Brit. J. Pharmacol. (1949), **4**, 168.

SOME OBSERVATIONS UPON THE PHARMACOLOGICAL ACTIVITY OF DI-*ISO*PROPYL FLUOROPHOSPHONATE

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(Received February 4, 1949)

Interest in the di-alkyl fluorophosphonates arose mainly out of the work of Adrian, Feldberg, and Kilby (1946, 1947), who showed that the most active member of the group was di-*iso*propyl fluorophosphonate, or DFP as it is usually termed. The work of others confirming and enlarging these original observations has been reviewed (Quilliam, 1947). DFP is of considerable clinical importance; for example, recent studies have shown it to be a powerful weapon for combating post-operative paralytic ileus (Quilliam and Quilliam, 1947; Grob, Lilienthal, and Harvey, 1947). The object of this paper is to record details of some of the pharmacological actions of DFP preliminarily reported by Quilliam and Strong (1947a and b, 1949).

The Isolated Rabbit Heart

Method

The hearts of rabbits were perfused with oxygenated Ringer-Locke solution by the usual Martin-Langendorff method. The duration of complete cessation of the heart beat (followed by a resumption of cardiac activity) was adopted as a means of comparing the sensitivity of preparations to acetylcholine at the various phases of each experiment. Control injections of redistilled propylene glycol, the solvent used for the DFP, were made in order to exclude sensitivity changes arising from this source. Solutions of DFP in propylene glycol, containing 10, 1.0, 0.1, or 0.01 g. per 100 ml., retained their activity for at least two months provided care was taken to keep the container firmly stoppered and to exclude entry of water to the stock solution by using carefully dried pipettes and syringes to withdraw samples of DFP solutions. For most experiments, a 1 g./100 ml. solution of DFP was used and as small volumes as possible were injected—e.g., 0.025 ml.—with a micro-syringe.

Results

Action of DFP upon the heart

If more than 25 mg. of pure DFP were injected into the perfusion fluid, the heart beat ceased immediately in diastole and recovery was never seen. When 10 to 25 mg. pure DFP were administered, the substance still appeared to be very toxic and the heart was arrested at once. Occasionally, however, after an interval of five to ten minutes the heart started to beat again feebly, the amplitude being extremely small and the recovery very incomplete. Within the dosage range 2.5 to 10 mg. DFP, there was an immediate cessation of the heart beat which was usually followed by a somewhat greater degree of recovery. In the range 0.25 to 2.5 mg., DFP caused a partial and transient reduction of the amplitude of the beat from which the heart recovered fully or very nearly so in the space of two minutes (Figs. 2 and 3).

With quantities of DFP less than 0.25 mg., the transient reduction of the beat was less marked and complete recovery correspondingly accelerated (Fig. 1). Reduction of the heart rate after an injection of 0.25 mg. to 2.5 mg. DFP was conspicuous by its absence, having been seen only once in a large number of experiments, and the heart rate remained constant throughout the period during which the heart was exposed to this dosage range of DFP alone. The partial depression of the heart beat due to an injection of DFP was uninfluenced by prior treatment of the heart with sufficient atropine to render it insensitive to acetylcholine; moreover, such a heart remained unresponsive to acetylcholine after DFP, no reversal of the full effect of atropine being seen. If the heart was showing signs of returning sensitivity to acetylcholine, usually some time after treatment with atropine, then DFP tended to accelerate the return of full sensitivity to the action of acetylcholine. Control injections of 0.025 ml. of propylene glycol failed to alter the sensitivities to acetylcholine of a number of preparations.

When a simple siphon with an electronic flow recorder was used (Quilliam, 1949), no change in coronary flow was detected during any period of

action of DFP alone on the heart. In electrocardiographic studies, Knox, Quilliam, and Strong (1949) showed that the slight prolongation of the P–R interval, the transient broadening and splintering of the P or QRS complexes of small extent, and the slight changes in the amplitude of T, which occur after injections of DFP (0.25 to 0.5 mg.) into the isolated heart, could be attributed to the action of the propylene glycol used as a solvent and not to the DFP itself.

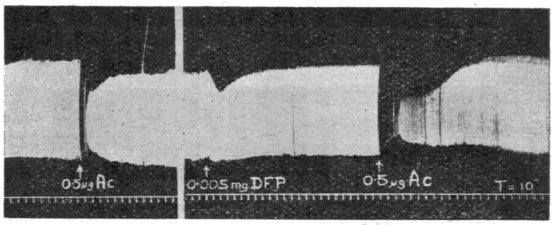

FIG. 1.—The action of 0.5 μg. of acetylcholine (Ac) upon the isolated perfused rabbit's heart before and after a very small dose of DFP (0.005 mg.) is illustrated and a marked potentiation of the effect of the second dose of acetylcholine is demonstrated. (All tracings are to be read from left to right.)

