TONUS AND INHIBITION IN UNSTRIATED MUSCLE

By Inderjit Singh, F.A.Sc.

(From the Physiological Laboratory, Medical College, Agra)

Received July 23, 1948

In previous papers, it has been shown that there are two kinds of tone, one that requires oxygen and the other that does not (Rao and Singh, 1940; Singh and Singh, 1947 *a*, *b*, 1948). The question arises as to the source of energy for anærobic tone. It is quite possible that the muscle may not be consuming oxygen and yet may be deriving energy from some anærobic mechanism. These points have been investigated in the present research.

The question of inhibition is closely related to that of tonus, as the two being of opposite characters; the study of each will help in the elucidation of the other. It has been shown that there are two kinds of inhibitions in unstriated muscle; during one the oxygen consumption increases and during the other it decreases (Rao and Singh, 1940). This follows from experiments on tonus. If the oxygen consumption decreases during tonic contraction, then during relaxation it should increase and if it increases during contraction it should decrease during inhibition.

Experimental

These experiments were performed on pieces of frog's stomach (Singh, 1939), dog's stomach (Singh, 1940) and fowl's gut (Singh, Singh and Muthana, 1947). If the muscle did not show much tone, then 10 to 20 per cent. of the sodium of the saline was replaced with potassium; this procedure is useful also in giving an idea of the relaxation of the muscle. Resting tone, with a little error, can be considered as possessing zero tension and the tension at any other time can be expressed as percentage of the maximal tension produced by potassium. Other experimental procedures were as in previous papers (Singh and Singh, 1948).

Results

Tonus

Aerobic tone.—This is increased by adding 0·1 per cent. glucose to the saline. This shows that the energy is derived from the production of lactic acid and its subsequent oxidation. If the muscle is poisoned with iodoacetic acid (1 in 20,000), tone still persists, showing that energy is derived

from other sources; in frog's stomach however there may be a marked fall of tonus. In the absence of oxygen the tone rapidly declines. In these respects, therefore, energy for the maintenance of tone is derived from sources similar to those for twitch.

Further analysis shows that the ærobic mechanisms for tone and twitch are not identical. In dog's stomach, as in frog's stomach, there is asphyxial increase of excitability; on reintroduction of oxygen, the response to twitch is depressed as in the frog's stomach, but tone increases (Fig. 1). This

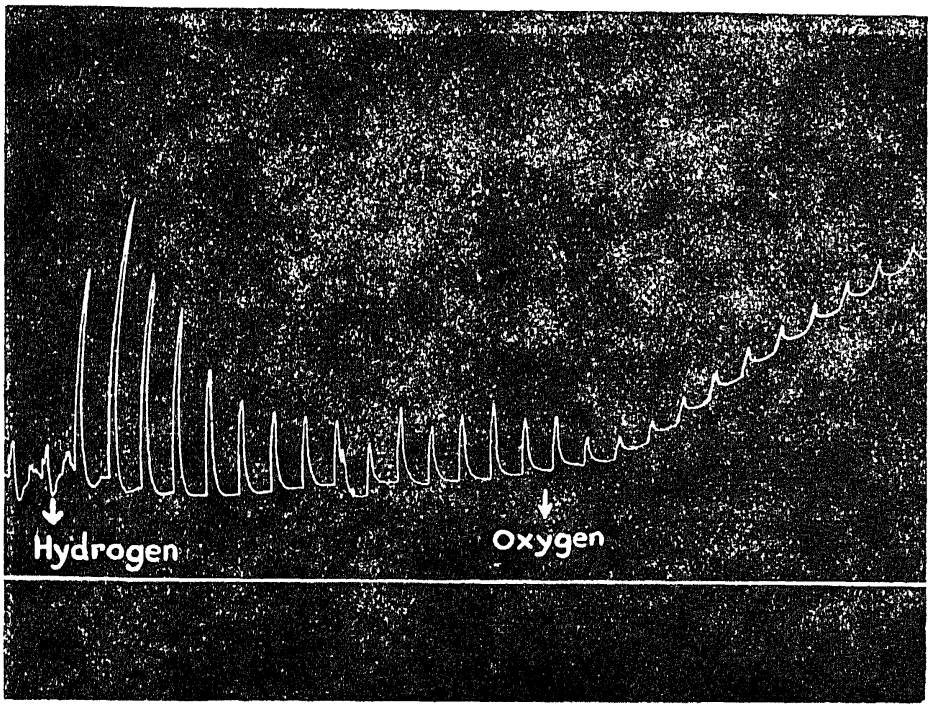

FIG. 1. *Dog's stomach.*—Stimulation by alternating current 12 volts for 12 seconds per minute.

experiment further shows that after asphyxia the muscle becomes more sensitive to oxygen. This is probably due to opening of reserve oxidative channels. This effect of oxygen is similar to that on the chemoreceptors of the carotid body. Watt, Dumke and Comroe (1943) found that the inhalation of pure oxygen by unanæsthetised dogs caused a reduction in breathing but in some animals, the respirations, after an initial reduction, were restored to their original value, although pure oxygen was being breathed.

Results, similar to those described above, are also obtained if the muscle is asphyxiated. The tone as well as the twitch produced by alternating current or acetylcholine decline, but the former declines more rapidly than the latter. The twitch may increase prior to declining (Fig. 2).

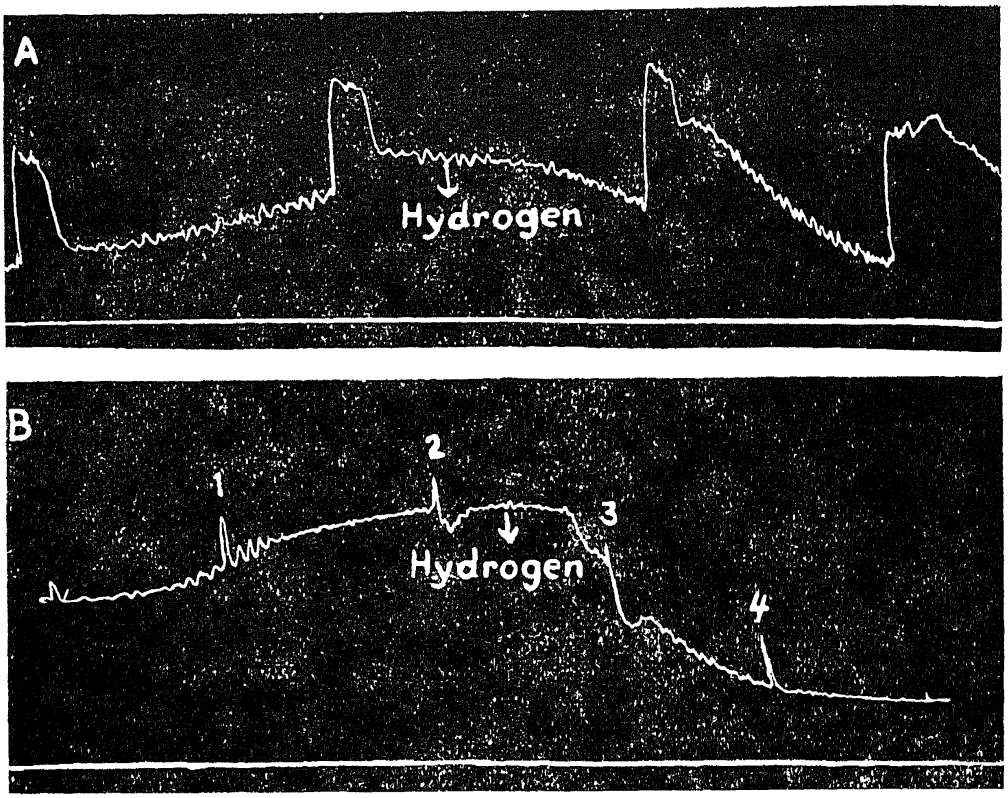

FIG. 2. *Fowl's gut.—A.* Stimulation by acetylcholine, 1 in 10^5. *B.* Stimulation by alternating current at 1, 2, 3 and 4.

Different muscles vary in their sensitivity to oxygen lack; dog's stomach muscle is more resistant than the frog's stomach or the fowl's gut. The latter is very susceptible. In the frog's stomach, there is individual variation. The coronary arteries are known to dilate more than the others as a result of anoxæmia.

