47 min.	1·10 ± 0·04 (9)	0·52 ± 0·01* (9)	0·08 ± 0·01* (9)	0·02 ± 0·004‡ (8)
Ischaemia	30	Ill	34 min.	0·99 ± 0·03 (4)	0·31 ± 0·01* (4)	0·16 ± 0·01* (4)	0·03 ± 0·005 (4)
Ischaemia	30	Death	1 hr. 23 min.	0·95 ± 0·03 (11)	0·47 ± 0·01* (11)	0·08 ± 0·01* (11)	N.D. (6)

* Significantly different from corresponding control mean at $P<0·01$.
‡ Significantly different from corresponding control mean at $P<0·05$.
† Range.
N.D. Not detectable (see text).

1952), probably because the rats were larger than the size (40 g.) recommended by Richter (1952) and because of the short period of anaesthesia used. These departures from the optimum conditions were imposed by the nature of the experiments.

RESULTS

Phosphate distribution in nucleotide shock

The phosphate levels in the muscle of normal and adrenalectomized rats in fatal nucleotide shock are shown in Table 2. The changes in the total acid-soluble and inorganic P fractions may reflect extracellular changes as the plasma inorganic P in the ATP-treated rats, at 20°, was very high; 56·7 ± 0·27 mg./100 ml. (controls, 6·50 ± 0·27). The effect of nucleotide shock on P distribution in the brain and liver is shown in Tables 3 and 4.

Phosphate distribution in ischaemic shock

The effect of hind-limb ischaemia on phosphate distribution in the fore-limb muscle is shown in Table 5. The apparently progressive changes in the total acid-soluble P, inorganic P and PC-P levels could only be proved statistically in the case of inorganic P where the difference between the level in the ill animals and those at death was significant at $P<0{\cdot}01$.

The mean values for the brain (Table 3) suggest that at 20° phosphate distribution was unaltered by limb ischaemia. The true picture is obscured by pooling the results in this way. Certainly no change was found in the ill rats examined before death, but those which died fell into two groups, those dying within 5 hr. 10 min. of removing the tourniquets and those which were in shock much longer and survived for from 12 hr. 45 min. to 21 hr. The different chemical findings in these two groups are shown in Table 6.

In all but two of the rats dying in ischaemic shock at 30° (Table 3) the PC and ATP levels were below the threshold for the methods used; and in these two animals the PC-P levels were 0·04 and 0·06 mg. P/g. wet wt. and the level of the terminal P of ATP was 0·03 mg. P/g. wet wt.

The liver changes are shown in Table 4 and their correlation with survival time at 20° in Table 6. This latter is not as obvious as in the brain but there is indication that a similar process is at work. Again ATP could only be detected in two rats dying at 30° where terminal P levels of 0·01 and 0·02 mg. P/g. wet wt. were found.

DISCUSSION

The only evidence of 'energy-depletion' in the muscle in shock is the progressive fall in PC-P in the undamaged muscle in ishaemic shock. These results resemble those of Bollman & Flock (1944). The fall

Table 5. *Phosphate distribution in the fore-limb and pectoral muscles of normal rats and rats after a 4 hr. period of bilateral hind-limb ischaemia*

Number of observations in each case shown in parentheses.

Treatment	Environmental temperature (°)	Clinical state	Time after release of tourniquets (average)	Total acid-soluble P (mg. P/g. wet wt.)	Inorganic P (mg. P/g. wet wt.)	Phosphocreatine P (mg. P/g. wet wt.)	Δ 7 min. P (mg. P/g. wet wt.)
Controls	20	—	—	1·63 ± 0·03 (10)	0·34 ± 0·02 (10)	0·57 ± 0·03 (10)	0·40 ± 0·02 (10)
Controls	30	—	—	1·89 ± 0·05 (22)	0·47 ± 0·02 (18)	0·54 ± 0·03 (17)	0·42 ± 0·01 (22)
Ischaemia	20	Ill	3 hr. 30 min.	1·77 ± 0·03 (7)	0·43 ± 0·02* (7)	0·50 ± 0·01 (7)	0·42 ± 0·02 (7)
Ischaemia	20	Death	6 hr. 15 min.	1·79 ± 0·02† (5)	0·58 ± 0·04* (5)	0·40 ± 0·03* (5)	0·42 ± 0·01 (5)
Ischaemia	30	Ill	34 min.	1·81 ± 0·03 (12)	0·58 ± 0·05† (10)	0·41 ± 0·04 (9)	0·39 ± 0·02 (12)
Ischaemia	30	Death	1 hr. 23 min.	1·93 ± 0·06 (16)	0·73 ± 0·02* (15)	0·28 ± 0·04* (15)	0·44 ± 0·02 (16)

* Significantly different from corresponding control mean at $P<0{\cdot}01$.
† Significantly different from corresponding control mean at $P<0{\cdot}05$.

Table 6. *The concentrations of Δ7 min. P and P liberated from ATP by potato pyrophosphatase in the brain and liver of rats at death related to their survival time after a 4 hr. period of bilateral hind-limb ischaemia*

Environmental temperature, 20°. Number of observations in each group shown in parentheses.