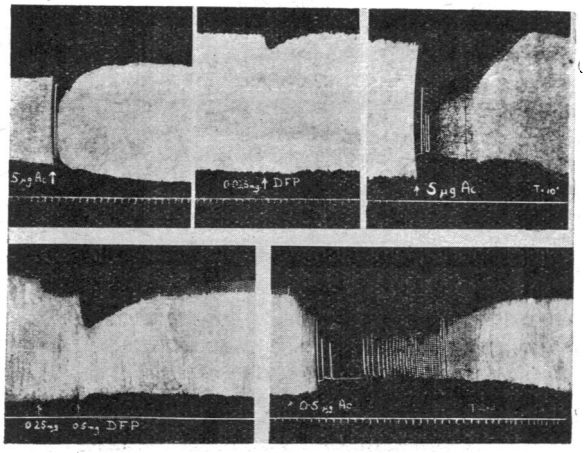

FIG. 2.—The action of 5 μg. of acetylcholine (Ac) is markedly potentiated by 0.025 mg. of DFP—i.e., partial sensitization of the heart. To sensitize the preparation fully, 0.25 mg. and then 0.5 mg. DFP were injected and afterwards the heart showed a great increase in sensitivity to acetylcholine (Ac): a dose of 0.5 μg. now gave a marked complete inhibition followed by a prolonged slowing. The sensitization was considerably greater than ten times. The slow onset of the acetylcholine effect after DFP seen in the above tracing sometimes occurred at the end of a long experiment.

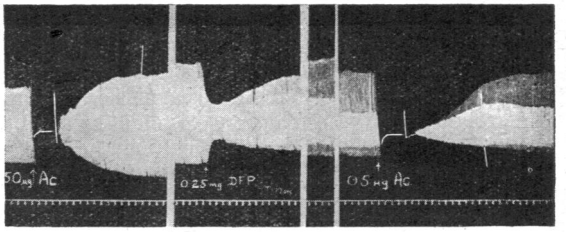

FIG. 3.—In this record, an isolated rabbit heart required 50 μg. acetylcholine (Ac) to produce a marked arrest of the beat. When the transient depression caused by the injection of 0.25 mg. DFP had passed off, the effect of 0.5 μg. acetylcholine will be seen to be somewhat greater than the action of the initial 50 μg. of acetylcholine before DFP. The sensitization to acetylcholine produced in the heart by DFP is thus a little greater than one-hundredfold.

Changes in the sensitivity of the heart to acetylcholine after DFP

With small quantities—e.g., 0.005 mg.—of DFP there was a clear potentiation of the action of acetylcholine in concentrations in which it had previously had an effect (Fig. 1). With a larger dose—e.g., 0.025 mg.—of DFP the potentiation of a given dose of acetylcholine was more marked. In order to demonstrate the full sensitizing power of DFP, a still larger dose had to be injected—e.g., 0.25 mg. + 0.5 mg. DFP—after which the sensitivity of the heart was increased between ten and one-hundredfold (Fig. 2). With a dose of 0.25 mg. DFP in a fresh preparation (Fig. 3), it was often possible to obtain more than a hundredfold sensitization of the heart to acetylcholine. The action of acetylcholine after DFP was prolonged compared with its evanescent action in the normal isolated heart. The heart usually recovered completely from the partial reduction in amplitude of the beat about two minutes after an injection of 0.25 mg. DFP, and at this point the sensitization of the preparation to acetylcholine appeared to be fully developed. This sensitization was so resistant to prolonged perfusion with normal Ringer-Locke fluid that it was considered to be permanent. While there were some individual variations, maximal sensitivity was usually developed after a single injection of 0.25 mg. to 0.5 mg. DFP.

The action of DFP on the heart after eserine or prostigmine

If an isolated rabbit heart was perfused with Ringer-Locke solution containing eserine sulphate (1/100,000), the usual sensitization to acetylcholine was observed. An injection of 0.25 mg. DFP now produced but a very slight increase in the sensitivity of the preparation. When normal Ringer-Locke

fluid was restored to the heart, the removal of the eserine resulted in a marked decrease in sensitivity to acetylcholine. If the heart was then subjected to a further 0.25 mg. DFP, there was a marked increase in response to the reference dose of acetylcholine (Fig. 4).