Anærobic tone.—In the absence of oxygen, the tone declines to about 50% of its original value in the dog's stomach and almost completely in the fowl's gut. Before declining there may be a phase of increment in the tonus, comparable to the asphyxial increase of excitability to alternating current, potassium and acetylcholine (Fig. 3).

In alkaline solutions, the addition of glucose, 0·1 per cent., causes a sharp recovery which is about 20 to 40 per cent. of the original tension; this can be maintained for several hours (Fig. 4). In fowl's gut, the action of glucose is immediate; contraction is produced as if a stimulant was added. This shows that the action of glucose is on the surface of the cells, and therefore there are enzyme systems on such surfaces which subserve metabolism. This may be important in connexion with the surface action of drugs, hormones and ions which may activate or modify the action of enzymes on such surfaces and so produce their effects. The presence of enzymes on surfaces may account for Clark's active patches on cell surfaces.

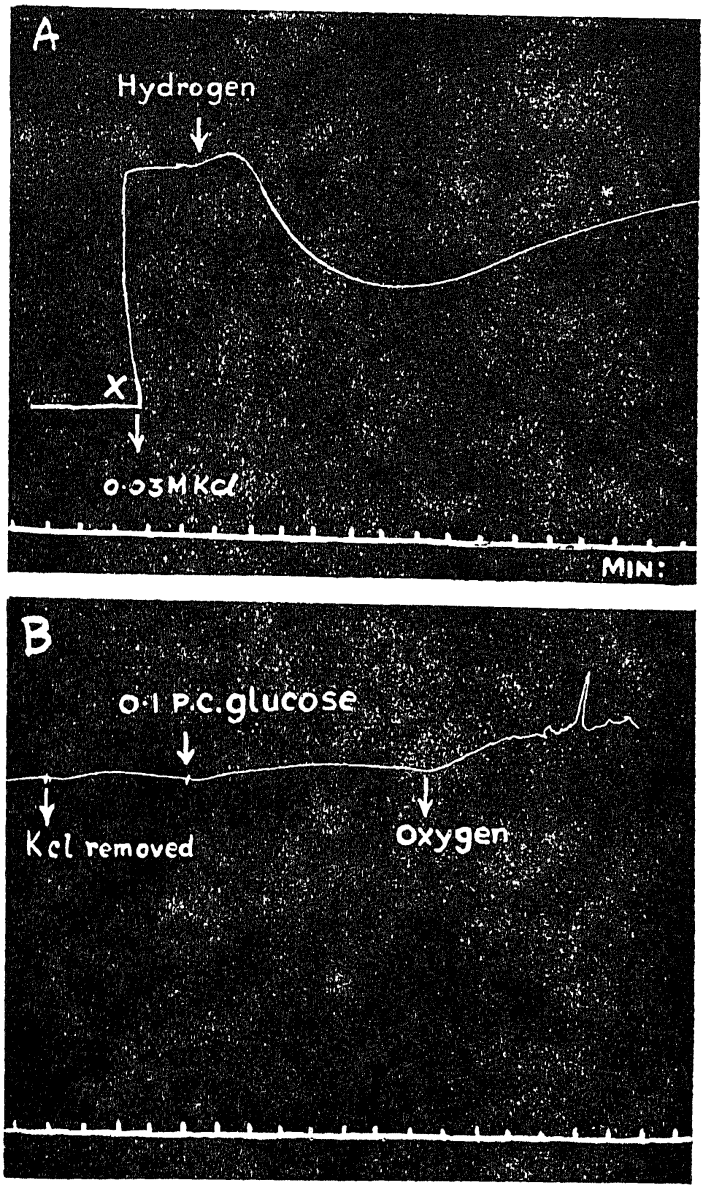

FIG. 3. *Dog's stomach.*—Effect of asphyxia on tone. *B.* Continuation of *A.* At *X*, drum stationary till maximum tension produced.

In the frog's stomach, the action of glucose was not found to be so dramatic (Singh and Singh, 1948). This slower action suggests that glucose is also metabolised inside the muscle fibres. There is also a natural variation in sensitivity of the muscle to glucose just as with other substances.

The sensitivity of unstriated muscle to glucose increases after asphyxia. This is shown by the following experiment on dog's stomach. Addition of glucose in the presence of oxygen hardly causes any increase of tone; asphyxiated muscle may however show a great increase of tone, during which the response to alternating current is depressed (Fig. 5). This shows the difference between the anaerobic metabolisms of tone and twitch. The increased sensitivity to glucose in asphyxia suggests opening of reserve anærobic channels, thus accounting for asphyxial hyperexcitability.

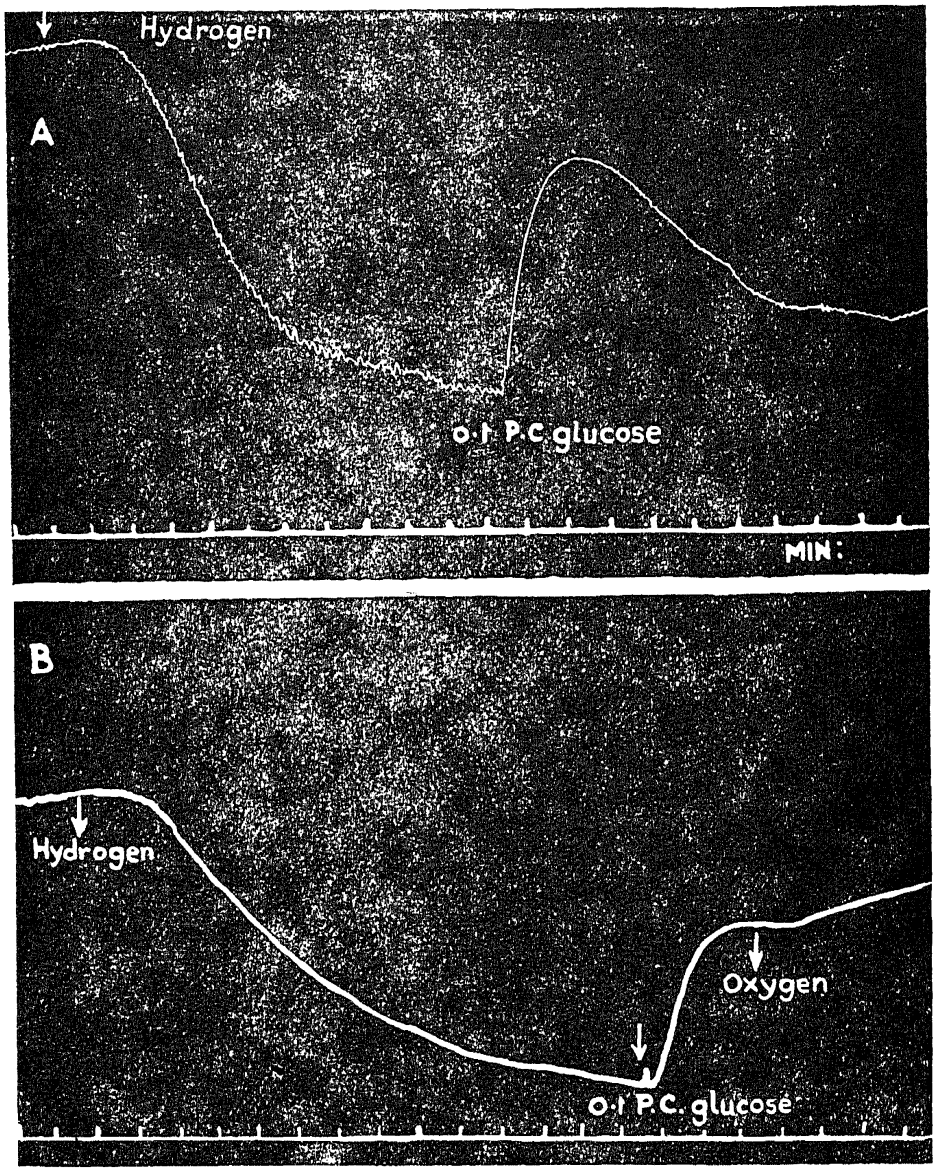

FIG. 4. *Fowl's gut.*—Effect of asphyxia, glucose and oxygen on tone.

If the asphyxia is continued for some time (beyond 10–20 minutes) then the tone again begins to increase. This is the asphyxial contraction described previously (Singh and Singh, 1947, 1948), which is rapidly abolished by oxygen in the frog's stomach, but very slowly in the dog's stomach. In the fowl's gut, this asphyxial contraction is hardly noticeable. Asphyxia thus causes two kinds of contractions; one immediately, and the other after some time. The first asphyxial contraction is usually absent. Hereafter, the second contraction will be referred to as the asphyxial contraction.

