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SOME EFFECTS OF LOCAL ANAESTHETICS ON CELL METABOLISM

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Abstract—Some effects of local anaesthetics (cocaine, procaine, amethocaine, and cinchocaine) have been investigated. In this study, the local anaesthetics inhibited citrate synthesis in intact cells of *Escherichia coli*, brain slices, and homogenates. The drugs also inhibited significantly the oxidation of α-oxoglutarate while having no appreciable effect upon the other isolated steps of the tricarboxylic acid cycle. The local anaesthetics also inhibited malate synthesis in intact cells of *E. coli*, acetoacetate synthesis, and sulfanilamide acetylation in tissue homogenates.

In rats subjected to chronic cocaine treatment, there was no effect upon citrate synthesis *in vivo* nor were there any changes seen in serum and urine lactate levels and serum Ca^{2+} levels. The drugs had no effect upon any of the metabolic reactions in cellular subfractions or on mitochondrial respiration. The drugs had no effect upon Mg^{2+}-stimulated ATPase from rat nerve or on the Na^{+}-sensitive ATPase from rat brain.

The results support the probable implication of membrane structure as the site of action of local anaesthetics.

THE effects of local anaesthetics on cellular respiration have been investigated by several workers.[1–4] In this laboratory, Ryman and Walsh[5] showed that cocaine depressed cellular respiration by inhibiting citrate synthesis and probably by blocking the entry of acetyl coenzyme A into the tricarboxylic acid cycle. Geddes and Quastel[6] demonstrated that local anaesthetics at pharmacologically active concentrations inhibited the K^{+}-stimulated respiration of slices of rat brain cortex in the presence of glucose but had no effect upon the unstimulated respiration.

Bollard and McIlwain[7] showed that cocaine in as low as 0·05 mM concentration caused a significant diminution in respiration and glycolysis in electrically stimulated cerebral tissues. More recent work by Kini and Quastel[8] has shown that cocaine exerts a twofold effect upon the aerobic conversion of glucose to amino acids in rat brain slices. Thus one effect of cocaine was to diminish the ratio K^{+}/Ca^{2+} and a second was to diminish the breakdown of pyruvate and oxaloacetate so that more of these acids are available for transamination.

The present work describes a detailed investigation of the effects of local anaesthetics on certain aspects of cell metabolism and also attempts to locate the precise site of action of these drugs.

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MATERIALS AND METHODS

Chemicals and reagents. Acetyl phosphate, adenosine triphosphate (Na salt), coenzyme A, NAD^+, cytochrome *c*, glyoxylate (Na salt), and oxaloacetate (Na salt) were obtained from Sigma Co.

All other chemicals were of pure grade. Sodium pyruvate was prepared by the method of Price and Levintow.[9] The local anaesthetics cocaine, procaine, amethocaine, and cinchocaine were used in the form of their hydrochlorides and were dissolved and neutralised to the required pH before use.

Radioactive 1-^{14}C-acetate was obtained from the Radiochemical Centre, Amersham, England.

Tissue preparations. Tissues (rat brain cortex or kidneys) were homogenized in cold Krebs-Ringer phosphate saline[10] to give suspensions containing 100 mg tissue/ml. Homogenization was carried out in glass homogenizers of the Potter-Elvehjem type, the tubes being cooled in ice. Cortex slices from guinea-pig brain were prepared by the method of McIlwain and Rodnight.[11]

Manometric determinations. All the manometric determinations were made in Warburg flasks at 37°, pH 7·2, with air as gas phase and KOH (20% w/v, 0·2 ml) in the centre well. When we were studying the isolated steps of the tricarboxylic acid cycle, the substrates were added from the side arm after preliminary incubation for 30 min. The step: α-oxoglutarate $\rightleftharpoons$ succinate was studied in rat brain homogenates with α-oxoglutarate as substrate in the presence of sodium malonate (0·033 M) to block the further metabolism of succinate. The step: succinate $\rightleftharpoons$ fumarate $\rightleftharpoons$ malate was investigated in rat brain homogenates with succinate as substrate in the presence of sodium arsenite (0·0033 M) which blocks the further metabolism of malate. The step: fumarate $\rightleftharpoons$ oxaloacetate was studied according to the procedure of Mann and Quastel,[12] in kidney homogenates.