Similar results were obtained in a fresh rabbit heart perfused with Ringer-Locke solution containing prostigmine (1/100,000); 0.25 mg. DFP now produced little change in the sensitivity of the preparation, but when normal Ringer-Locke fluid was restored the sensitivity of the heart was markedly reduced and could be enhanced subsequently by 0.5 mg. DFP.

If the sensitization produced by eserine or prostigmine was not maximal, a dose of DFP caused additional sensitization which could not be removed by perfusion with normal Ringer-Locke solution.

The action of eserine and prostigmine on the heart after DFP

If a heart was fully sensitized to acetylcholine with DFP and then perfused with eserine sulphate

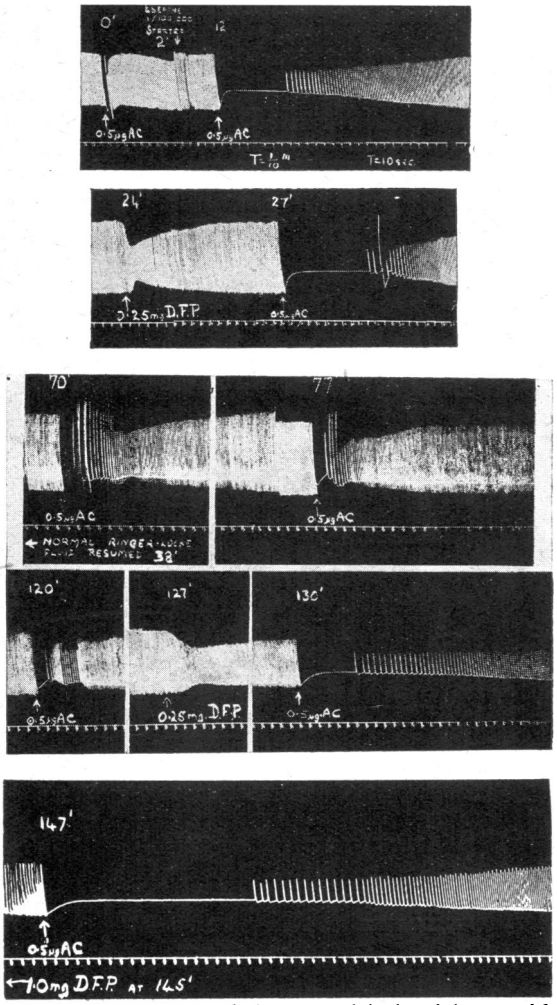

FIG. 4.—Treatment of the normal isolated heart with eserine led to the usual sensitization to acetylcholine (Ac) at 12 min. An injection of 0.25 mg. DFP at 24 min. but little affected the sensitivity. A period of perfusion with normal Ringer-Locke fluid reduced the sensitivity of the preparation to the test dose of 0.5 μg. of acetylcholine (Ac) at 70, 77, and 120 min. Subsequent doses of 0.25 mg. at 127 min. and 1.0 mg. at 145 min. DFP markedly sensitized the heart. Time marker: 10 sec.

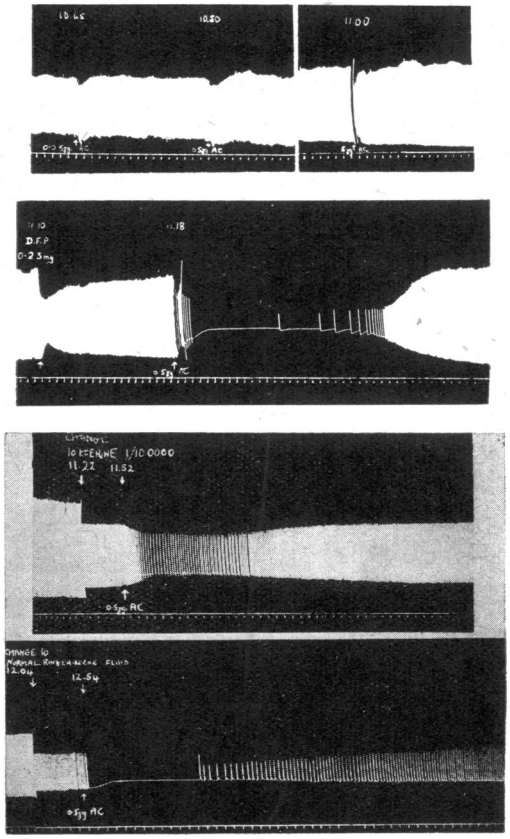

FIG. 5.—The effects of 0.05 μg., 0.5 μg. and 5.0 μg. of acetylcholine (Ac) are shown on a fresh isolated heart at 10.45, 10.50, and 11.00 respectively. A dose of 0.25 mg. DFP at 11.10 sensitized it markedly. The addition of eserine (1/100,000) at 11.22 to the perfusion fluid markedly decreased the sensitivity of the preparation to acetylcholine at 11.52, and this "atropine-like" effect was removed by continued perfusion with normal Ringer-Locke fluid as seen at 12.54.