The asphyxial contraction has very interesting properties. Glucose, like oxygen, has now inhibitory action. Glucose may be added to the saline just when the tension begins to rise, or when it has risen half way fully. In the former case glucose may prevent the asphyxial contraction (Fig. 6)

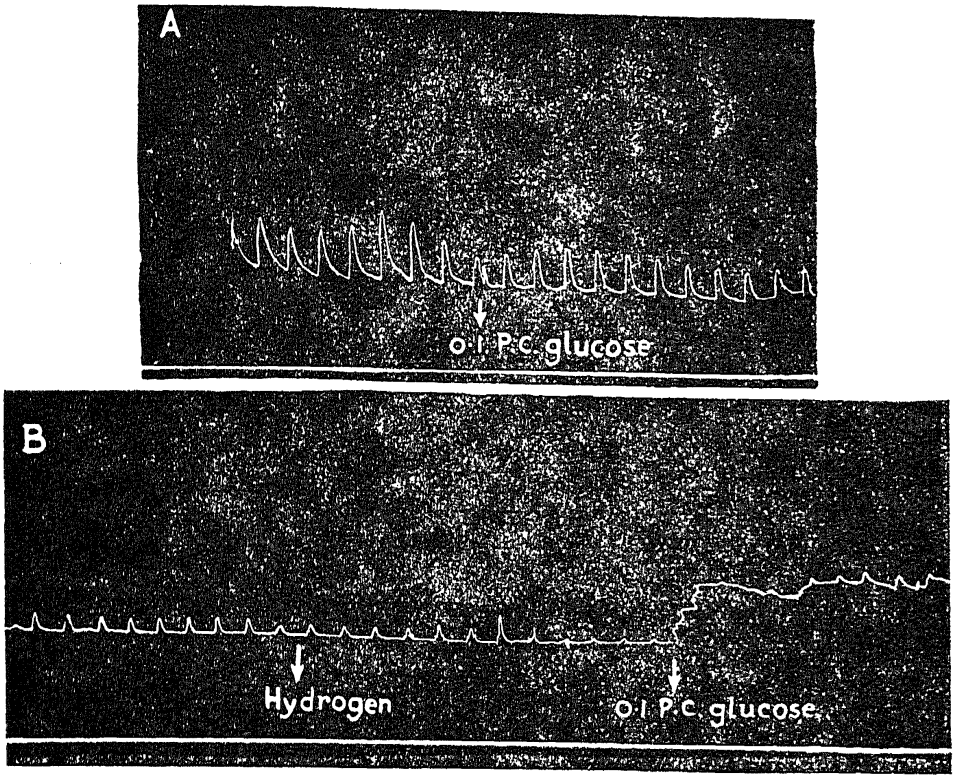

FIG. 5. *Dog's stomach.*—*A.* Effect of glucose in the presence of oxygen. *B.* Effect of glucose in the absence of oxygen.

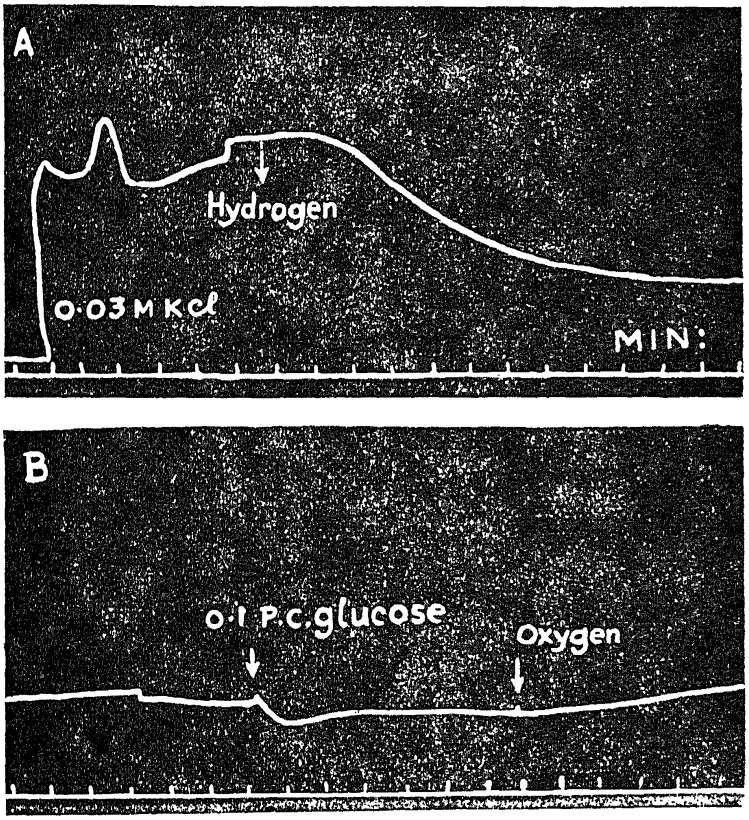

FIG. 6. *Fowl's gut.*—Effect of glucose on alactic tone. *B.* Continuation of *A.* Note contrast with Fig. 4.

and in the latter case, it diminishes the rate of rise of tension (Fig. 7) and if the tension has fully developed, it causes slight relaxation (Fig. 8). Glucose thus antagonises the asphyxial contraction but not very powerfully. This is important, otherwise the glucose normally present in the blood of an intact animal would prevent the asphyxial contraction.

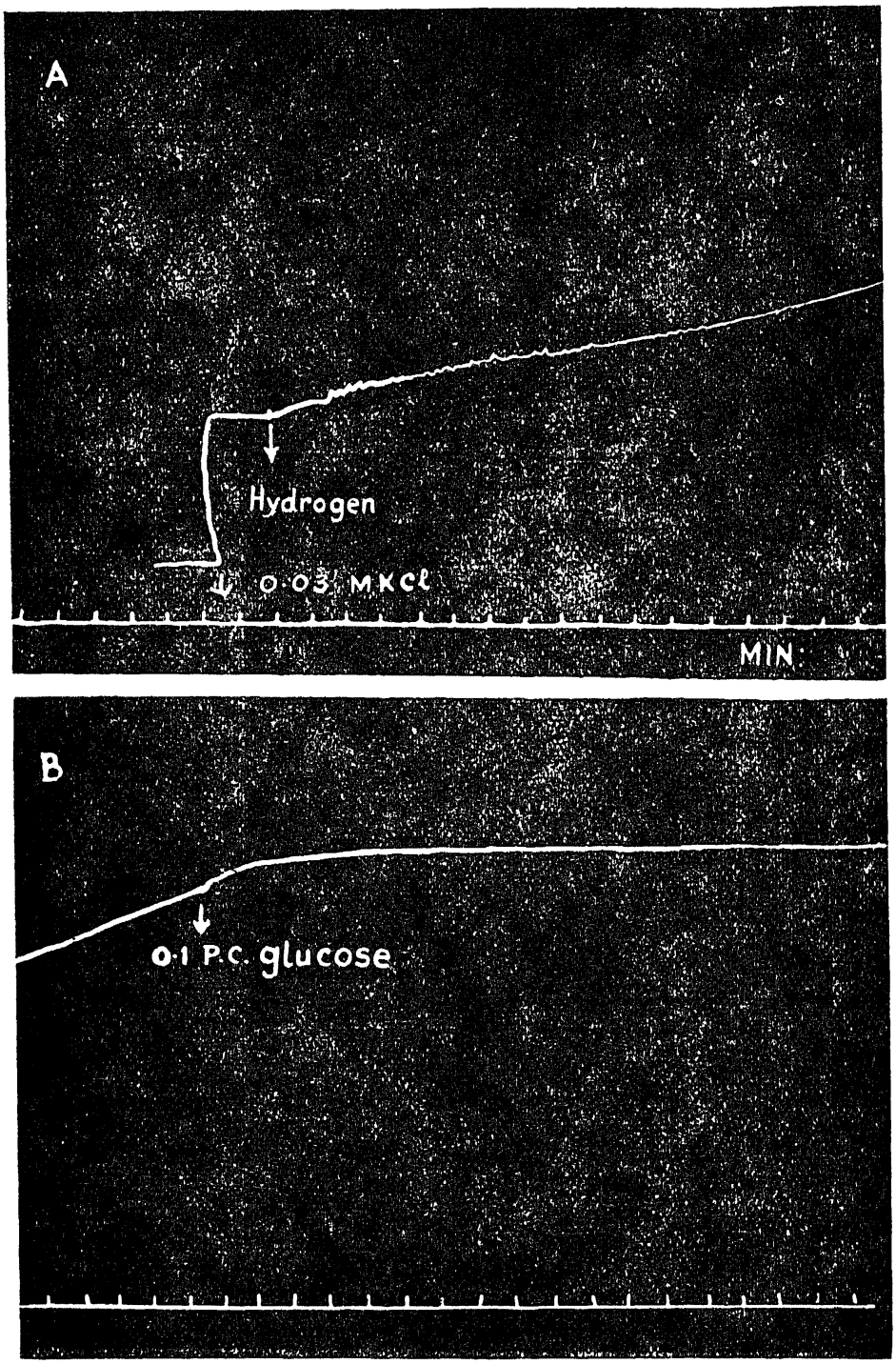

FIG. 7. *Dog's stomach.*—Effect of asphyxia and glucose on tone. *B.* Continuation of *A*. In *A*, drum stationary when potassium is added, till maximum tension results.