Collection of blood and urine samples. Blood was collected by cardiac puncture from rats anaesthetized lightly with ether. Urine was collected by keeping the rat for short periods on a large funnel covered with a perforated lid. The freshly voided urine was used immediately.

Preparation of cell subfractions

(*i*) *Yeast.* Cell subfractions of yeast were prepared by grinding fresh baker's yeast (5 g) with an equal weight of ballotini glass beads (0·1 mm diameter, grade 15, English Glass Co. Ltd.), extracting with 0·16 M $NaHCO_3$ solution (10 ml) in a mortar, and centrifuging the viscous suspension at 3000 *g* for 30 min.

(*ii*) *E. coli.* were grown on 2% w/v agar slopes at 30° for 24 hr. Stock cultures of the organism were subcultured every fortnight and stored at 4°. For experiments involving citrate synthesis, freshly cultured *E. coli* were grown on a medium containing acetate as the chief source of carbon, with constant aeration at 30° for 16–18 hr. Cells were then harvested by centrifuging at 1800 *g* for 30 min and suspended in distilled water. For experiments on malate synthesis, *E. coli* were grown on a medium suggested by Umbreit and Gunsalus,[13] without aeration at 37° for 40 hr. The cells were centrifuged down at 20,000 *g* for 15 min and washed thrice with distilled water (10-ml aliquots). The washed cells were suspended in distilled water (10 ml) and used in subsequent experiments. For experiments involving cell subfractions, *E. coli* grown on acetate medium were used.

Cell subfractions of *E. coli* were prepared from an acetone dried powder; 0·2 g of dry *E. coli* powder was ground with an equal weight of "microid polishing alumina" (Griffin and George Ltd.; grade 3/50) and extracted with 0·25 M potassium phosphate buffer, pH 7·4 (4·0 ml). The resulting viscous suspension was centrifuged at 3000 *g* for 30 min.

Citrate synthesis in the cell subfractions of yeast and *E. coli* was studied by the method of Novelli and Lipmann.[14] Malate synthesis in *E. coli* subfractions was studied by the procedure of Kornberg and Madson.[15]

(*iii*) *Brain and liver.* Cell subfractions of brain were prepared by rubbing 50 mg of acetone dry brain powder with 0·1 M potassium phosphate buffer, pH 7·4 (2·0 ml) and centrifuging at 14,000 *g* for 30 min. Pigeon liver subfractions were prepared by the method of Lipmann and Soodak[16] for studying acetoacetate synthesis; those prepared by the procedure of Johnson and Quastel[17] were used in the experiments on sulfanilamide acetylation. Citrate synthesis in brain subfractions was studied by the procedure used for yeast and *E. coli* subfractions.

Rat brain mitochondria. The method of Brody and Bain[18] was used for preparing rat brain mitochondria and studying the mitochondrial respiration.

Enzyme preparations. The method of Abood and Gerard[19] was used for the preparation of Mg^{2+}-activated adenosine triphosphatase from rat nerve and studying its activity. The method of Aldridge[20] was used for preparing Na^{+}-sensitive adenosine triphosphatase from rat brain and studying its activity.

Separation of cholesterol and fatty acid fractions. The cholesterol and fatty acid fractions of rat liver were separated by the method of Francis *et al.*[21] ^{14}C Content of these fractions was determined by the infinite thickness technique in a thin-window bell-type counter.

Analytical determinations. Citrate was determined by the method of Natelson *et al.*,[22] malate by the fluorimetric method of Hummel,[23] acetoacetate by the method of Barkulis and Lehninger,[24] sulfanilamide by the method of Bratten and Marshall.[25] Lactate was determined by the method of Barker and Summerson,[26] calcium by the method of Clark and Collip,[27] and inorganic phosphate by the method of Fiske and SubbaRow.[28] In all the experiments involving synthesis, the results were corrected for the endogenous levels. A Unicam-500 spectrophotometer was used for the colorimetric and fluorimetric determinations.

EXPERIMENTAL AND RESULTS

Effects of local anaesthetics on the synthesis of citrate from acetate in E. coli. These effects were studied essentially in the same manner as that previously employed for yeast.[5] The results (Table 1) indicate that local anaesthetics inhibited citrate synthesis significantly. Of the anaesthetics tested, cinchocaine appeared to be the most potent inhibitor.

