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# *Effects of Procaine Concentration and Duration of Contact on Oxygen Consumption in Bovine Dental Pulp*

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A previous preliminary study in our laboratory on the effects of various dental drugs and materials on respiration in bovine dental pulp indicated that procaine exerted a depressant action on the rate of oxygen consumption in this tissue.<sup>1</sup> Subsequently, Kozam and Burnett showed that procaine, Xylocaine, and Primacaine depressed oxygen consumption in rabbit incisor pulp and that significantly different effects of concentration were noted only between the highest and lowest concentrations of the anesthetics used in their experiments.<sup>2</sup> The present study was conducted to explore the effect of procaine concentration on pulpal respiration by a somewhat different method from that employed by Kozam and Burnett and also to investigate how much respiration would be reduced by prolonged contact with the anesthetic agent. It was believed that if the effect of various concentrations of procaine were studied over a period of several hours, behavioral trends might become more apparent than if a series of data from only a single period of observation was utilized.

The matter of the prolonged effects of procaine was also of interest because of scattered clinical impressions that surgical wound healing was often complicated where local infiltration anesthesia was employed. It has been held that tissue necrosis sometimes apparently related to infiltration anesthesia is attributable to anoxia produced by the action of vasoconstrictors, but rarely has the role of the anesthetic agent itself been questioned. Although it was recognized that the relation of procaine to pulp tissue *in vitro* would differ in many respects from that injected into the tissues of intact animals, it was also believed that *in vitro* experiments would provide some information as to the basic action of this anesthetic agent on a fairly homogeneous connective tissue structure.

## MATERIALS AND METHODS

Pulp tissue was obtained from the molar teeth of freshly slaughtered cattle. An earlier study<sup>3</sup> has shown that the level of respiratory activity of pulp is related to the developmental status of the tooth. Therefore, each sample of pulp tissue slices exposed to the action of procaine was matched with a control sample of pulp slices from the identical tooth. The slices were approximately 0.5 mm. in thickness. The suspending

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medium was Krebs-Ringer-phosphate solution buffered to pH 7.4. Tetracycline hydrochloride was added to the suspending solution in a 1:100,000 ratio to inhibit bacterial activity, which is likely to become quite noticeable after 3–4 hours.

In those preparations in which the effects of procaine were to be studied, procaine hydrochloride crystals were dissolved in the suspending solution just described to achieve concentrations of 0.25, 0.50, 1.00, 2.00, and 4.00 per cent, respectively for the five series of experimental samples. The control samples, of course, were suspended in the buffered Krebs-Ringer-phosphate-tetracycline solution only.

Oxygen consumption was measured, as in our previous studies, by the direct method of Warburg.<sup>4</sup> The gas phase was oxygen, and the temperature of the water bath was 38° C. Manometric readings were made at hourly intervals after the beginning of the experiments for 5 consecutive hours. After manometric observations were completed, gas-volume determinations were accomplished for each flask-manometer system by the method of Lazarow.<sup>5</sup> The tissue samples were then transferred to individual aluminum-foil weighing baskets, dried for 24 hours at 105° C., and weighed. The  $Q_{O_2}$  ( $\mu$ l. of  $O_2$  consumed per mg. of dried tissue per hour) was calculated for each tissue sample for each of the five consecutive 1-hour periods.

Each  $Q_{O_2}$  value of each sample subjected to the action of procaine was then compared with the corresponding  $Q_{O_2}$  of its control sample sliced from the same pulp. Using the  $Q_{O_2}$  of the control sample as the standard, the  $Q_{O_2}$  of the matched experimental sample was expressed as a percentage of the control value. The percentage values thus obtained were the data subsequently analyzed.

Extensive studies in our laboratory have shown that the water content of dental pulp amounts approximately to 90 per cent of its total weight. This being the case, it seemed evident that the tissue samples would vary in dry weight in accordance with the concentrations of the procaine solutions in which they were immersed for several hours. It is true that the ingredients dissolved in the Krebs-Ringer-phosphate-tetracycline solution would also contribute to the dry weight of the specimens. However, this latter factor pertains to all specimens, constituting a small, uniform proportion of the dry weight that can be ignored in comparative studies.

