

EFFECT OF TRITURATED DILUTIONS OF ETHANOL ON THE PERISTALTIC CONTRACTIONS OF ISOLATED RABBIT INTESTINE

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ABSTRACT

Effect of triturated dilutions of ethanol from 10^{-3} to 10^{-24} were studied on the penstaltic contractions of isolated rabbit intestinal pieces. Triturated dilutions of ethanol were applied in two doses. It was generally observed that the addition of first dose was followed by a small increase of 1.1 gms. in the resting tension which occurred at a slower rate in about 36.5 sec. The second doses however, increased the resting tension by 2.4 gms, in about 21 Sec. In addition, the active tensions showed large variations on the application of first doses of triturated ethanol, while the second doses always decreased this parameter below the first dose levels. The rate of contractions was however, unaltered by both the first and second doses of ethanol, although an increase the contraction times was found to be balanced by a simultaneous decrease either in the peak duration or relaxation times. The results are discussed in terms of electrophysiological, active state and free ionic Ca^{++} release phenomena.

Introduction

Physiologists and homoeopathic physicians generally divide drug dilutions in two main categories. Dilutions less than 10^{-24} are called microdilutions. For many decades, microdilutions as high as $10^{-1000000}$ have been found often to have a specific ability to relieve symptoms of illness. In spite of this clinical evidence, there have been few investigations of high dilutions using standard laboratory techniques. This lack of experimental investigation probably is because the action of substances in dilutions beyond 10^{-24} violates two firmly held principles of physical chemistry, Avogadro's law and the doctrine of the non-specificity of some atomic particles. According to Avogadro, the molecular weight of any substance expressed in grams contains 6.2×10^{23} molecules. Theoretically therefore, any substance diluted beyond 10^{-24} molecules, will contain molecules of the original material. Similarly, the specific action of dilutions greater than 10^{-24} can not be attributed to electrons which become separated from the diluted arterial and remain in the solution since the electrons from one atom do not differ from those of others. Therefore, all high dilutions of electrons would have a similar or non specific action. The apparently specific action of substances at dilutions greater than 10^{-24} has not as yet been explained, although many theories have been advanced to account for it (Stephenson, 1973). The finding of various investigators into the action of diluted solutions has been arranged into five main groups, each group being determined by the

methods of experimental investigation used, i.e. bacteriological (Bunker, 1928; Paterson and Boyd, 1941), biochemical (Persson, 1930, 1938) botanical (Roy, 1932; Stephenson, 1973), physiological (Boyd, 1968) and zoological (Krawkow, 1923; Konig, 1927; Vondracek, 1929).

Junker (1928) seems to be the one who has ever used an alcohol for microdilution study. He added various substances like atropine sulphate, a sodium salt, potassium olcate, octyl alcohol, a vitamin and orange juice in dilutions upto 10^{-27} to cultures of paramecium. Many sinusoidal curves were obtained, with rhythmic alterations of maximum and minimum effects. He obtained significant changes in the growth of paramecia in cultures having orange juice 10^{-24} , a sodium salt 10^{-26} , octyl alcohol 10^{-24} and 10^{-25} , atropine sulphate 10^{-26} , potassium olcate 10^{-26} and nonylic acid 10^{-24} and 10^{-27} . Later, Paterson and Boyd (1947) demonstrated the alteration of the Schick test from positive to negative following the administration by mouth of alum precipitated toxoid 10^{-26} or Diphtherinum 10^{-402} . The results showed that out of 33 children with positive Schick reaction, 20 or 61% became Schick negative after they received dilutions of alum precipitated toxoid 10^{-60} or diphtherinum 10^{-402} . These results were found to be significant.

The smooth muscles have long been used as a tool for the study of pharmacological effects of the drugs. Generally, the drugs are employed in dilutions not exceeding 10^{-6} . However, in homoeopathic system of medicine, the drugs are generally prepared in dilutions greater than 10^{-4} , i.e. beyond Avogadro's number. The homoeopathic system of medicine is very widely used throughout the world and is well known for its effective treatment of various diseases. This system of medicine uses very high dilutions of ethanol-extracted triturated drugs and is based upon the assumption that increasing degrees of triturated dilutions increases the potency of drugs, with the effect of the diluted drugs being just opposite to those of the parent drugs.

Boyd (1968), was the first physiologist who, being basically a physician approached the problem of diluted drugs more scientifically with clear cut experimental evidences. He prepared dilutions of various drugs for upto 10^{-19} and observed that these drugs produced opposite effects when used in such high dilutions. The studies of Boyd (1968) seem to be the only one of their kind in the recent years which have provided experimental evidence in support of the functions of homoeopathic drugs. Since almost all the homoeopathic drugs are prepared in alcohol, it was our desire to investigate in the first instance, the effect of triturated dilutions of ethanol itself. The present work was therefore, carried out to investigate whether the triturated dilutions of ethanol for upto 10^{-96} could reverse already reported effects of ethanol on the contraction parameters of smooth muscles (Nasreen *et al.*, 1983a).

Material and Methods

1. Animals and Solutions:

Male and female rabbits of oryctologus cuniculus species, weighing from 0.7 to 1.7 Kg were used through out the experiments reported here. These animals were obtained either from our own breeding house or from the local Karachi market. For recording the contractile activity, a slightly modified Kreb's-Hensliet solution

(Winegrade and Shanes, 1962) was used which had the following composition: NaCl, 118mM; KCl 4.8mM; CaCl₂, 1mM; MgSO₄, 1.2mM; KH₂PO₄, 0.8mM; NaHCO₃, 25mM; Glucose, 5.55mM and pH 7.4. The chemicals used were obtained either from Merck, Germany or from AnalaR and were of analytical grade. In general, this solution was made in a 10 times concentrated stock which had all the ingredients with the exception of NaHCO₃ and glucose. Whenever needed, 100ml of this stock solution were diluted to 1 liter and NaHCO₃ and glucose were added in amounts given above. The general experimental setup and the technique used for recording intestinal contraction parameters were the same as described earlier (Nasreen *et al.* 1983a).