Effects of local anaesthetics on the synthesis of citrate from pyruvate in rat brain homogenates and guinea pig brain slices. The citrate synthesis in brain homogenates and slices was studied in the presence of barium chloride, which blocks the further metabolism of citrate (Weiland and Rosenthal[29]). It is seen (Table 2) that local anaesthetics inhibited the synthesis of citrate from pyruvate in brain homogenates as well as in slices.

Effects of local anaesthetics on some isolated steps of the tricarboxylic acid cycle. These experiments were carried out in order to see whether the local anaesthetics had any effect upon any of the steps of the tricarboxylic acid cycle other than citrate synthesis. The results (Table 3) indicate that all the local anaesthetics (4 m-moles) inhibited significantly the step: α-oxoglutarate ⇌ succinate. The maximal effect was

TABLE 1. EFFECTS OF LOCAL ANAESTHETICS ON THE SYNTHESIS OF CITRATE FROM ACETATE IN *E. coli*

Anaesthetic (1 m-mole)	Citrate synthesised (μg/ml suspension/hr)		Inhibition (%)
	Control	Anaesthetic	
Cocaine	200	76	62
Procaine	225	104	53
Amethocaine	238	107	55
Cinchocaine	246	46	81

Equal volumes of *E. coli* suspensions were incubated with magnesium acetate (0·4 M) with and without anaesthetic in a total volume of 15·0 ml. The mixtures were aerated at 30° and pH 7·0 in tubes fitted with bubblers. After 2 hr, samples (1·0 ml) were withdrawn from the reaction mixtures and deproteinised with 30% (w/v) TCA (1·0 ml). The mixtures were centrifuged and the clear supernatant (1·0 ml) was used for the determination of citrate.

TABLE 2. EFFECTS OF LOCAL ANAESTHETICS ON THE SYNTHESIS OF CITRATE FROM PYRUVATE IN RAT BRAIN HOMOGENATES AND GUINEA PIG SLICES

Anaesthetic (4 m-moles)	Citrate synthesised (μg/100 mg brain/hr)	Inhibition (%)
(i) Homogenates		
Control	60	
Cocaine	42	30
Procaine	32	46
Amethocaine	19	68
Cinchocaine	8	86
(ii) Slices		
Control	145	
Cocaine	65	55

(i) The reaction was studied in Warburg flasks at 37° and pH 7·2. After preliminary incubation for 15 min, the substrates in the side arm (pyruvate and oxaloacetate in phosphate saline buffer, 0·1 M final concentration) were added to the main compartment which contained phosphate saline ± anaesthetic (4 m-moles), 1·0 ml rat brain homogenate (= 100 mg tissue), and barium chloride (0·033 M). Total volume 3·0 ml. After incubation for 1 hr, the reaction was terminated by the addition of 30% (w/v) TCA (1·5 ml), centrifuged, and an aliquot of the supernatant (1·0 ml) analysed for citrate.

(ii) In guinea pig brain slices a similar procedure was employed. Potassium chloride (0·1 M) was included in the medium to stimulate respiration, and slices weighing 60–80 mg each were used.

seen with cinchocaine (96% inhibition). The step: succinate ⇌ fumarate ⇌ malate was inhibited only by cinchocaine (4 m-moles) by 64%. The step: fumarate ⇌ oxaloacetate was inhibited appreciably by only amethocaine (4 m-moles) and cinchocaine (4 m-moles). Thus amethocaine inhibited the reaction by 37 per cent while cinchocaine inhibited by 50 per cent.

Further studies of the effects of local anaesthetics on acetate- and pyruvate-utilising systems. In these experiments the effects of local anaesthetics on four different systems were investigated. The synthesis of malate from acetate is an important reaction of the so-called glyoxylate cycle operating in many bacteria[30] and also resembles closely the synthesis of citrate from acetate. Cocaine (1 m-mole) significantly inhibited this

TABLE 3. EFFECTS OF LOCAL ANAESTHETICS ON THE STEP α-OXOGLUTARATE ⇌ SUCCINATE

Anaesthetic (4 m-moles)	O_2 uptake (μl/100 mg wet wt. tissue/90 min)	Inhibition (%)
Control	122	
Cocaine	46	62
Procaine	93	23
Control	111	
Amethocaine	10	91
Cinchocaine	5	96

Each Warburg flask contained rat brain homogenate (100 mg tissue) in Krebs-Ringer phosphate saline, pH 7·2, α-oxoglutarate (0·02 M), sodium malonate (0·033 M) ± anaesthetic (4 m-moles) in a total volume of 3·0 ml.

reaction in the respiring cells of *E. coli* (Table 4). The local anaesthetics inhibited the synthesis of acetoacetate from pyruvate in respiring rat liver homogenates and also acetylation of sulfanilamide in the presence of pyruvate in a system composed of pigeon liver extract and respiring rat brain homogenate (Table 4). The effect of cocaine on the synthesis of cholesterol and fatty acids was studied *in vivo* with ^{14}C-labeled acetate. In these experiments (Table 5) there was a marked decrease in the incorporation of ^{14}C-labeled acetate into the cholesterol and fatty acid fractions in cocaine-treated animals.