The amounts of procaine hydrochloride that would be dissolved in the tissue of a series of hypothetical samples of pulp slices immersed in solutions of suspending medium with 0.25, 0.50, 1.00, 2.00, and 4.00 per cent procaine hydrochloride, respectively, were calculated. In order to check the validity of the foregoing assumption and the calculations based on it, two series of pulp-slice samples of closely similar wet weights were suspended for several hours in Krebs-Ringer-phosphate-tetracycline and in Krebs-Ringer-phosphate-tetracycline–4 per cent procaine solutions, respectively. The wet weights of both series of samples were then determined. Following this, all samples were immersed in four successive fresh baths of Krebs-Ringer-phosphate-tetracycline solution to leach out the procaine hydrochloride, and the wet weights were again determined. Comparison of the two sets of mean weights showed that both groups of samples had lost some of their masses, presumably because of detachment of superficial cells and fibers. However, the difference between the mean weight loss of the control and that of the procaine-treated specimens indicated that the greater loss by the latter group represented the amount of procaine hydrochloride that had been absorbed in the tissue. The mean difference in weight loss agreed within 5 per cent of the calculated

amount of procaine hydrochloride that the tissue had contained. Therefore, all mean  $Q_{O_2}$  values originally obtained were corrected to reflect the dry weights minus the dissolved procaine hydrochloride, and these provide a second set of basic data.

## RESULTS

Oxygen-consumption measurements were made on 16 samples of pulp slices in 0.25 per cent procaine solution, on 24 samples in 0.50 per cent solution, on 23 samples in 1.00 per cent solution, on 24 samples in 2.00 per cent solution, and on 23 pulp samples in 4.00 per cent solution. Similar measurements were made on the same number of control samples. Oxygen-consumption measurements were made at the ends of five successive 1-hour periods. Thus a total of 1,100  $Q_{O_2}$  values was obtained.

TABLE 1\*  
EFFECT OF PROCAINE HYDROCHLORIDE ON RATE OF OXYGEN  
UPTAKE IN BOVINE DENTAL PULP

| Per Cent Procaine Concentration | No. of Samples |        | 1st Hour | 2d Hour | 3d Hour | 4th Hour | 5th Hour |
|---------------------------------|----------------|--------|----------|---------|---------|----------|----------|
| 0.25.....                       | 16             | { Mean | 99.5     | 94.6    | 91.2    | 79.8     | 75.1     |
|                                 |                | { S.D. | 50.6     | 21.3    | 28.3    | 12.8     | 25.8     |
| 0.50.....                       | 24             | { Mean | 92.8     | 80.0    | 69.1    | 56.9     | 46.7     |
|                                 |                | { S.D. | 29.1     | 21.9    | 19.4    | 12.3     | 18.0     |
| 1.00.....                       | 23             | { Mean | 75.0     | 64.9    | 48.9    | 44.0     | 33.9     |
|                                 |                | { S.D. | 24.6     | 20.6    | 17.2    | 12.7     | 18.1     |
| 2.00.....                       | 24             | { Mean | 75.2     | 58.7    | 48.8    | 39.3     | 29.9     |
|                                 |                | { S.D. | 32.7     | 28.7    | 18.1    | 16.1     | 13.7     |
| 4.00.....                       | 23             | { Mean | 32.0     | 22.1    | 14.2    | 14.8     | 7.9      |
|                                 |                | { S.D. | 23.5     | 12.6    | 10.8    | 10.6     | 5.4      |

\* Mean values are percentages of control  $Q_{O_2}$  and are not corrected for weights of absorbed procaine.

The results of these observations, derived from  $Q_{O_2}$  values that were not corrected for the weight of the procaine that was contained in the samples, are shown in Table 1. Corresponding results obtained by correction of the mean  $Q_{O_2}$  values for the weight of absorbed procaine hydrochloride are shown in Table 2. The information contained in Table 2 is presented graphically in Figure 1.

It will be observed that procaine depressed oxygen utilization in pulp tissue somewhat in proportion to the concentration of the drug. An unexplained exception to this mode was the lack of distinction between the effects of the 1.00 and 2.00 per cent solutions. It will be noted, also, that procaine exerted its effect in a continuous manner, the depression of respiration becoming more profound with the duration of its association with the tissue.