2. Preparation of Triturated Dilutions of Ethanol:

For trituration purposes, commercial ethanol was first distilled in a pyrex glass distiller and then in a rotary vacuum evaporator, obtained from, Rikakikai Co. Ltd. Tokyo, Japan. The distilled ethanol was 98-99% concentrated. To begin with the preparation of triturated dilutions, 15ml of distilled ethanol were taken in a 250ml stoppered volumetric flask containing 135ml deionized distilled water. This constituted the first and initial dilution of 10⁻⁴. This first dilution was then triturated (shaken) for 2 hours in a mechanical shaker obtained from Presicion Scientific Co., USA. After the completion of 2 hours shaking, 15ml of this triturated dilution were taken in another flask and 135ml of deionized distilled water were added. This constituted the dilution of 10⁻¹². This dilution was again triturated for 2 hours and 15ml of the dilution were again used to prepare the next higher dilution. This procedure was continued for preparing dilutions of upto 10⁻²⁴. All the triturated dilutions were stored in air tight bottles to avoid evaporation.

Results

For the study of the effects of various triturated dilutions of ethanol, two doses (each of 1 ml) were applied to the contracting intestine. Care was taken to apply the second dose only after the observed maximum effect of the first dose. The values of the first dose are represented as % of the initial (normal), while the values of the second dose are given as % of the first dose.

1. Effect on the Rate of Contractions:

Changes in the rate of contraction were studied for 22 triturated dilutions of ethanol. The details of these results are shown in Fig 1A&B. The first dose of ethanol did not produce any marked effect on the rate of contractions for upto triturations 10⁻²⁰. Thereafter, the first dose markedly decreased the contraction rate in triturations 10⁻²¹ and 10⁻²² (Fig. 1A). An increase in the rate of contractions was also observed in trituration 10⁻²⁴. The second dose however, decreased the contraction rate markedly in triturations 10⁻⁹, 10⁻¹¹, and 10⁻¹⁵ (Fig 1B). Further, the increase in the rate of contractions produced by any of the two doses of ethanol, did not exceed to a maximum of 16%. Similarly, the maximum decrease in the rate of contractions did not exceed a maximum value of 20% in

the two doses. No definite sequential relationship could be observed between various triturations of ethanol and the rate of contractions.

2. Effect on the Resting Tensions

The results of increase in resting tensions alter the addition of first doses of ethanol are shown in Fig.2. The results showed small variations in resting tensions in all the triturated ethanol from 10^{-3} to 10^{-15} without there being any significant change. It however, increased markedly after the addition of trituration 10^{-16} , and it attained a peak value of about 4 gms. in 10^{-17} trituration. A similar increase in resting tension was also observed in trituration of 10^{-21} which continuously increased to a peak value in 10^{-24} trituration.

The effect of second dose of ethanol was calculated by subtracting the effect of first dose from the total effect. These results are also given in Fig. 2. The results showed very large variations in the resting tensions. The increasing resting tension developed prominent peaks at trituration 10^{-5} , 10^{-8} , 10^{-13} , 10^{-17} , 10^{-21} and 10^{-22} . The graph in Fig. 2 also demonstrated that the second doses of ethanol produced very prominent increase in resting tensions in almost all the triturations. It was further noted that the greatest effect was produced by ethanol triturations of 10^{-21} and 10^{-22} showing a 4 times increase in resting tension. There was however, no direct relation-ship between the increase in resting tensions and ethanol triturations.

3. Effect on the Time of Maximum Change in Resting Tensions:

The time required for the increase in resting tensions to peak values was also measured. These measurements are plotted in Fig. 3. The results showed very large variations in this parameter after the addition of first and second doses of triturated ethanol. It was a general observation that the resting tensions increased to peak values in longer time periods after the addition of first doses of triturated ethanol. After second doses however, the resting tensions increased in smaller time periods. These results demonstrated that the addition of first doses of triturated ethanol was followed by a lesser increase in the resting tensions which also occurred at a slower rate. On the other hand, the second doses increased the resting tensions to a greater extent and at faster rate with the exception of triturations 10^{-1} and 10^{-20} where the resting tensions increased at a very slow rate.

4. *Effect on the Active Tensions:*

The active tension results showed that the first doses of most of the triturated ethanol produced large variations in the active tensions which changed without any sequence (Fig. 4). A maximum increase of about 83% of the initial occurred in trituration of 10^{-24} . Similarly, a maximum decrease occurred in triturations 10^{-23} and 10^{-24} which equaled about 74% of the initial. In addition, the second doses were always found to decrease the active tensions even below the level of the first doses (Fig. 5), the decreases generally being 65%-85% of the first dose. There was no definite relationship between ethanol triturations and the decrease in active tensions.

5. *Effect on the Contraction Times:*

Measurements of intestinal contraction times showed that after the application of first doses of ethanol, it increased in most of the triturations, the increases ranging in between 10% and 38% of the initial (Fig. 6A). The contraction times were also found to be decreased by 11% and 19% in triturations 10^{-10} and 10^{-20} respectively. When second doses of ethanol were applied, the contraction times decreased in most of the triturations. These decreases ranged in between 10% and 22% of the first dose, with the maximum decrease occurring in trituration 10^{-4} (Fig. 6B). In addition, trituration 10^{-23} was also found to increase the contraction times by about 22% of the first dose. These results clearly showed that the first doses of triturated ethanol increased the contraction times while the second doses decreased this parameter in most of the cases.