Experiments in vivo. These experiments were carried out in order to see whether the local anaesthetics inhibited the acetate- and pyruvate-utilising reactions *in vivo*. For this purpose, the effect of chronic treatment in rats on the citrate synthesis *in vivo* was investigated.

Chronic cocaine treatment of rats with cocaine. Two groups of Wistar strain rats (12 in each group), 7–8 weeks old and weighing roughly 100 g each, were used. The experimental group received sterile cocaine (1·5 mg in distilled water) for 21 days. The control group received an equivalent volume of distilled water.

Effect of cocaine treatment on the citrate-synthesising ability of brain. In these experiments the citrate synthesis in brain homogenates from control and the chronically cocaine-treated rats was compared. The results (Table 6) indicate that there was a significant increase in the citrate-synthesising ability of brain homogenates from the cocaine-treated animals.

Effect of cocaine treatment on the citrate levels of blood and kidneys in rats. In these experiments the chronically cocaine-treated rats were injected with a large dose of

TABLE 4. THE EFFECTS OF LOCAL ANAESTHETICS ON THE PYRUVATE- AND ACETATE-UTILISING REACTIONS

System	Anaesthetic (m-moles)		Inhibition (%)
(i) Acetate → malate		μg malate synthesised/ml suspension/hr	
	Control	38	
	Cocaine (1)	11	71
(ii) Pyruvate acetoacetate		μmoles acetoacetate synthesised/100 mg liver/hr	
	Control	0·437	
	Cocaine (4)	0·354	19
	Procaine (1)	0·340	22
	Amethocaine (1)	0·300	31
	Cinchocaine (1)	0·247	40
(iii) Sulfanilamide acetylation from pyruvate		μg sulfanilamide acetylated/90 min	
	Control	56	
	Cocaine (1)	50	11
	Amethocaine (4)	42	25
	Cinchocaine (4)	33	38

(i) Acetate → malate. Equal volumes of *E. coli* cell suspension and 0·4 M magnesium acetate (total vol. 20·0 ml) were aerated at 30° and pH 7·0 with and without anaesthetic in the manner described in Table 1. Aliquots (1·0 ml) were withdrawn after 2 hr, deproteinised with 30% (w/v) TCA (1·0 ml), centrifuged, and the supernatant (1·0 ml) used for malate determination.

(ii) Pyruvate → acetoacetate. The mixtures (3·0 ml) with and without anaesthetic and containing pyruvate (0·02 M), phosphate buffer pH 7·4 (0·01 M), and 2·0 ml rat liver homogenate were incubated at 37° for 1 hr. The reaction was terminated by the addition of 12% (w/v) TCA (1·0 ml); 1·5 ml of the clear supernatant after centrifugation was used for the acetoacetate determination.

(iii) Sulfanilamide acetylation. Each Warburg flask contained sodium pyruvate (0·02 M), phosphate buffer pH 7·2 (0·02 M), sulfanilamide (100 μg), pigeon liver extract (1·4 ml = 84 mg dry powder), rat brain homogenate (0·5 ml = 100 mg tissue), ± anaesthetic; total vol. 3·0 ml. Incubation in air at 37°. After 90 min, aliquots (1·0 ml) deproteinised with 30% (w/v) TCA (2·0 ml), centrifuged, and the supernatant used for the determination of sulfanilamide. The amount of sulfanilamide acetylated was calculated by difference from the amount initially present.