	Brain		Liver	
Survival time	Δ 7 min. P (mg. P/g. wet wt.)	Terminal P of ATP (mg. P/g. wet wt.)	Δ 7 min. P (mg. P/g. wet wt.)	Terminal P of ATP (mg. P/g. wet wt.)
2 hr. 25 min.–5 hr. 10 min.	0·04 ± 0·01‡* (4)	0·02 ± 0·008§* (3)	0·06 ± 0·01§* (5)	0·02 ± 0·007 (4)
12 hr. 45 min.–21 hr.	0·19 ± 0·003* (4)	0·05 ± 0·007 (3)	0·11 ± 0·007* (4)	0·02 ± 0·003† (4)
Controls (Tables 3 and 4)	0·15 ± 0·005 (16)	0·05 ± 0·003 (16)	0·18 ± 0·04 (12)	0·04 ± 0·02 (12)

Comparison with control means * = different at $P<0{\cdot}01$; † = different at $P<0{\cdot}05$.
Comparison with means of rats with long survival time ‡ = different at $P<0{\cdot}01$; § = different at $P<0{\cdot}05$.

in PC was not observed by Goranson *et al.* (1948) but they do not seem to have examined their rats at death when it is most pronounced. This change closely resembles that found in diphtheritic toxaemia by Pinchot & Bloom (1950). There was no evidence of the fall in ATP content reported by Kovách *et al.* (1952).

Loss of ~P by muscle is often accompanied by rigidity (Erdös, 1943; Bendall, 1951) but the change is insufficient for that, as the muscles were completely flacid. The chemical differences between the muscles in shock and in death after 'uncoupling' by 3:5-dinitro-*o*-cresol have been mentioned before (Stoner *et al.* 1952*b*) and the changes reported here were less than those found by Parker (1954) after sublethal doses of 3:5-dinitro-*o*-cresol which produced little toxic effect on the rat.

Before attaching much metabolic significance to the fall in PC the possibility that it follows local anoxia due to vascular spasm must be considered. Its occurrence in ischaemic shock and not in nucleotide shock favours this view as distant vascular spasm has been found after the former type of injury (Barnes & Trueta, 1942; Green, Stoner, Whiteley & Eglin, 1951). In unpublished work with Dr M. Bielschowsky on unilateral hind-limb ischaemia in the rat, it was found that changes of this type occurred in the uninjured contralateral limb and these changes could be prevented by denervation of either limb. However, one would hardly expect such a mechanism to be accentuated by a rise in environmental temperature (but see Barcroft, Dornhurst, McClatchey & Tanner, 1952).

In the brain the ~P level only fell when the survival time was short or when the environmental temperature was raised to 30°. Then the fall in ATP content was similar to that reported by LePage (1946*a*) and Kovách *et al.* (1952) but these changes are terminal and may be related to the convulsion which often occurs at death under these conditions. The ~P level of the brain falls in convulsions and anoxia (Klein & Olsen, 1947) and according to Richter (1952), 'as much as 50% of the total brain phosphocreatine was lost in brains fixed in liquid air after 1 sec. of electrical stimulation'. Consequently, the changes observed hardly support an 'energy-depletion' hypothesis, but it must be admitted that functional change can occur in the brain before detectable chemical change (Klein & Olsen, 1947) and that studies on whole brain cannot exclude the possibility of 'energy depletion' in a few vital cells.

In nucleotide shock the ATP level in the liver was only reduced when shock was aggravated by raising the environmental temperature. In ischaemic shock the liver ATP level was low at death at both environmental temperatures but the fall was more striking at 30°. Even then it appeared to be a terminal event. The fall was similar to that found by Kovách *et al.* (1952) but it is difficult to say what effect it would have on hepatic function. It is also impossible to say how far these changes follow alterations in the blood supply to the liver. In haemorrhagic shock there are profound changes in hepatic circulation (Engel, Harrison & Long, 1944) and ~P stores (LePage, 1946*b*) and the survival time can be prolonged by preventing anoxic liver damage (Delorme, 1951). Less is known about the hepatic circulation in ischaemic shock and this is being studied.

The ~P level in the heart has not been studied because the technical difficulties involved (Pollack, Flock & Bollman, 1934; Davies, Francis & Stoner, 1947) made it unlikely that accurate results could be obtained under the present experimental conditions. However, when a rat dies in shock, respiration ceases first and the heart continues to beat slowly, but regularly, and would seem to have sufficient energy for its diminished requirements. The ATP content of the heart is said to be increased in ischaemic shock (Denson, Gray & Jensen, 1953)

but a 48 % reduction was reported by Kovách *et al.* (1952)—probably insufficient to cause cardiac arrest (Chang, 1938).

A simple 'energy-depletion' hypothesis will not account for the appearance of the animal after injury. A rat can obviously exist for many hours in severe shock without a generalized progressive fall in $\sim$P. Such changes as occur may, perhaps, be interpreted as follows:

At 20°, shock, in the rat, is accompanied by a fall in oxygen consumption and body temperature, interpreted as representing a decrease in energy production (Tabor & Rosenthal, 1947). With the fall in energy production the lower body temperature will decrease the need for energy and in this way some sort of balance between $\sim$P production and utilization can be achieved which permits the animal to persist in a state of shock for a long time. Clearly (Table 6) some rats die before this balance is attained, but the biochemical cause of death in both these rats and those which survive much longer is not very apparent.