6. *Effect on the Relaxation Times:*

The relaxation times were also measured from the same contraction cycle records which were previously used for the measurement of contraction times. These results are shown graphically in Fig. 7A&B. The graph showed that the first doses of most of the triturated ethanol decreased relaxation times, the decreases generally ranging from 2% to 27% of the initial. The most prominent decreases were observed in triturations 10^{-4} , 10^{-11} , 10^{-19} and 10^{-23} (Fig. 7A). In addition, an increase in relaxation times was also observed in triturations of 10^{-4} , 10^{-10} and 10^{-19} which amounted to a maximum of 38% of the initial. When second doses of ethanol were applied, these markedly increased the relaxation times in triturations 10^{-4} , 10^{-8} and 10^{-19} , with the maximum increase occurring in 10^{-19} , i.e. 53% above the level of first doses (Fig. 7B). The second doses of various triturated ethanol were also found to decrease the relaxation times and the most marked effect was observed in 10^{-5} where the decrease was about 22% of first dose. These results have demonstrated that the first doses of ethanol decreased the relaxation times in most of the cases while the second doses increased them in only four triturations.

7. *Effect on the Peak Durations:*

The effect of various triturated ethanol on the peak durations of contraction cycle of rabbit intestine are graphically represented in Fig. 8A&B. The results showed that after the application of first doses, the peak durations were highly prolonged in triturations 10^{-3} and 10^{-23} , the increases being 1.4 to 2 times the initial values (Fig. 8A). In rest of the triturations, the change in peak durations was small, the maximum increases and decreases being 40% and 50% of the initial respectively. The addition of second dose further increased the peak duration by 70% of the first dose in 10^{-9} trituration (Fig. 8B). In addition, the second doses also increased peak durations markedly in triturations 10^{-3} and 10^{-15} , the increases being about 300% of the first dose in the later. In the remaining ethanol trituration, there was no significant change in the peak durations. These results demonstrated that the peak durations were always increased by the first and second doses of triturated ethanol and that there was never a significant decrease in this parameter.

8. *Effect on Duration of Contraction Cycles:*

For the study of the effects of various triturated ethanol on the duration of contraction cycles, all the results described in paragraphs 5 to 7 were considered. It was noted that first doses of ethanol produced marked changes in the duration of contraction cycles only in trituration 10^{-5} and 10^{-11} , both representing a 15% increase and decrease respectively (Fig.9A). A 13% increase was also observed in trituration 10^{-22} , while the remaining triturations showed no effect on this parameter. Addition of second doses, produced a prominent increase in the duration of contraction cycles in trituration 10^{-5} only where the increase was about 17% of first dose (Fig. 9B). A 13% decrease was observed in trituration 10^{-5} . Thus, no sequential relationship could be observed between various triturated ethanol and changes in the duration of contraction cycles.

9. *Effect on the Duration Between Individual Contractions:*

In all the experiments using triturated ethanol, two successive contraction cycles were recorded at fast moving paper and the duration between these individual contractions was measured. These results are illustrated in Fig. 10 A&B. The results showed that after the addition of first doses of ethanol, duration between individual contractions decreased markedly in all the cases except in 10^{-33} trituration where it increased by 2.5 times the initial value (Fig. 10A). On the other hand, addition of second doses of ethanol increased this parameter very prominently in triturations 10^{-4} , 10^{-9} , 10^{-10} and 10^{-15} (Fig. 10B). In rest of the triturations, no change was observed. It was therefore, dear that first doses of triturated ethanol always decreased the duration between individual contractions while the second doses increased it in many of the triturations.

Discussion

Trituration is a process in which a solution is put to continuous mechanical shaking for a given time period. Our results showed that the most marked effect of various triturated ethanol was on the resting tension which increased in all the trituration after the addition of both first and second doses. However, the main difference between the two doses was in the time required to produce maximum change in resting tension, which was comparatively longer for the first doses and much smaller in the second, for almost all the ethanol triturations. The general observation was that the addition of first doses of triturated ethanol produced a smaller increase in resting tension and this occurred at a slower rate. The results in Fig. 2 showed that, with the exception of one or two occasional peaks, the resting tension was increased to more or less an average value of about 1.1 gms, and it occurred in an average time period of about 36.5 sec. These results seem to be slightly different from those described earlier for non-trituated ethanol (Nasreen *et al.*, 1983a). Further, this lesser tension was produced at such a slow rate that it took almost the double time as that required by absolute ethanol. It is very difficult to explain these results at this stage. However, we suggest that the resting tension development is directly related to the concentration of ethanol. On electrophysiological bases, it can be stated that lesser concentration of ethanol produced smaller membrane depolarizations at a slower rate. It is also probable that a lesser entry of ethanol into the smooth muscle cells may have resulted in a smaller release of free ionic Ca^{++} into the sarcoplasm causing a lesser tension development. This hypothesis is supported by the use of the second doses of ethanol which caused a tension development of about 2.4 gms, in about 26.9 sec. These results were very similar to those obtained with absolute ethanol (Nasreen *et al.*, 1983a). The larger effect of second doses is probably due to summation effect of both the doses. It is to be remembered that the second doses of ethanol were applied to intestinal pieces which already had ethanol of the first doses in their surrounding medium. Thus, on electrophysiological grounds, it can be stated that the membrane depolarizations caused by the second doses were superimposed on the membrane depolarization produced by the first ones. This resulted in larger depolarizations responsible for not only higher tension development but also for a faster rate. This phenomena however, does not explain the occurrence of occasional peaks of larger tension development, as observed for triturations 10^{-5} , 10^{-8} , 10^{-13} , 10^{-17} , 10^{-21} and 10^{-20} . It requires further investigation whether these occasional peaks are also dependent on larger membrane depolarizations or on some other phenomena directly involved with the contractile mechanisms.

Regarding the active tension development, the application of first doses of triturated ethanol was followed by very large fluctuations in this parameter. The active tensions increased or decreased in a random fashion and these were not found to be related with the degree of trituration. Similarly, the active tensions could not be related with the passive or resting tensions of the intestine, as was expected. In earlier studies, active tensions have been shown to be related to the number of spikes present in an action potential (Bulbring, 1955; Bulbring *et al.*, 1958). It may therefore, be suggested that 'low' concentrations of ethanol or the degree of trituration may have affected the discharge pattern of the pacemaker cells located in the Auerbach plexus in such a way that the number of spikes in action potentials varied from trituration to trituration.