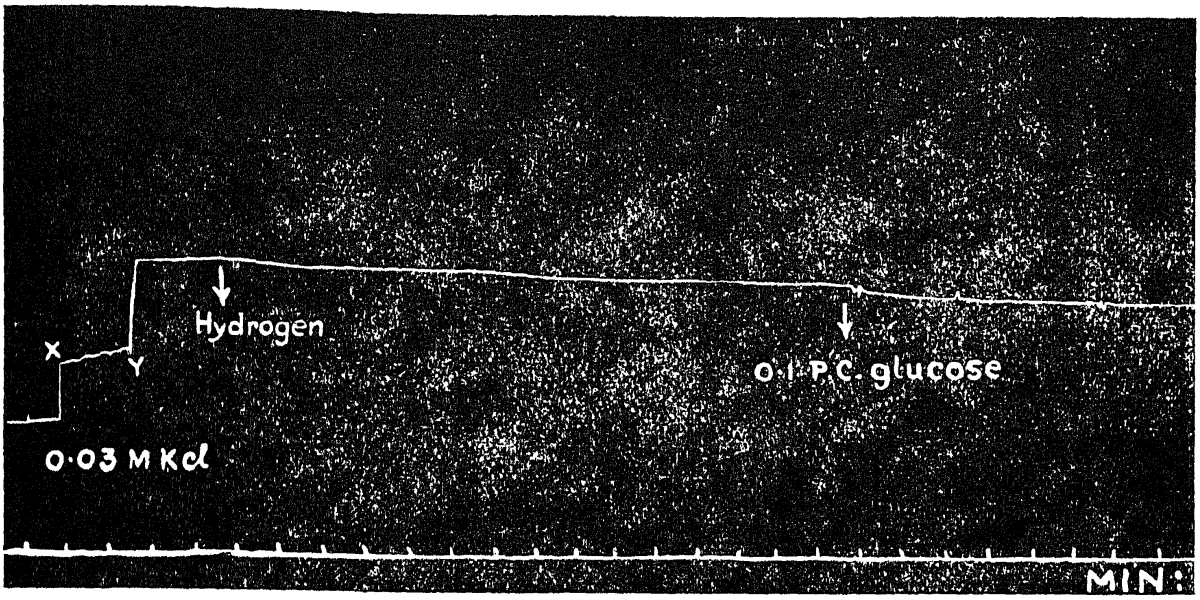

FIG. 8. *Dog's stomach.*—Effect of asphyxia and glucose on tone. Drum stationary at *X* and *Y*.

The above experiment shows that the normal state of the myosin molecule is that of shortening, but is kept extended by energy derived from the production of lactic acid. This is further shown by the action of iodoacetic acid. When the muscle is poisoned with the drug the tone in dog stomach increases. This shows that normally there is a state of tonic inhibition and upon this background further contraction is produced (Fig. 9).

Glucose changes the properties of the asphyxial contraction. In the absence of glucose oxygen inhibits the contraction, but in its presences, oxygen augments the tone. Glucose thus causes some fundamental change in the properties of the muscle. These states of the muscle may be termed as lactic and alactic asphyxial tones in the presence and absence of glucose respectively. The former tone consumes energy while the latter does not.

The change produced by glucose from the alactic to the lactic stage may be very rapid (Fig. 3) or may take about a minute or longer (Fig. 10). Thus the alactic tone may be changed into the lactic tone immediately, so that the second mechanism takes over from the first without interruption in the tension or the process may take about a minute or longer. The taking over by the second mechanism is shown by the effect of oxygen as mentioned above. The inhibitory action of glucose, thus may not be apparent, owing to the rapidity of change of state of the muscle. An interesting point is that if now the muscle is asphyxiated, it loses the tension that has been developed by the alactic mechanism.

An important property of the muscle in the asphyxial contraction is shown by the effect of potassium. If potassium had been added previously

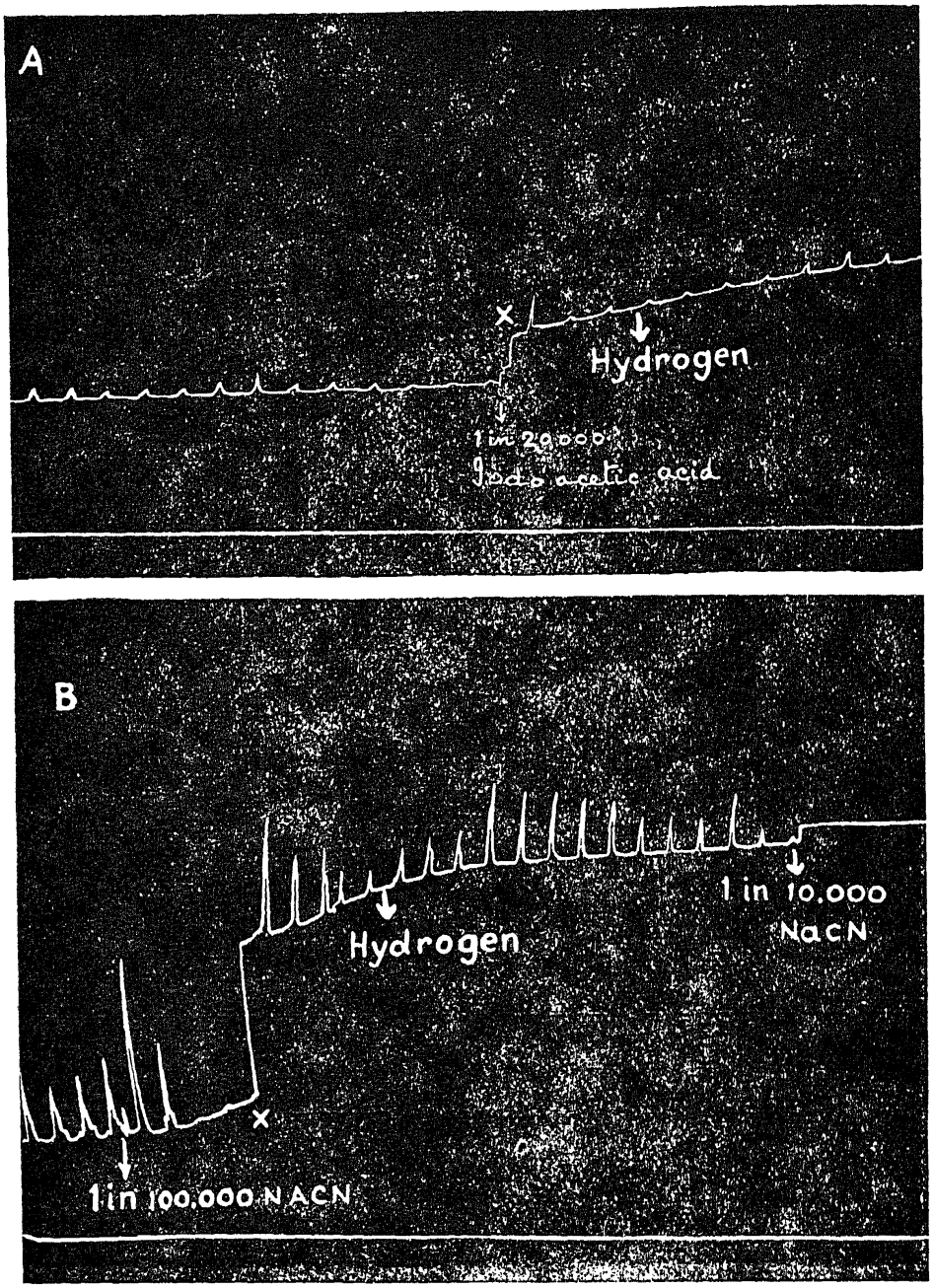

FIG. 9. *Dog's stomach.—A.* Effect of iodoacetic acid (1 in 20,000) on tone. On adding I.A.A. drum stationary for a while at *X*. *B.* Effect of 1 in 10^5 NaCN on tone, 5 minutes after adding, drum stationary for a while at *X*.

to the saline, its removal has now no effect, that is, the muscle does not relax, which it does ordinarily under ærobic conditions. This state in which the muscle does not relax, though the stimulant is withdrawn has been described previously (Gokhale and Singh, 1945; Singh 1946). The asphyxial contraction is therefore independent of the stimulant. The muscle now relaxes slowly (Fig. 11).