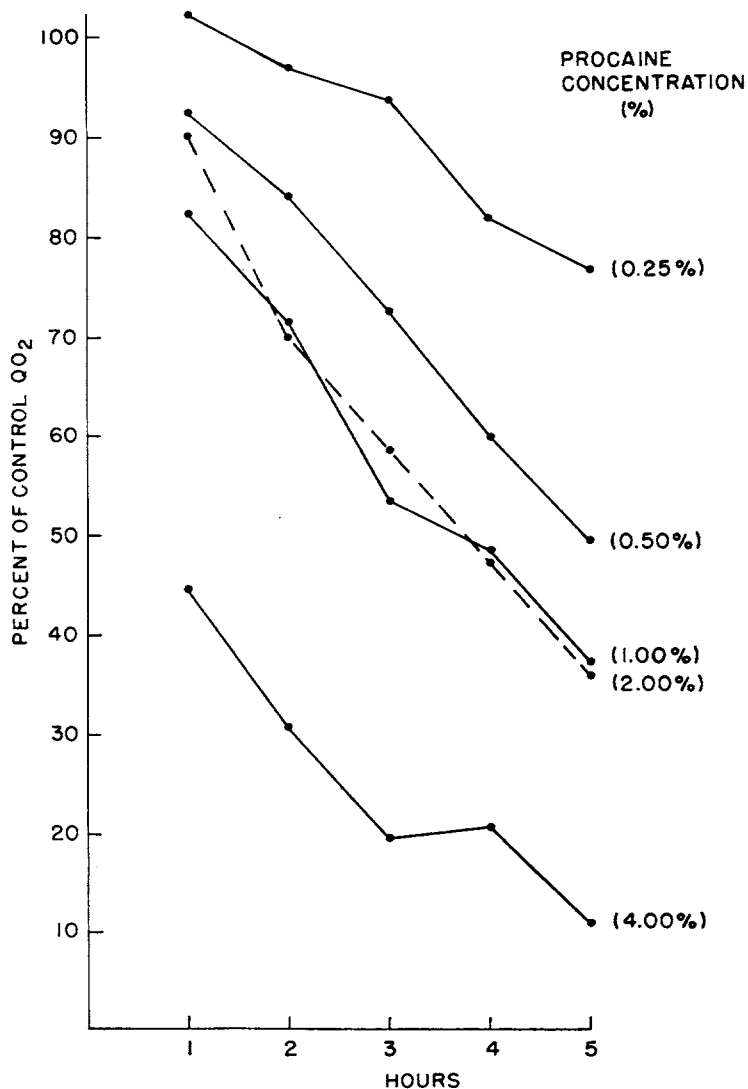
## DISCUSSION

Perusal of Table 1, with special attention to the standard deviations of the mean values, might lead one to suspect that many of the values are of doubtful significance. However, when the mean values are plotted graphically, as in Figure 1, it is highly questionable that the relative positions of most of the 25 points upon which the five

**TABLE 2\***  
**EFFECT OF PROCAINE HYDROCHLORIDE ON MEAN RATE OF**  
**OXYGEN UPTAKE IN BOVINE DENTAL PULP**

| Per Cent Procaine Concentration | No. of Samples | 1st Hour | 2d Hour | 3d Hour | 4th Hour | 5th Hour |
|---------------------------------|----------------|----------|---------|---------|----------|----------|
| 0.25.....                       | 16             | 102.4    | 96.9    | 93.4    | 81.8     | 76.9     |
| 0.50.....                       | 24             | 97.4     | 84.0    | 72.5    | 59.7     | 49.0     |
| 1.00.....                       | 23             | 82.5     | 71.3    | 53.7    | 48.4     | 37.2     |
| 2.00.....                       | 24             | 90.2     | 70.4    | 58.5    | 47.1     | 35.8     |
| 4.00.....                       | 23             | 44.8     | 30.9    | 19.8    | 20.7     | 11.0     |

\* Mean values are percentages of control  $Q_{O_2}$  and are corrected for weights of absorbed procaine.



**FIG. 1.**—Effect of procaine hydrochloride on the rate of oxygen consumption in bovine dental pulp

curves are based is an expression of chance. Extensive experience in respiratory studies of the dental pulp has convinced us that the rate of oxygen consumption in this tissue is quite variable. This variation is caused, in part, by peculiarities in physiologic status to which previous reference has been made.<sup>3</sup> The influences of other physiologic differences between pulps are becoming apparent, although detailed analyses of the basis of the differences have not been completed. An even more intriguing problem has been presented by the realization that the rate of oxygen consumption in individual dental pulps *in vitro* is not uniform over a period of 1 or 2 hours. We have mentioned this in a previous paper.<sup>6</sup> The fluctuating rate, together with the inherent differences of individual pulps, can therefore needlessly disturb confidence in apparent trends if overzealous statistical treatment of data derived from single-period observations is attempted.

The decline in the rate of oxygen consumption ascribed to the various concentrations of procaine in this investigation cannot be attributed to *in vitro* degeneration of the tissue. Previous studies in our laboratory have shown that pulp can continue to respire for more than 24 hours *in vitro* without exogenous nutritive support, although evidence of decline becomes apparent after about 11 hours.<sup>3</sup> In the present study the tissues were observed *in vitro* for only 5 hours. In fact, we have shown previously that when endogenous respiration is observed over a period of several hours, the pulpal rate of oxygen consumption slowly increases as the respiratory quotient drops.<sup>6</sup> This we have attributed to a shift to protein and lipid utilization as endogenous supplies of glucose become exhausted. Further support for the belief that the continuing suppression of oxygen consumption in the tissues exposed to the various concentrations of procaine is caused by the drug itself is the fact that the rate of oxygen consumption in the control samples did not drop during the experimental period.