TABLE 5. EFFECT OF COCAINE ON THE INCORPORATION OF 1-^{14}C-ACETATE INTO THE CHOLESTEROL AND FATTY ACIDS IN RAT LIVER

Experiment	% ^{14}C incorporated into cholesterol		Inhibition (%)	% ^{14}C incorporated into fatty acids		Inhibition (%)
	Control	Treated		Control	Treated	
1	0·156	0·080	48·7	0·762	0·050	93·5
2	0·164	0·096	41·2	0·582	0·116	80

Rats of the same age, sex, and strain and nearly the same weight were employed. Each experimental rat was injected i.p., 1·0 ml sterile cocaine (3·0 mg/ml); the control rat was injected with 1·0 ml sterile 0·9% (w/v) saline. After 4 hr each rat was injected i.p. with 1·0 ml of 1-^{14}C-acetate in 0·9% (w/v) saline (containing 68 μg of 1-^{14}C-acetate and 10 μc of ^{14}C). The experimental rat was also injected with an additional 1·0 ml cocaine (3·0 mg/ml) and the control rat, a similar volume of saline; 16 hr after the injection of 1-^{14}C-acetate, the rats were sacrificed, livers quickly removed and weighed, and the cholesterol and fatty acid fractions separated (see methods) and ^{14}C content determined.

cocaine (10 mg) intraperitoneally. After 5 min, the animals from both the control and experimental group (3 in each group) were sacrificed. Blood from each animal (5·0 ml) was deproteinized with 10% (w/v) TCA (1·5 ml), centrifuged, and the clear supernatant analysed for citrate content. The freshly excised kidneys were homogenised in 10% (w/v) TCA (5·0 ml). After centrifugation the supernatant was used for citrate analysis.

TABLE 6. EFFECT OF CHRONIC COCAINE TREATMENT ON THE CITRATE-SYNTHESISING ABILITY OF BRAIN HOMOGENATES

Experiment	Citrate synthesised (μg/100 mg brain/hr)		Increase over control (%)
	Control	Treated	
1	42	58	38·1
2	36	50	38·9
3	36	52	44·4

Components of the system were as described in Table 2. After incubation at 37° for 1 hr, aliquots (0·5 ml) were withdrawn and deproteinised with 30% (w/v) TCA (1·0 ml), centrifuged, and supernatant (1·0 ml) was used for citrate determination.

The citrate levels of blood (mg/100 ml) for the control group were in the range 2·03–2·54 and in the range 1·83–2·54 for the cocaine-treated group. The citrate levels (mg/100 ml) for kidneys from the control rats varied between 15·2 and 20·1, and between 16·5 and 22·1 for the cocaine-treated rats. Thus it appeared that the cocaine treatment of rats did not appreciably alter the citrate levels of their blood and kidneys.

Effect of cocaine treatment on lactate levels of blood and urine in rats. The lactate levels of blood and urine in rats subjected to chronic cocaine treatment were determined in order to investigate whether the local anaesthetics inhibited pyruvate utilization *in vivo*.

In these experiments the lactate levels (mg/100 ml) for the control rats varied between 36·0 and 55·0, and between 36·0 and 57·5 for the cocaine-treated group (7 rats in each group). The lactate levels of urine (mg/100 ml) for the control rats fell in the range 3·0–4·3, and in the range 2·8–7·4 for the cocaine-treated group. These results indicated that the chronic cocaine treatment in rats had no appreciable effect upon the lactate levels of their blood and urine.

Experiments with cellular subfractions. These experiments were designed to see whether the local anaesthetics exerted their demonstrated effects at the enzymic level. For this purpose the systems previously studied in intact cells, homogenates, and slices were reinvestigated with cellular subfractions.

The local anaesthetics (1 m-mole) had no effect upon citrate synthesis in cellular subfractions of *E. coli* and brain. Thus, in the case of *E. coli*, the citrate synthesised (μg/ml subfraction/hr) was in the range 348–367 for the controls and 350–373 in the presence of anaesthetics. For brain subfractions, the citrate synthesised (μg/ml subfraction/hr) was in the range 112–135 for the controls and 112–133 in the presence of the anaesthetics. The drugs had no effect upon malate synthesis in *E. coli* subfractions.

Thus the malate synthesised (μg/ml subfraction/hr) varied between 444 and 490 for the controls and between 438 and 484 in the presence of anaesthetics (1 m-mole). The drugs also had no effect upon acetoacetate synthesis in subfractions of pigeon liver. Thus the acetoacetate synthesised (μmoles/g liver/90 min) was in the range 5·6–6·5 for the controls and in the range 5·5–6·5 in the presence of local anaesthetics (1 m-mole).