The slow relaxation of asphyxiated muscle is due to contraction and not the result if increase in viscosity. This is shown by the observation

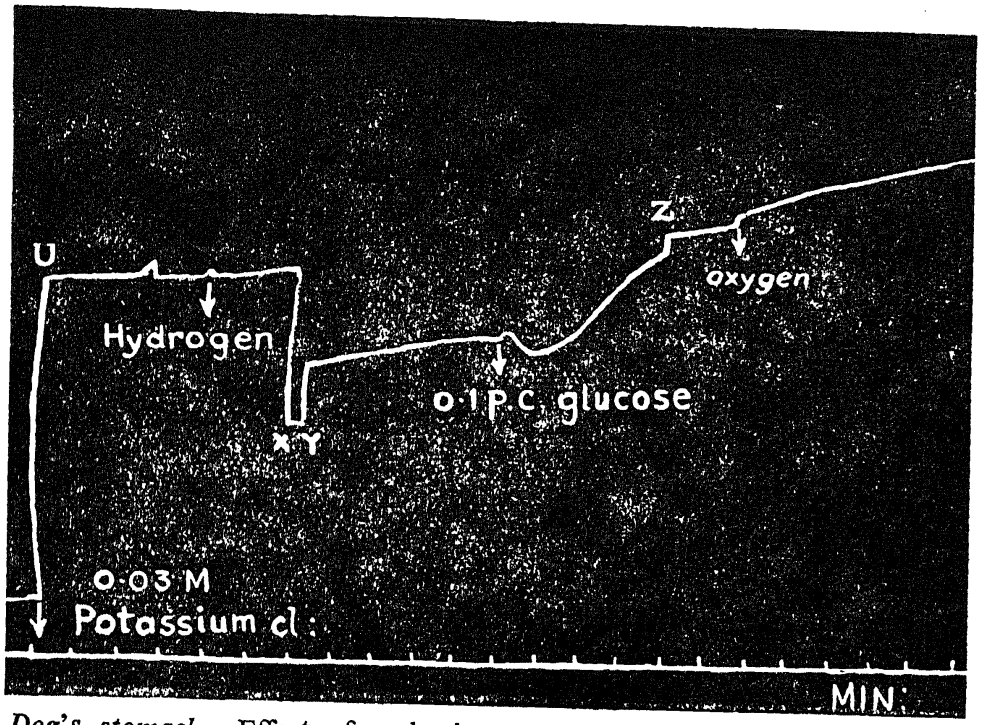

FIG. 10. *Dog's stomach.*—Effect of asphyxia on tone. Drum stationary at *U, X, Y* and *Z*.

that once the asphyxial contraction begins, withdrawal of potassium does not stop the contraction (Fig. 12).

The asphyxial contraction is hastened by prior stimulation either by potassium or by alternating current. This is due to exhaustion of the muscle by such stimulation. Sometimes on asphyxiation, the contraction begins immediately, the energy reserves presumably being very low. The asphyxial contraction is hastened by iodoacetic acid, which deprives the muscle of energy released from production of lactic acid; pH 6 has similar action.

During the asphyxial contraction the muscle is not completely exhausted of all energy reserves, as shown by the fact that the muscle responds to alternating current. It appears, therefore, that the muscle is kept relaxed by the lactic acid mechanism, at least in the dog's muscle, as shown by the specific effects of glucose. When this mechanism is exhausted, then the asphyxial contraction occurs, though energy is still available for contraction through other metabolic mechanisms. This is in agreement with the view that *rigor mortis* occurs in striated muscle when glycogen disappears (McDowall, 1946). *Rigor mortis* of striated muscle, and the asphyxial contraction of unstriated muscle, are therefore related. Normally when unstriated muscle dies, it does not show any contraction like *rigor mortis* of striated muscle, but the asphyxial contraction passes into such a state on death of the muscle.

Does the asphyxial contraction occur normally ?—The asphyxial contraction is not a terminal phenomenon, as it does not occur in a muscle dying in

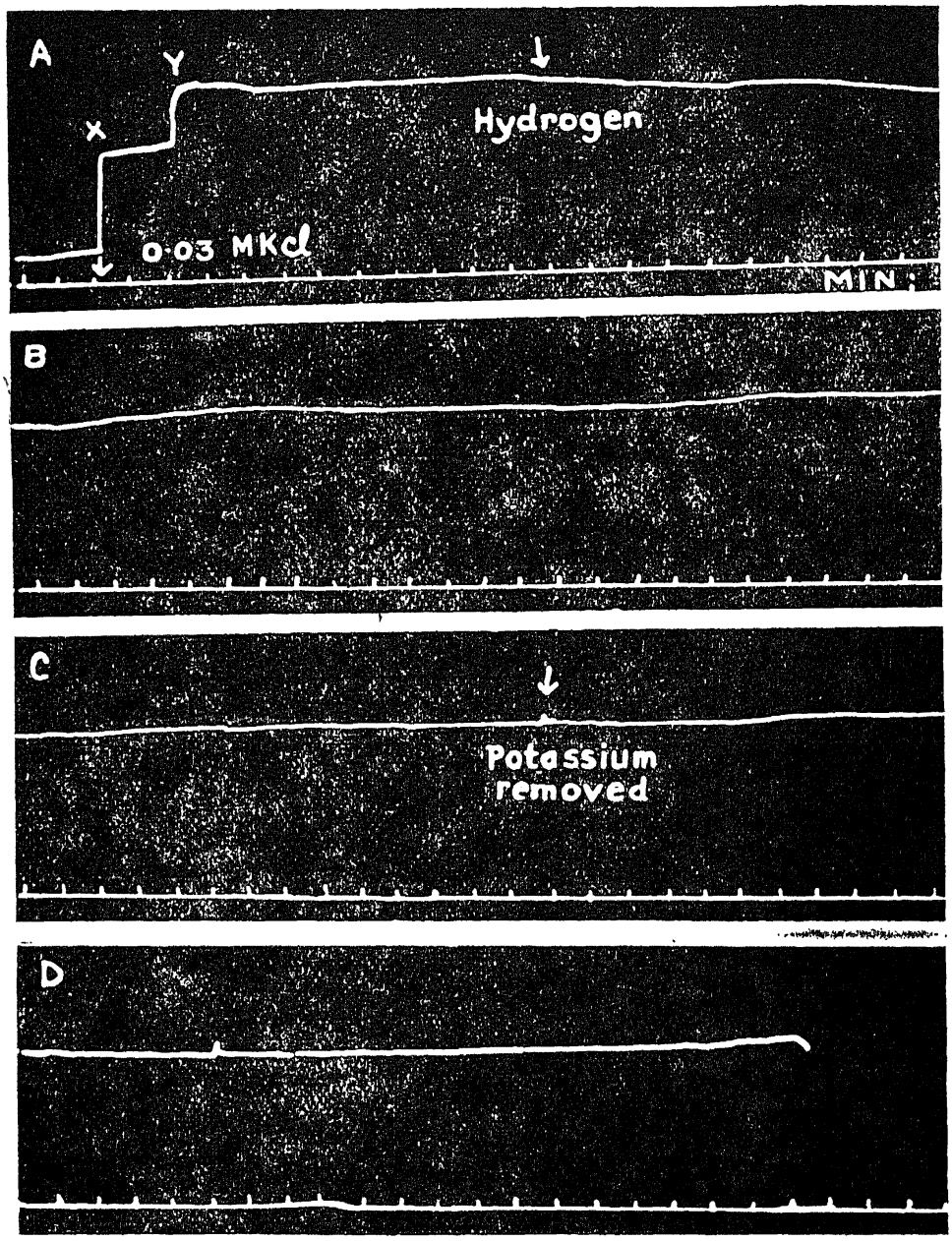

FIG. 11. *Dog's stomach.*—Effect of asphyxia on tone. Drum stationary at *X* and *Y*. *A, B, C, D* are continuous records.

saline. The chief properties of the asphyxial contraction are (1) that it does not diminish on exclusion of oxygen or addition of sodium cyanide, 1 in 10,000 (Fig. 9). (2) It is converted into ordinary tone by glucose. These two properties are shown by muscle removed fresh from an animal, so that the asphyxial contraction appears to be a normal phenomenon. It is also produced by stimulants such as potassium, barium, acetylcholine, iodide, nitrate, thiocyanate, *even in the presence of oxygen* (Figs. 7, 8, 11, 12). This is also proved by oxygen consumption experiments (Lovalt Evans, 1926; Rao and Singh, 1940). Thus the state of the muscle as found in the asphyxial contraction can be produced by the agencies other than asphyxia.