Another possibility for these large variations in active tensions may be the release of some substances from the plastic and rubber tubings used for various connections, as has been suggested earlier by Boyd (1968). However, the second doses of triturated ethanol were always found to decrease the active tensions below the first dose levels. Since resting tensions were always found to be increased in all the second doses of triturated ethanol, our previous assumption supported the idea that the increase in resting tension was associated with a simultaneous decrease in active tension (Nasreen *et al.*, 1983a).

In the present study, the rate of intestinal motility was rarely found to be changed by different triturated dilutions. It is well known that the rate of contractions depends upon the number of contraction cycles occurring per unit time and also on the duration between individual contraction cycles (Nasreen *et al.*, 1985). Since each individual contraction cycle is by itself composed of three different phases, i.e. contraction phase, relaxation phase and the phase of peak duration, there is a possibility that variations may occur in any one or more of these phases in such a way that the total duration of contraction cycle is not altered and the rate of contraction remains constant. Our investigations showed very interesting results in this respect. It was generally noted that the first and second doses of many of the ethanol trituration caused either an increase or a decrease in the contraction times, without there being any sequential relationship between the contraction times and the degree of trituration. Whenever, the contraction times were increased, these were balanced by either a decrease in the relaxation times or a decrease in peak durations, so that the duration of contraction cycles remained the same. Similarly, a decrease in contraction times was followed by a simultaneous increase either in the relaxation times or peak durations. It was further observed that in cases where the above mentioned three factors could not balance each other, the duration between individual contractions was increased to compensate the difference.

Comparatively lesser work has been done on the mechanical contraction properties of visceral muscles. However, the whole understanding of contractile activity in visceral muscles is based upon the concept of active state as given by Hill (1950). The most physiological meaningful definition of active state in terms of contraction of smooth muscle fibres is to say that as long as the contractile elements are shortened from the resting conditions, then the muscle fibres are in the active state. Thus, contractions and relaxations are simply the measures of the transient prolongation or reduction of the active state. A shortening or prolongation of contraction phase, as observed in our experiments, would therefore, be explained on the basis of a shortening or a prolongation in the duration of active state. In addition, the duration of active state has also been shown to be directly related to the release of free ionic Ca^{++} in the sarcoplasm (Florey, 1966). In most of our experiments, contraction times were found to be increased in duration after the addition of first doses of various triturated ethanol. This increase could have been possible only if there was an increase in the duration of active state. We therefore, suggest that triturated ethanol may have produced membrane depolarization at a slower rate which resulted in a slow rise of free ionic Ca^{++} level in the sarcoplasm and this in turn produced a very slow development of the active state. This slow rate of active state development may have then increased the contraction times in our experiments. The addition of second doses of triturated ethanol must have followed by a faster rate of membrane depolarization, a faster rate of free ionic Ca^{++} release and a faster rate of active state development, all of which resulted in a shortening of contraction time. Similarly, an

increase or decrease in the peak durations would mean a prolonged or shorter interaction between the contractile proteins, actin and myosin. In addition, peak durations would also depend upon the number of spikes present in the individual action potentials. It is however, not probable that in our experiments an increase of peak duration was due to ethanol- produced train of spikes in the action potentials because this would have increased the active tensions as well, which was not the case observed. We therefore, believe that in our experiments peak duration was increased due to an increase in the duration of active state. The relaxation times are generally dependent upon the rate at which the sarcoplasmic free ionic Ca^{++} is resequestered back to the intracellular binding sites. If this occurs at a faster rate, relaxation time would be smaller while a slower rate of Ca^{++} resequestration would increase the relaxation time. It is therefore, probable that triturated ethanol may have altered any one or more of the above described phenomena in such a way that the total duration of contraction cycle remained more or less the same and thus the rate of contraction.

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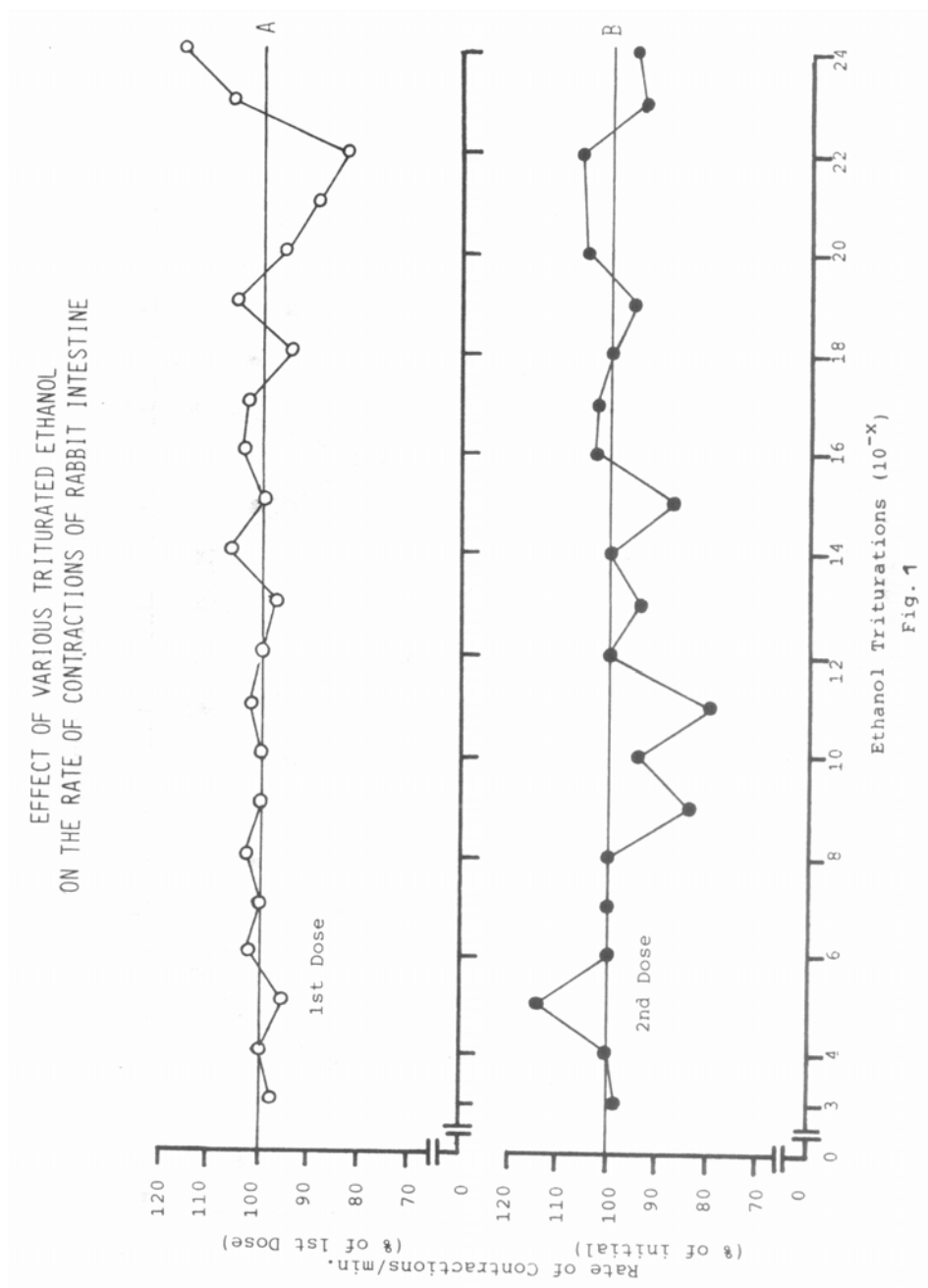
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EFFECT OF VARIOUS TRITURATED ETHANOL
ON THE RESTING TENSIONS OF RABBIT INTESTINE