Effects of local anaesthetics on mitochondrial respiration. The experiments were carried out to see whether the local anaesthetics affected the cell metabolism at the mitochondrial level. For this purpose, the effects of local anaesthetics upon the respiration of brain mitochondria were investigated in the presence of various tricarboxylic acid cycle intermediates and glutamate. In these experiments, both cocaine and procaine (2 m-moles) had no appreciable effect upon the oxidation of pyruvate, α-oxoglutarate, or glutamate. Amethocaine (2 m-moles) inhibited pyruvate oxidation by 30 per cent and glutamate oxidation by 50 per cent, while having no significant effect upon α-oxoglutarate oxidation. Cinchocaine (2 m-moles) inhibited pyruvate oxidation by 74 per cent, α-oxoglutarate oxidation by 69 per cent, and glutamate oxidation by 83 per cent. However, none of the drugs had any effect upon the succinate oxidation.

Effects of local anaesthetics on the adenosine triphosphatase from nerve and brain. These experiments were designed to see whether local anaesthetics impaired the transport of ions, particularly Na^+ and K^+, across the cell membrane by inhibiting the adenosine triphosphatase activity. In these experiments, both cocaine and procaine (1 m-mole each) had no effect upon the Mg^{2+}-stimulated rat nerve ATPase, whereas amethocaine (1 m-mole) inhibited the enzyme by 24 per cent and cinchocaine (1 m-mole) by 62 per cent. None of the anaesthetics had any effect upon the Na^+-sensitive ATPase from rat brain.

Effect of chronic cocaine treatment on the serum Ca^{2+} levels in rats. These experiments were carried out to learn whether any changes in Ca^{2+} concentration at the membrane level might be reflected in altered levels of serum Ca^{2+}. In these experiments serum Ca^{2+} levels of cocaine-treated rats were compared with those in normal rats. The chronic cocaine treatment of rats was as described earlier. In these experiments (8 rats in each group) the serum Ca^{2+} levels for the controls ranged from 10·02 to 11·52 (mg/100 ml) and from 9·04 to 12·17 for the cocaine-treated rats. Thus it is apparent that chronic cocaine treatment in rats caused no alteration in their serum Ca^{2+} levels.

DISCUSSION

The work described in this paper concerns the effects of local anaesthetics on various aspects of cell metabolism. Our preliminary experiments indicated that the local anaesthetics inhibited synthesis of citrate from acetate or pyruvate in intact cells of *E. coli*, and slices and homogenates of brain, thereby confirming and extending the earlier findings.[5] The conversion of pyruvate into citrate is an important reaction controlling the cellular activity and, by inhibiting this reaction, local anaesthetics could impair both energy production and certain synthetic pathways in the cell. It might also be emphasized that citrate too plays an important metabolic role, for it not only stimulates fatty acid synthesis in the presence of biotin-dependent acetyl CoA carboxylase[31] but can function as an acetyl donor in certain acetylation reactions

in the presence of citrate cleavage enzyme. As a result of impairment in citrate synthesis, it might be assumed that local anaesthetics could affect the above mechanisms as well.

The local anaesthetics significantly inhibited the oxidation of α-oxoglutarate to succinate (Table 3), while having little or no effect upon the other reactions of the tricarboxylic acid cycle studied.

It was also possible to demonstrate that the local anaesthetics inhibited various metabolic reactions in which acetate or pyruvate was involved. Thus the drugs inhibited synthesis of malate from acetate in intact cells of *E. coli*, synthesis of acetoacetate from pyruvate in liver homogenates (Table 4), the incorporation *in vivo* of 1-^{14}C-acetate into the cholesterol and fatty acid fractions of liver (Table 5), and acetylation of sulfanilamide in a system composed of pigeon liver extracts and respiring brain homogenates (Table 4).

Attempts were also made in this study to see whether local anaesthetics inhibited any of the above metabolic reactions *in vivo*. Experiments with cocaine *in vivo* showed that even in chronically cocaine-treated rats little or no change in citrate levels of blood and kidneys was seen. On the other hand, a consistent and significant increase in the citrate-synthesising ability of brain homogenates from cocaine-treated animals was observed, an effect which could be attributed to general cortical stimulation by cocaine. There was little or no change in lactate levels of blood and urine in rats even after chronic cocaine treatment, indicating no possible impairment of pyruvate utilization *in vivo*. These observations were also contradictory to earlier reports that lactate accumulated in urine of frogs and rabbits treated with cocaine.[32]

The failure to observe any metabolic effect of cocaine *in vivo* could perhaps be partly accounted for by low concentrations of drug.