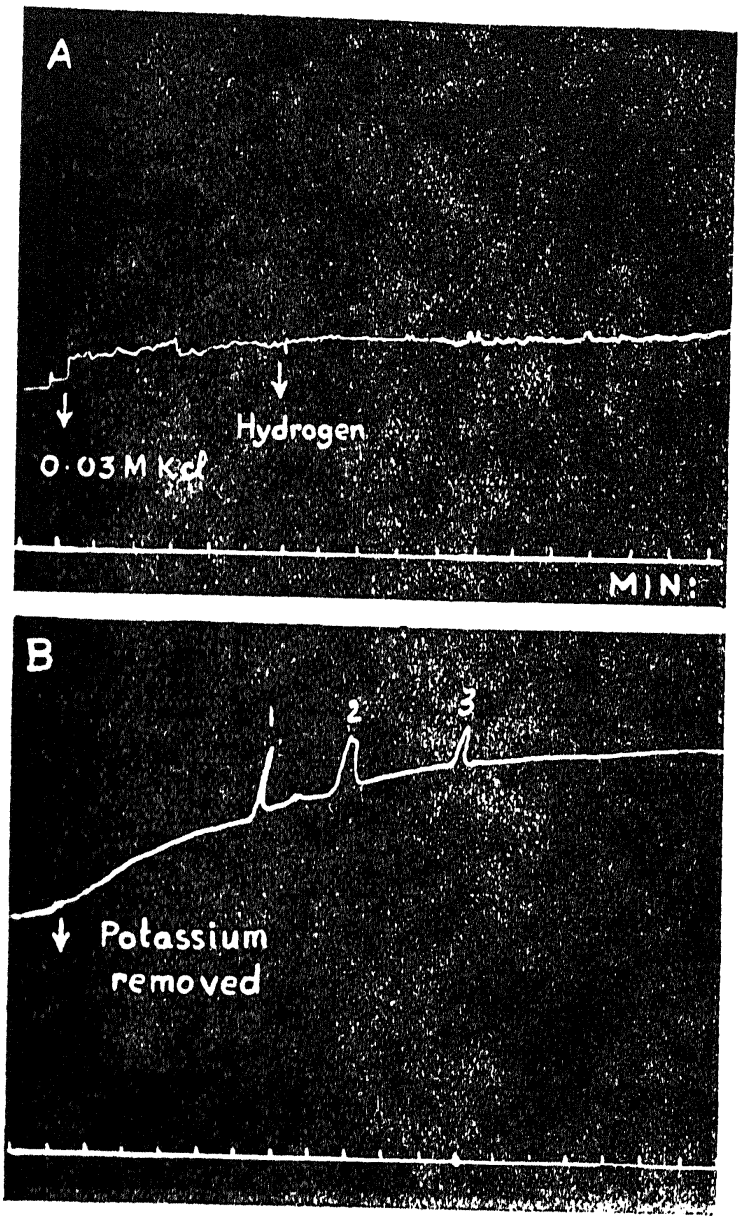

FIG. 12. *Dog's stomach.*—Effect of asphyxia or tone ; stimulation by alternating current at 1, 2, 3. *B.* Continuation of *A.*

Similar mechanism, as described for unstriated muscle, appears to exist in striated muscle. Thus Langley (1913) showed that striated muscle may continue to be contracted, though the stimulant is withdrawn.

Some muscles, such as rabbit's and fowl's gut, show predominantly the non-asphyxial tone while others, such as dog's stomach, show the asphyxial one. Usually, there is a mixture. Ordinary twitch contractions and non-asphyxial tone are superposed upon the asphyxial one, the degree of the latter being variable.

Effect of cyanide.—Sodium cyanide produces effects similar to those of asphyxia 1 in 10,000 NaCN, produces immediate fall of tonus, which may be followed by partial recovery and then a further fall of tonus (Fig. 13). The

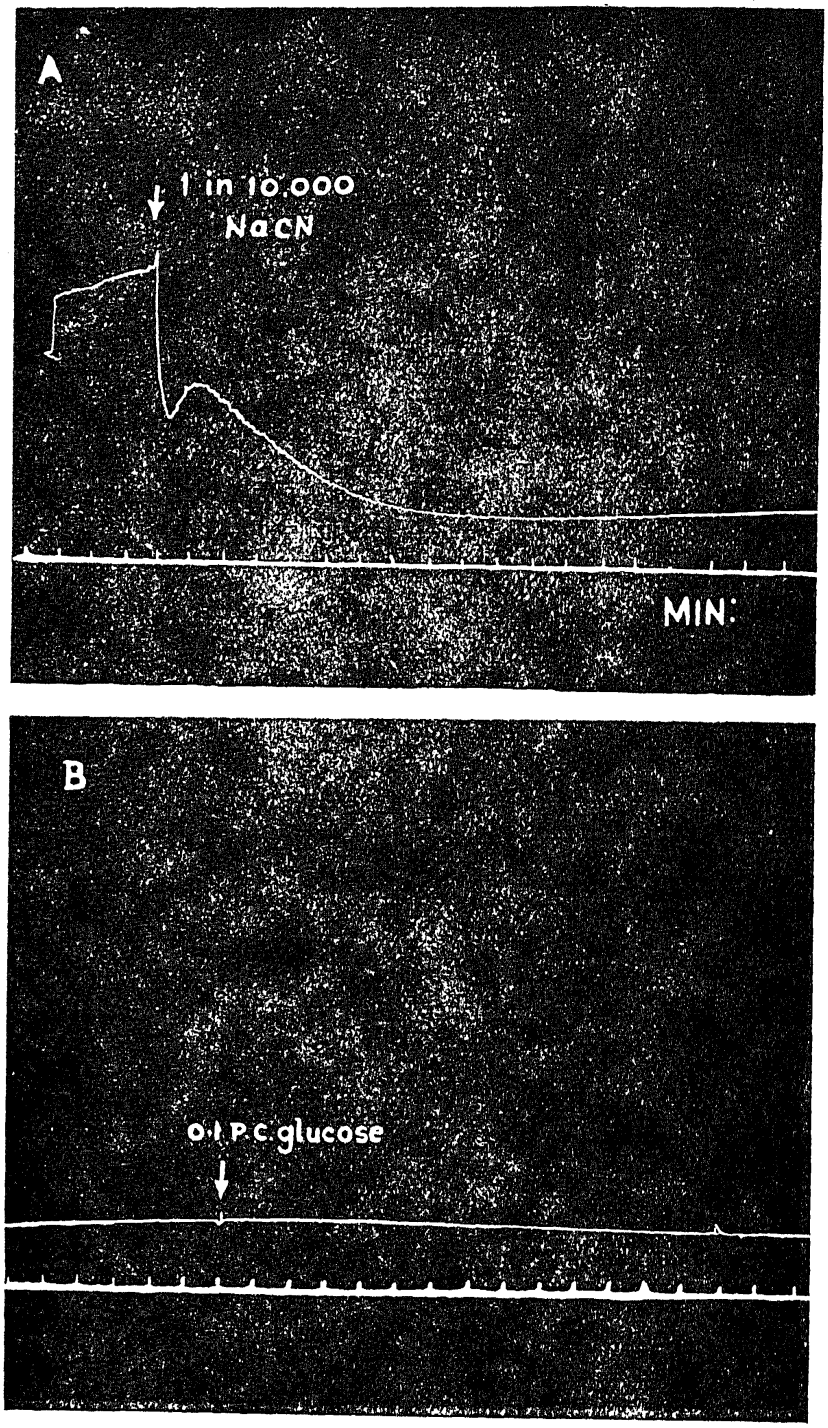

FIG. 13. *Fowl's gut.*—Effect of cyanide on tone. *B* Continuation of *A*.

partial recovery appears to be the same as the asphyxial increase in excitability. Like asphyxia, the effect of cyanide is variable. Some muscles relax greatly, while others are hardly affected. After relaxation, the muscle begins to contract. This contraction, as with asphyxia, is variable. It may contract immediately on addition of cyanide (Fig. 9) or may show only a very slight contraction (Fig. 13). As with asphyxia, glucose has a dual effect on the muscle treated with cyanide. Shortly after addition of cyanide, the

effect is stimulatory. After about 10 minutes, this effect is lost and the action may become inhibitory. Glucose then changes the alactic to lactic tone, as evidenced by the increase of tone and revival of excitability.