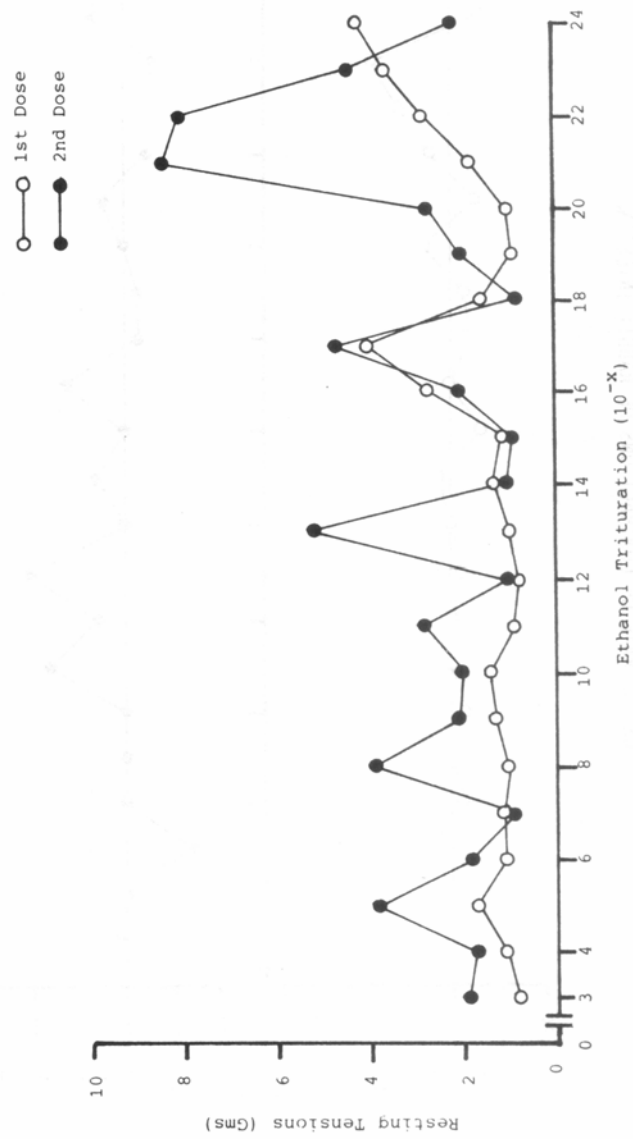


Fig.2

EFFECT OF VARIOUS TRITURATED ETHANOL ON THE TIME
OF MAXIMUM CHANGE IN RESTING TENSIONS OF RABBIT INTESTINE

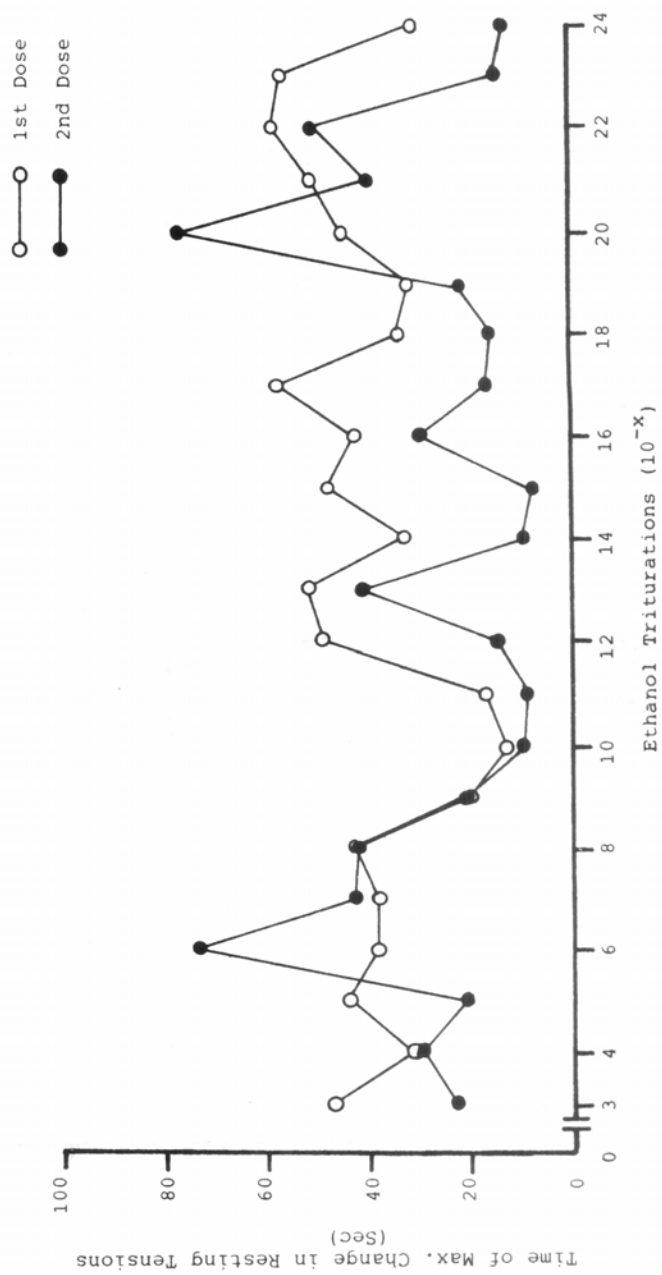


Fig. 3

EFFECT OF VARIOUS TRITURATED ETHANOL