Further experiments indicated that none of the acetate- and pyruvate-utilising reactions previously studied in intact cells or homogenates were inhibited by local anaesthetics in cellular subfractions. These results suggested that the drugs did not inhibit the above reactions at the enzymic level.

The local anaesthetics had no appreciable effect upon brain mitochondrial respiration in the presence of various intermediates of tricarboxylic acid cycle and glutamate. The marked inhibitory effects of cinchocaine and to a lesser degree of amethocaine in most of our experiments were probably due to their cytotoxic properties. Thus cocaine and procaine, while inhibiting respiration in brain slices and homogenates, had little or no effect upon mitochondrial respiration, and in this respect these drugs resembled compounds like alcohol and chlorpromazine.[33]

Very interesting findings have emerged from the work of Quastel on narcotics.[33] It has been shown that narcotics inhibit brain cortex respiration probably by altering certain equilibrium conditions at some site in the intact cell membrane. It is therefore likely that the local anaesthetics could act in this manner.

There is considerable evidence that local anaesthetics cause changes in permeability of cell membrane, involving the movement of certain ions during the process of blocking nerve conduction.[34–38] It appeared therefore that the metabolic effects of these drugs seen in intact cells or homogenates might indeed have been secondary to these membrane changes.

At this point it seems necessary to consider different conditions under which our experiments have been carried out. In all the experiments *in vitro* involving intact

cells or homogenates, the substrates acetate and pyruvate were added to systems where cellular structure remained in an intact or semi-intact condition. In such cases, the substrates had to penetrate the membrane barrier before they could be metabolized, and here significant inhibition by local anaesthetics was seen. In most of the experiments *in vivo* except when 1-^{14}C-acetate was used, no substrates were administered to rats, and here no effect of cocaine was observed. Likewise in experiments where cell subfractions were employed, involving no penetration by substrates across the cell membrane, no inhibition by drugs was seen.

It was therefore interesting to speculate whether the local anaesthetics blocked the entry of acetate and pyruvate into the cell, thereby exerting their metabolic effects. It is also fair to assume that the entry of the acetate and pyruvate into the cell might be dependent upon the movement of ions, particularly Na^+ and K^+. If this were true, the local anaesthetics could block the entry of the above substrates into the cell by impairing the movement of Na^+ and K^+.

Another interesting possibility regarding the action of local anaesthetics emerges from the recent work of Kini and Quastel[8] that the ratio K^+/Ca^{2+} could be a rate-limiting factor for the conversion of pyruvate into acetyl CoA. The local anaesthetics might alter the ratio K^+/Ca^{2+} within the cell matrix, possibly by impairing certain membrane ion transport mechanisms and thereby affecting the pyruvate metabolism.

Various mechanisms for cation transport across cell membrane have been suggested.[39–41] Of particular interest are the cation-stimulated ATPases shown to be involved in cation transport across cell membrane.[39] In the present study, the local anaesthetics had no effect upon either the Mg^{2+}-stimulated ATPase from rat nerve[19] or the Na^+-sensitive ATPase from rat brain.[20] It is perhaps necessary to examine the effects of these drugs upon certain other specific mechanisms of cation transport before any firm conclusions can be drawn.

There is considerable evidence that Ca^{2+} plays an important role in excitable tissues such as nerve.[42–44] There is also evidence that Ca^{2+} binds the sites in the membrane which are normally available to Na^+ and K^+ during excitation and that local anaesthetics block these sites upon which the transference of Na^+ and K^+ largely depends.[44]

The experiments of Kini and Quastel[8] have shown that cocaine (0·05 m-mole) diminishes the ratio K^+/Ca^{2+} in the cell by displacing Ca^{2+} from their sites in the membrane, probably by altering the ionic permeability, and thereby inhibits the breakdown of pyruvate and oxaloacetate so that more of these keto acids are available for transamination.

In our experiments little or no change in the serum Ca^{2+} levels of rats was seen even after chronic cocaine treatment. However, it is possible that the subtle changes at the membrane level might well not be reflected by changes in serum Ca^{2+} levels.

Summing up, it appears from the present study that local anaesthetics affect the cell metabolism probably at the membrane level, possibly by inducing certain changes in the cell membrane.

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