Small concentrations of cyanide (1 in 10^5) make the muscle more sensitive to oxygen lack, as shown by the asphyxial increase in excitability.

In the fowl's gut, 1 in 10,000 sodium cyanide causes relaxation and reduction of spontaneous contraction. These effects are further enhanced if oxygen is withheld. This shows that oxygen is also made available to the cells, by a system other than the cytochrome one (Fig. 15), or they contain a cyanide resistant cytochrome.

Inhibition

Gross and Clark (1923) observed that the asphyxiated gut did not respond to adrenaline. Prasad (1936) found that adrenaline had a powerful inhibitory effect on the activity of the gut muscle asphyxiated in presence of glucose or poisoned with iodoacetic acid. Further, Singh (1942) has shown that there are two kinds of inhibition, one produced by electric current and the other by drugs and ions; so far, the effect of asphyxia on the former inhibition has not been investigated.

Effect of asphyxia on electrical inhibition.—Electric current produces three kinds of inhibition, the first one during the flow of the current, the second on cessation of the current, and the third after the lapse of a certain interval after cessation (Singh, 1942). Any of these inhibitions can be studied. The third inhibition is due to a subnormal phase following contraction, and corresponds to indirect inhibition in the central nervous system; the first one corresponds to direct inhibition. In the guinea pig's uterus in alkaline solutions, stimulation with direct current produces beautiful inhibitory twitches; these can be produced by alternating current in the dog's stomach if the animal is killed with an overdose of ether and chloroform. In the fowl's gut, the third kind of inhibition is ordinarily produced. In the guinea pig's uterus, frequent stimulation may cause a gradual decrease of tonic contraction, just as frequent stimulation producing contraction may cause a rise in tonus.

When oxygen is excluded, the inhibitory twitches decline just as the contraction twitches and are similarly restored by glucose (Fig. 14). Glucose is ineffective in the presence of iodoacetic acid. When the muscle fatigues to electric current, introduction of adrenaline produces inhibition. Tonic contraction can also release energy not only for the twitch contraction (Singh and Singh, 1948), but also for the twitch inhibition, as shown by the

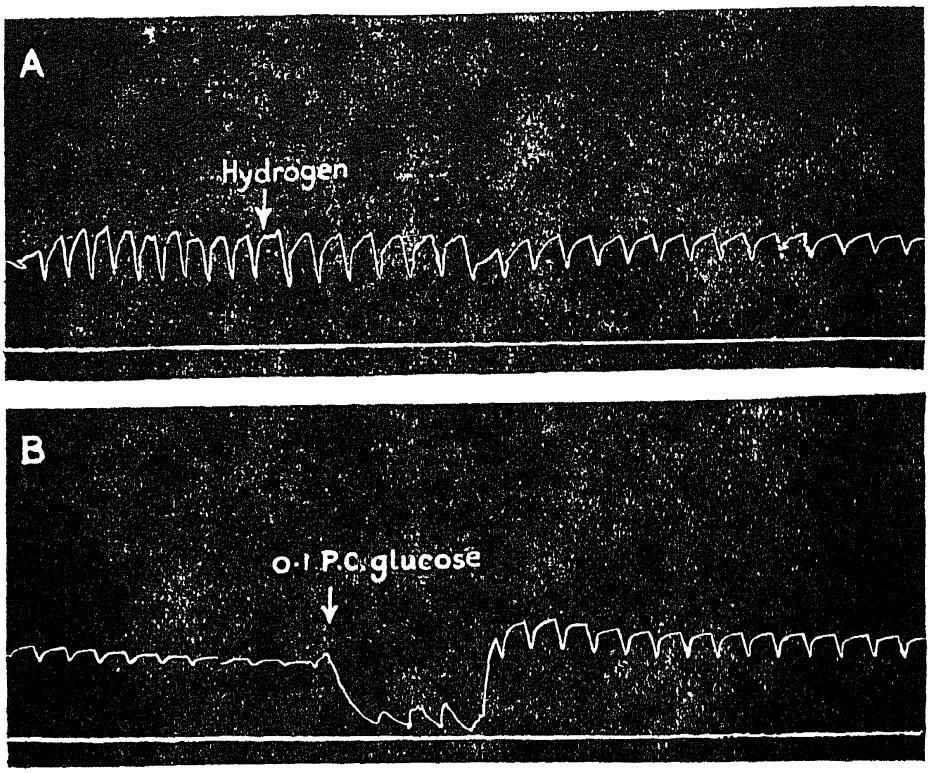

FIG. 14. *Guinea Pig's uterus.*—Inhibitory twitches by direct current 14 volts for 10 seconds per minute. *B.* Continuation of *A.*

fact that mild increase in tonus increases the inhibition (Singh, 1942). In the frog's stomach, the inhibition produced by adrenaline and ammonium decreases in the absence of oxygen and is restored by glucose. After asphyxia, on the reintroduction of oxygen, the inhibitory twitches, just as the contraction twitches, are temporarily suppressed (Fig. 15). The above results on inhibition resemble exactly those described for contraction. They are in agreement with the oxygen consumption experiments. These results suggest that the ærobic metabolic mechanisms for tone and inhibition are antagonistic.

When the muscle passes into alactic tone, then inhibition is not produced. Inhibition is produced however if the muscle is in the lactic tone; hence the beneficial effect of glucose. Thus the contradictory findings of various observers are reconciled. In the experiments of those observers in which the asphyxiated muscle did not respond, it must have passed into the second stage; in the experiments of other observers, in which the muscle responded, it must have been in the first stage.

Adaptation.—Adaptation is identical with inhibition (Singh, 1945), therefore it is affected identically as inhibition by asphyxia. It was found that cyanide was very powerful in diminishing adaptation (Singh, 1938). Asphyxia produces identical results. When the muscle is continuously