ON THE ACTIVE TENSIONS OF RABBIT INTESTINE

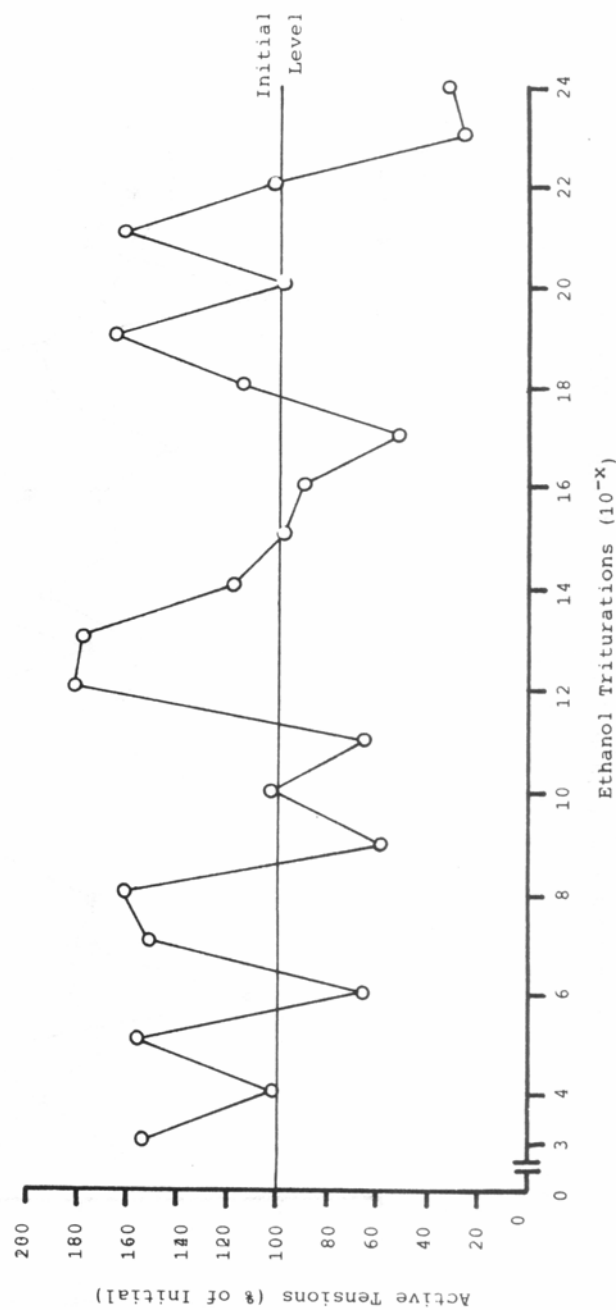


Fig.4

EFFECT OF VARIOUS TRITURATED ETHANOL ON
THE ACTIVE TENSIONS OF RABBIT INTESTINE

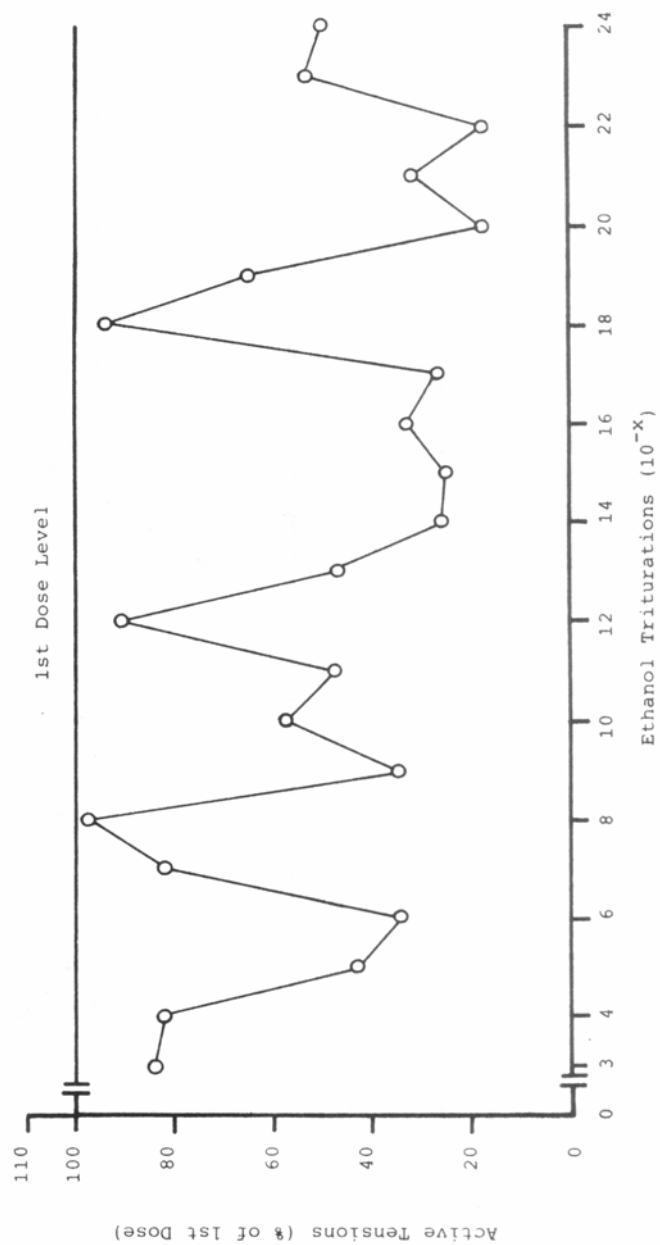


Fig. 5

EFFECT OF VARIOUS TRITURATED ETHANOL
ON THE CONTRACTION TIMES OF RABBIT INTESTINE