Raising the environmental temperature to 30° decreases the consumption of oxygen by the normal rat (Benedict & MacLeod, 1929), but by maintaining the body temperature of the shocked rat, it prevents the gross fall in oxygen consumption normally seen in shock (Tabor, personal communication). While energy production may not be so severely depressed, the higher temperature also preserves the demand for energy and, it would seem, to a disproportionate extent for the survival time is shortened and the fall in $\sim$P is exaggerated. Under these conditions no balance can be achieved and perhaps here the rat may be said to die from 'energy depletion' although the fundamental change is the fall in energy production.

SUMMARY

1. The phosphate distribution in the muscle, brain and liver has been studied in rats in shock produced by the injection of adenosine triphosphate and by bilateral hind-limb ischaemia.

2. The total acid-soluble and inorganic phosphate levels in the muscle were increased in nucleotide and ischaemic shock. The phosphocreatine and adenosine triphosphate concentrations were unaffected in nucleotide shock but the phosphocreatine level was reduced in the undamaged muscle in ischaemic shock especially when the environmental temperature was raised to 30°.

3. In the brain, the adenosine triphosphate concentration was reduced at death in nucleotide shock. In ischaemic shock this change was only seen, under ordinary environmental conditions, at death when the survival time was short or when the environmental temperature was increased to 30°. Under the latter conditions there was an increase in the total acid-soluble and inorganic phosphate levels and a pronounced fall in the phosphocreatine content. For the most part the changes in the acid-labile phosphate fraction (Δ 7 min. P) followed those in the adenosine triphosphate fraction except that this fraction was increased if the rat survived for a long time at an environmental temperature of 20°.

4. In the liver an increase in the inorganic phosphate was the only significant change in nucleotide shock at 20°, but at 30° there was also a reduction in the labile phosphate and adenosine triphosphate levels. In ischaemic shock these changes were seen at both environmental temperatures.

5. These results are discussed from the point of view of the 'energy depletion' hypothesis which has been advanced to explain the state of the animal after severe injury. It is concluded that this hypothesis can only be applied to death under certain conditions and that the results indicate a fundamental decrease in energy production.

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Leuco-Anthocyanins

1. DETECTION AND IDENTIFICATION OF ANTHOCYANIDINS FORMED FROM LEUCO-ANTHOCYANINS IN PLANT TISSUES

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A method for the detection of leuco-anthocyanins, and the chromatographic identification of the anthocyanidins formed from them by boiling with mineral acid, has been described in an earlier paper (Bate-Smith, 1953). This method has been improved and applied to the leaves and other tissues of numerous species of plants. In the present paper the results of identification of anthocyanidins produced from leuco-anthocyanins in some of these species are reported. In the paper which follows the systematic distribution of the leuco-anthocyanin reaction in leaves is discussed.

EXPERIMENTAL AND RESULTS

Paper chromatographic separation of anthocyanidins

The main problem is to obtain a solution of the anthocyanidins in sufficient concentration for paper chromatography, without at the same time concentrating irrelevant substances. This is accomplished by converting the leuco-anthocyanins into anthocyanidins in aqueous solution (rather than in methanol as described previously) and extracting the anthocyanidins with *iso*amyl alcohol.

Conveniently, 0·2–1·0 g. of tissue is covered with 2N-HCl (about 3 ml.) in a test tube, and heated in boiling water for 20 min. The aqueous solution is decanted (filtered if necessary) into a small narrow test tube and shaken with sufficient *iso*amyl alcohol (3-methylbutan-1-ol) to give a supernatant layer just deep enough to be drawn cleanly into a capillary tube, from which the solution is spotted on the starting line of the chromatogram. The applications are repeated, employing a current of hot air to accelerate drying, until the colour is deep enough to ensure visibility of the anthocyanidins on the developed chromatogram. A marker of known identity is applied on each paper.

In order to prevent the anthocyanidins from fading, it is necessary to maintain a low pH during chromatography. This was originally achieved (Bate-Smith & Westall, 1950) by using the upper phase of the mixture *n*-butanol–2N-HCl (1:1, v/v). A solvent brought to our notice by Forestal Laboratories has given better-defined spots and more consistent results. This 'Forestal solvent' consists of water–acetic acid–conc. HCl (10:30:3, v/v). Solvents containing *m*-cresol and HCl have also been tested, and one of these consisting of *m*-cresol–5·5N-HCl–acetic acid (1:1:1, v/v) (acetic acid is added in order to bring the aqueous and phenolic constituents into a single phase) has given promising results. As was found with the phenolic solvents employed in earlier work on the chromatography of flavonoid compounds (Bate-Smith, 1949), this solvent has the effect of suppressing the lyophilic properties of methoxyl groups to a greater extent than the aliphatic solvents, so that the order