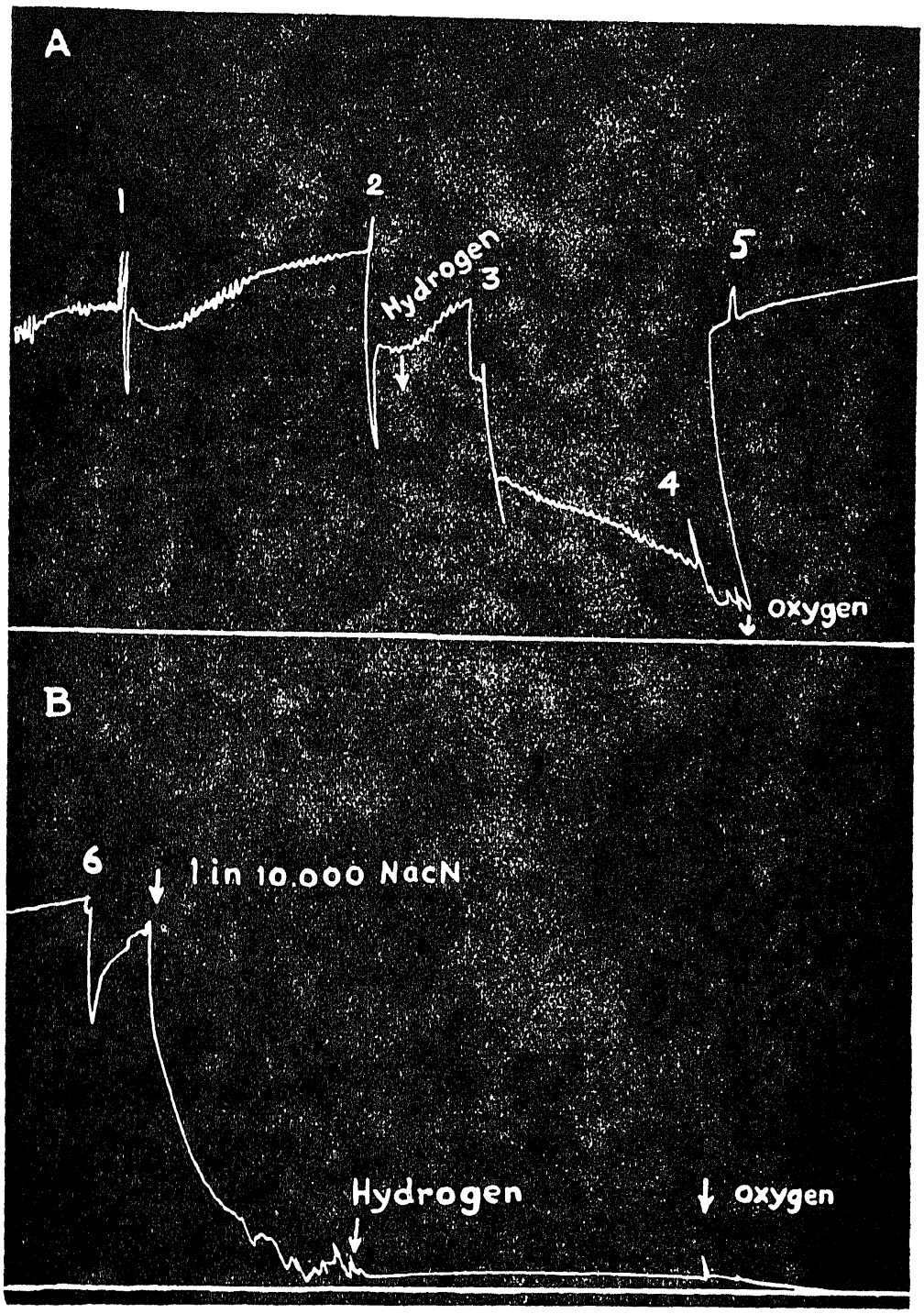

FIG. 15. *Fowl's gut.*—Inhibition following contraction by alternating current at 1, 2, 3, 4, 5, 6; note at 5 it is absent. Also effect of sodium cyanide and hydrogen. *B.* Continuation of *A.* When oxygen introduced drum stationary for 5 minutes in *A.*

stimulated with alternating current, potassium or acetylcholine, it fails to relax if in alactic tone (Fig. 16); as energy is required for the maintenance of tension, the opposite happens if in lactic tone. This is the first instance in which I have found that adaptation and fatigue are affected oppositely in unstriated muscle, that is, in the absence of oxygen.

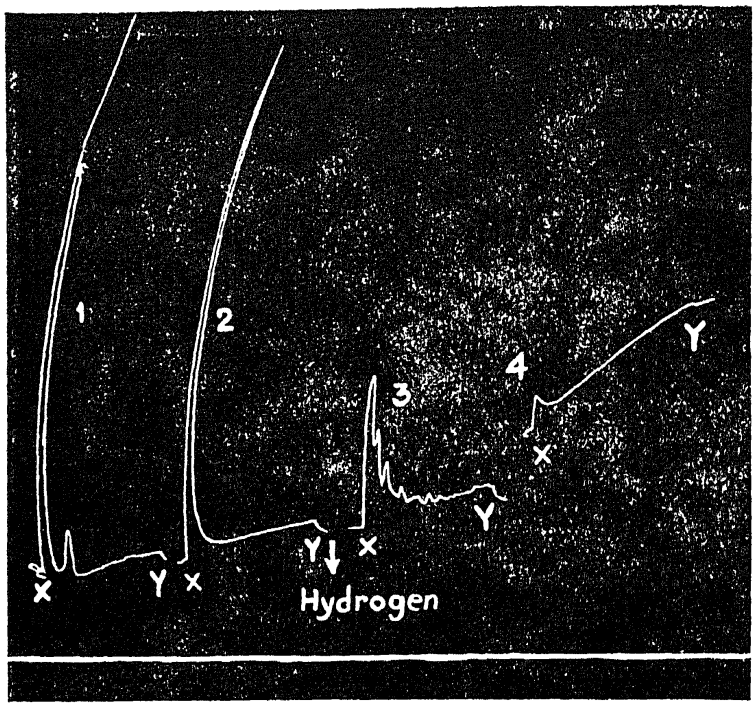

FIG. 16. *Dog's stomach.*—Continuous stimulation with alternating current from *X* to *Y* for 3 minutes. 1 and 2 normal contractions; 3 after 10 minutes of asphyxia; 4 after 20 minutes of asphyxia. Drum stationary in between contractions.

DISCUSSION

These experiments show that one of the functions of the glycogen lactic acid system is to keep the muscle relaxed. Then how does the same system cause contraction of myosin. Either there are two different kinds of contractile proteins, one of which is relaxed and the other contracted, or in the same protein, there are chemical groups which are acted upon differently by lactic acid. The other possibility is that the entire function of the energy liberation in muscle is to keep the muscle relaxed like imparting potential energy to a stretched spring. This process will have to be increased when the muscle tends to contract, hence the increased liberation of energy would not be due to the contraction *per se*, but due to increased tendency to relaxation while the muscle is contracting.

It would therefore appear that the normal state of the muscle whether in relaxation or contraction is a dynamic equilibrium between two opposing forces. This might perhaps account for the presence of a dual nerve supply to unstriated muscle. In cardiac muscle, it is known that both the sympathetic as well as the vagus are in a state of tonic activity simultaneously.

SUMMARY AND CONCLUSIONS

1. Unstriated muscle shows two kinds of tone, ærobic and anærobic.

2. The anærobic tone is further divided into two classes, one which requires energy and the other that does not; these have been termed lactic and alactic tones respectively.

3. The ærobic and anærobic mechanisms for tone and twitch are not identical.

4. Glucose has a stimulatory action on the lactic tones and inhibitory on the alactic tone.

5. Glucose converts the alactic into lactic tone.

6. Normally there is a state of tonic inhibition.

7. The asphyxial contraction is independent of the stimulant.

8. Tone similar to that produced by asphyxia, is also produced by other stimulants, so it appears that the asphyxial contraction is the mechanism by which the muscle can keep up tension without expenditure of energy normally.

9. Sodium cyanide produces results similar to those of asphyxia.

10. Sodium cyanide does not entirely abolish activity, which is further decreased by oxygen lack.

11. Inhibition is arrested by asphyxia; glucose causes restoration. On reintroduction of oxygen there is paralysis of inhibition.

12. Adaptation is diminished by asphyxia.

References

Gokhale, S. K., and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1945, **21**, 202.
Gross, L. and Clark, A. J. .. *J. Physiol.*, 1923, **57**, 457.
Langley, J. N. .. *Ibid.*, 1913, **47**, 159.
Evans, C. Lovatt .. *Physiol. Rev.*, 1926, **6**, 358.
McDowall, R. J. S. .. *Hand-book of Physiology and Biochemistry*, London, 1946, 45.
Prasad, B. N. .. *J. Physiol.*, 1936, **86**, 425.
Rao, M. S., and Singh, I. .. *Ibid.*, 1940, **98**, 12.
Singh, I. .. *Ibid.*, 1938, **92**, 241.
——— .. *Ibid.*, 1939, **96**, 367.
——— .. *Ibid.*, 1940, **98**, 155.
——— .. *Ind. Journal Med. Rev.*, 1942, **30**, 629.
——— .. *Proc. Ind. Acad. Sci.*, 1944, **20**, 195.
——— .. *Ibid.*, 1945, **22**, 123.
——— .. *Ibid.*, 1946, **23**, 58.
——— and Singh, S. I. .. *Ibid.*, 1947 *a*, **23**, 312.
——— .. *Curr. Sci.*, 1947 *b*, **16**, 259.
——— .. *Proc. Ind. Acad. Sci.*, 1948, **27**, 127.
———, and Singh, S. I., Muthana, M. C. .. *Ibid.*, 1947, **25**, 51.
Watt, Dumke and Comroe .. *Amer. J. Phy.*, 1943, **138**, 610. Quoted from Best, C. H. and Taylor, N. B. *Physiological Basis of Medical Practice*," London, 1945, 348.