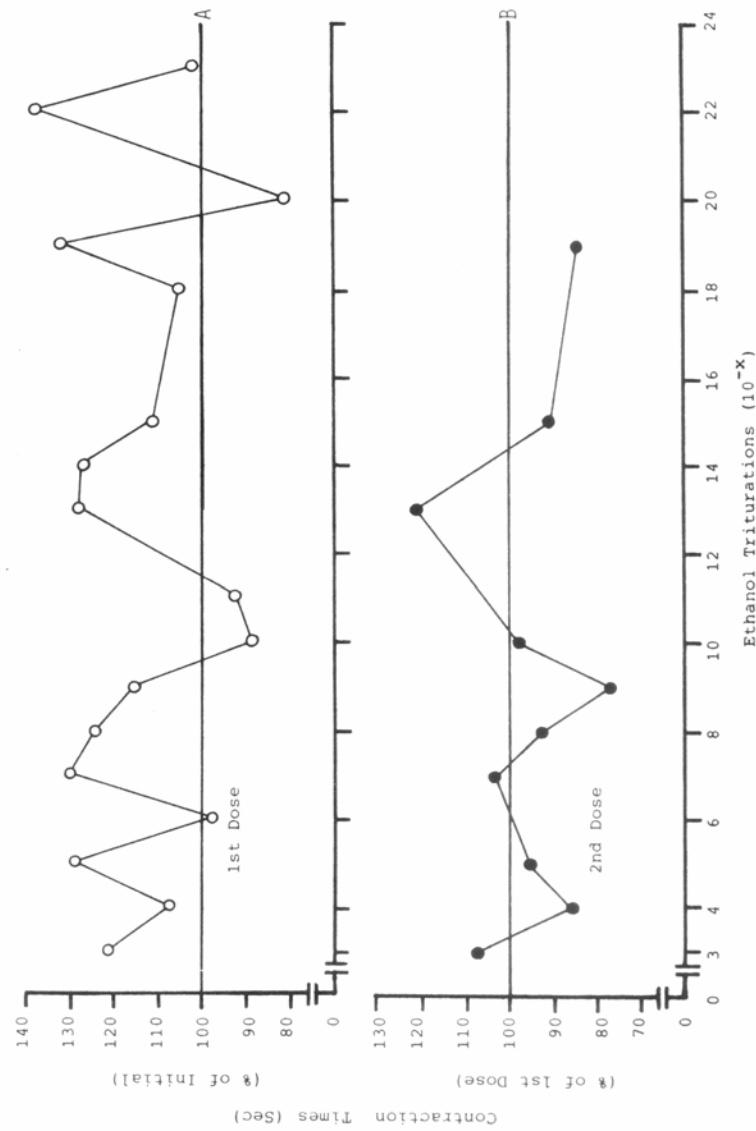


Fig.6

EFFECT OF VARIOUS TRITURATED ETHANOL
ON THE RELAXATION TIMES OF RABBIT INTESTINE

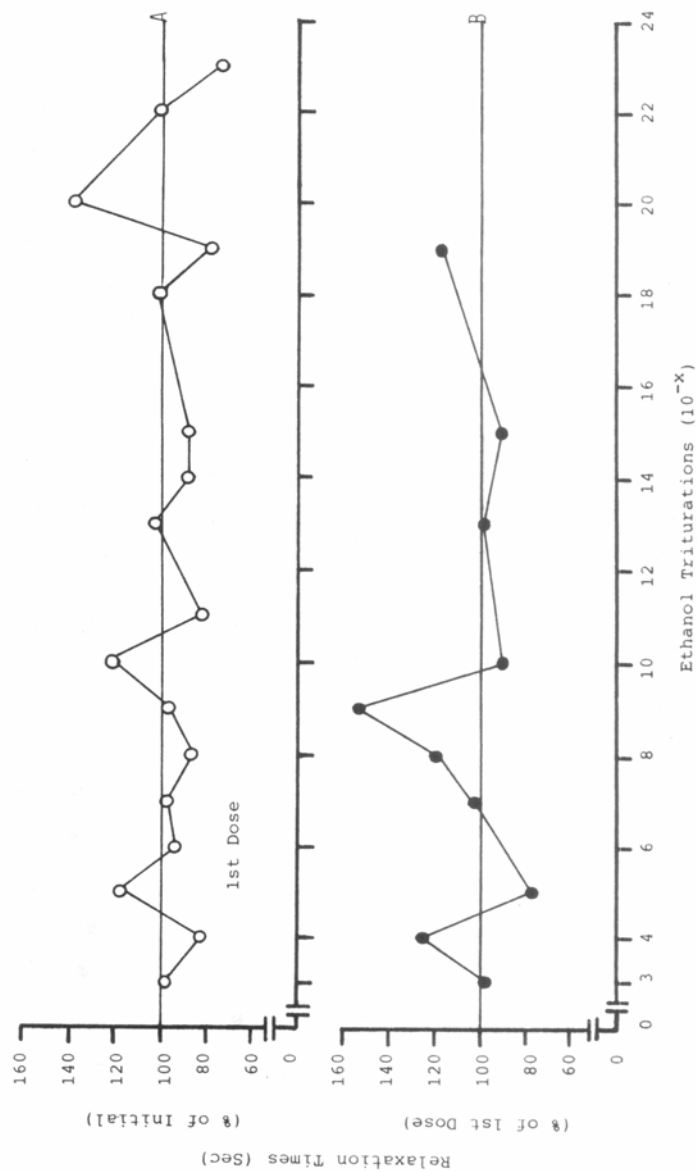


Fig.7

EFFECT OF VARIOUS TRITURATED ETHANOL ON THE