In examining the trends that are evident in Figure 1, one is tempted to believe that if the experimental period had been extended for a few hours, the procaine would have killed the tissue. Extrapolation of the data suggest that the 0.25 per cent solution of procaine would have been lethal in about 10 hours and the 4.00 per cent solution in about 6 hours. These speculations are supported by the studies of Zinner, Jablon, Sanders, and Saslow,<sup>7</sup> who showed that all of the several commercial preparations of dental local anesthetics were completely cytotoxic to tissue cultures of monkey kidney and HeLa cell tissue cultures in 2-3 hours.

The influence of various drugs on the rate of oxygen consumption in rabbit sciatic nerve was studied by Sherif.<sup>8</sup> He found that procaine borate inhibited oxygen uptake by this tissue in a manner similar to that of cocaine hydrochloride but that concentrations double those of cocaine were required to produce comparable effects. His data show that the inhibition becomes more profound as the concentration of the drug is increased. Cook and McDevitt reported that several local anesthetics, including procaine, depress respiration in yeast and in rat liver in a manner roughly proportional to the toxicity of the anesthetics.<sup>9</sup> Wollenberger investigated the action of various narcotics and local anesthetics on the respiration of heart muscle.<sup>10</sup> He found that many of these substances, including procaine, had an inhibitory effect on oxygen uptake that corresponded somewhat to the concentration of the drug. Thus the results of this study, with the puzzling exception of the indistinguishable effects of the 1.00 and 2.00 per cent procaine concentrations, are in general agreement with the finding of several

other workers in respect to the relation of procaine concentration to the degree of respiratory depression in various tissues.

Wollenberger also noted that procaine, tetracaine, and paraldehyde depressed anaerobic glycolysis in cardiac tissue, while cocaine had a slight stimulatory effect.<sup>10</sup> This reported difference between the action of procaine and cocaine is interesting because it has often been assumed that the general similarities between the anesthetic effects of these drugs might be due to certain similarities in chemical structure. Ryman and Walsh have presented evidence that cocaine inhibits cellular respiration by blocking the entry of acetate into the tricarboxylic acid cycle.<sup>11</sup> The capacity of cocaine and procaine to reduce oxygen consumption would suggest that both would have the same metabolic site of action. But Wollenberger's observation of their different effects on anaerobic glycolysis would seem to indicate either that procaine acts at a different metabolic site or that it acts, unlike cocaine, at more than one site.

The work of Wollenberger and of Ryman and Walsh help to explain the progressive decline in oxygen consumption in pulp tissue during prolonged contact with procaine. Oxygen is used in largest quantities in those reactions related to the tricarboxylic acid cycle. If procaine acts either to inhibit the formation of acetate or its precursors or to block its entry into the Krebs cycle, much of the substrate for anaerobic processes would be depleted at a rate commensurate with the metabolic level of the tissue. The significance of these changes is not in the decrease in the intracellular substrate or in the enforced alteration of oxygen requirement but rather in the consequent deterioration of energy-providing mechanisms that are dependent on the first two factors. It is quite possible that if the effect is excessively prolonged, some of the deterioration may become irreversible.

This explanation could account for the lethal effect of procaine and other local anesthetics on cells in tissue cultures reported by Zinner *et al.* It could also account for some of the degenerative or necrotizing changes that have occasionally been claimed to have been attributable to excessive infiltration anesthesia. In the latter instance the vasoconstricting drug contained in the anesthetic solution could have been the means that merely made it possible for the anesthetic agent to exert its antimetabolic effect over a longer period of time. Because this and other studies have shown that procaine reduces the rate of oxygen consumption in various tissues, it follows logically that there is little need for the normal oxygen supply during the time the procaine is exerting its action. Hence it is believed that the untoward tissue effects associated with procaine infiltration anesthesia probably are not traceable to local hypoxia produced by vasoconstriction.

#### SUMMARY

This investigation was concerned with the effects of 0.25, 0.50, 1.00, 2.00, and 4.00 per cent procaine hydrochloride solutions on the rate of oxygen consumption in bovine dental pulp over 5-hour periods. Except for the indistinguishable effects of the 1.00 and 2.00 per cent solutions, the rate of respiration was depressed approximately in proportion to the concentration of the drug. The continuing decline in respiration throughout the 5-hour period of the experiments with all concentrations of procaine employed suggest that this drug can be lethal to tissues if sufficient time is allowed for its action.

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