PEAK DURATION OF CONTRACTION CYCLES OF RABBIT INTESTINE

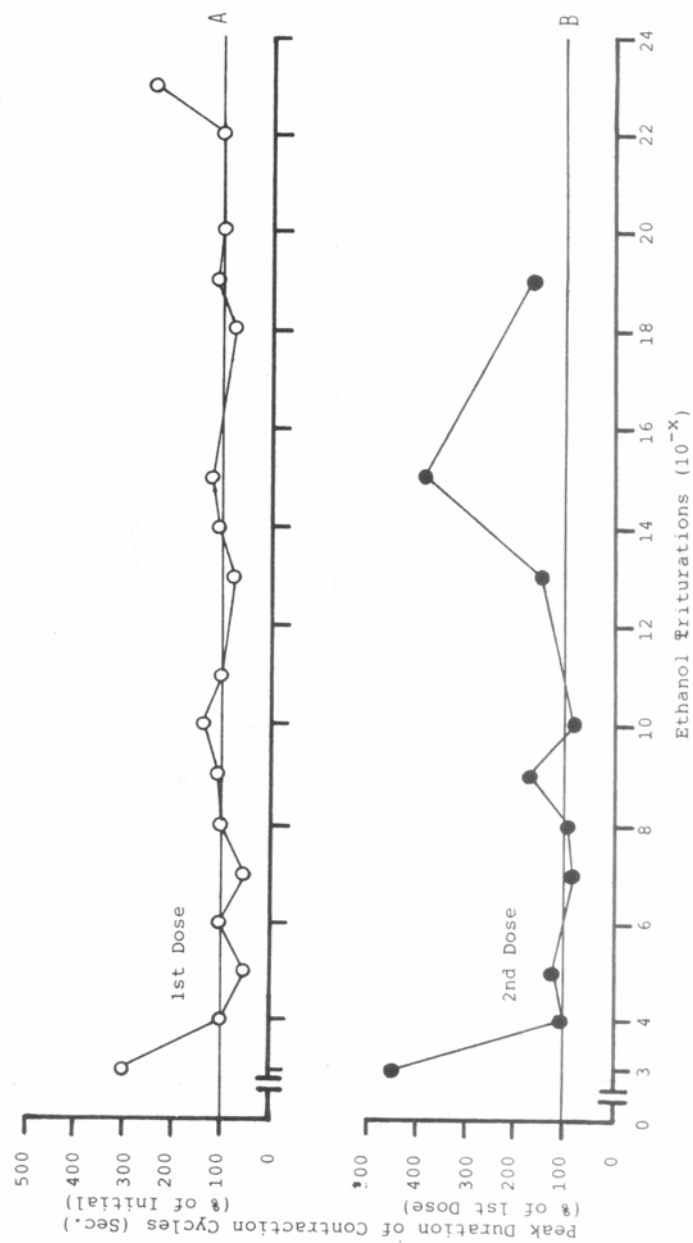
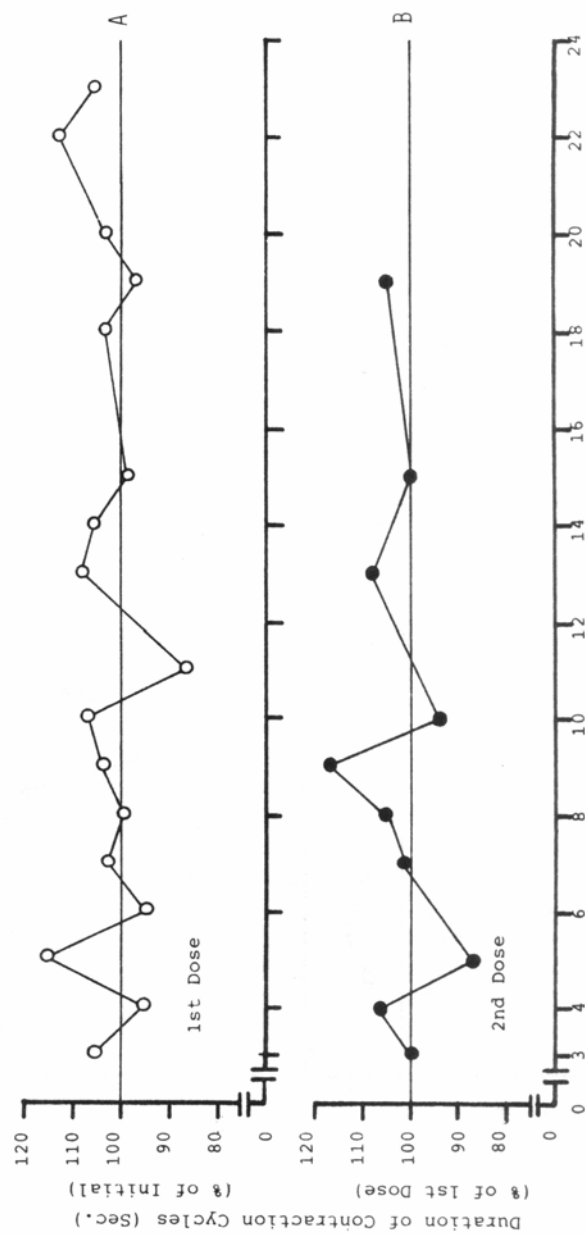


Fig. 8

EFFECT OF VARIOUS TRITURATED ETHANOL ON
THE DURATION OF CONTRACTION CYCLES OF RABBIT INTESTINE



Ethanol Triturations (10^{-x})

Fig. 9