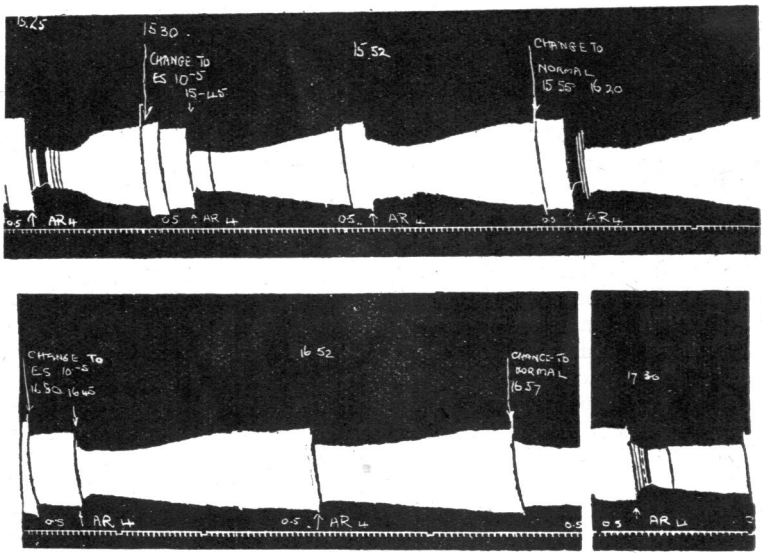

FIG. 6.—The response of the rabbit heart fully sensitized with DFP to 50 μg. arecoline (0.5AR4) is seen at 15.25. The addition of eserine (1/100,000) at 15.30 to the perfusion fluid markedly reduced the sensitivity of the preparation to the reference dose of arecoline (0.5 AR4) at 15.45 and at 15.52. When normal Ringer-Locke fluid was restored to the heart at 15.55, its sensitivity to arecoline had returned by 16.20 and the "atropine-like" effect of eserine was developed for the second time in the same preparation in the lower tracing.

(1/100,000 in Ringer-Locke solution) for a period, the depression of the heart beat produced by the reference dose of acetylcholine was greatly *reduced* compared with that produced immediately before perfusion with eserine, a change reminiscent of the effect of a small dose of atropine. If normal Ringer-Locke fluid was now restored to the heart the sensitivity of the preparation to acetylcholine returned approximately to the level obtained initially in the fully sensitized heart (Fig. 5).

A closely similar result was obtained with prostigmine: perfusion for as short a time as ten minutes with prostigmine (1/100,000 in Ringer-Locke solution) greatly reduced the sensitivity of a heart, already fully sensitized with DFP, to the reference dose of acetylcholine; then, after about an hour's perfusion with normal Ringer-Locke fluid, the sensitivity to acetylcholine returned to almost the initial post-DFP level.

If the sensitization with DFP was only partial, it was usually found that eserine or prostigmine had an additive effect, increasing sensitization already produced by a small dose of DFP. The "atropine-like" action of eserine or prostigmine was only observed in hearts which had been fully sensitized with DFP; it was, however, an unexpected finding, and the "atropine-like" action of eserine was investigated further. Normally, when the rabbit heart is fully sensitized with DFP, no change occurs in its sensitivity to pilocarpine or to arecoline. Both these drugs are powerfully antagonized by atropine in the heart, and consequently, if eserine has a true atropine-like action in a heart which has been fully sensitized with DFP, it should reduce not only the effect of acetylcholine but also the effects of pilocarpine and arecoline. Experimentally, this was found to be so, the atropine-like action of eserine being best demonstrated with arecoline (Fig. 6).

THE ISOLATED FROG RECTUS ABDOMINIS MUSCLE

METHOD

In some experiments the method of Chang and Gaddum (1933) was employed, a 40-ml. bath being used, but in most experiments the following procedure was adopted: the muscle was suspended in a 10-ml. bath of oxygenated frog Ringer solution in such a way that it could be stimulated through two platinum electrodes fixed to each end by ligatures. The muscle was stimulated maximally by a condenser discharge and the contractions recorded on a kymograph by a tangentially writing lever. This preparation was subjected to the action of test doses of acetylcholine for precisely 60 sec. and was stimulated every 50 sec. throughout the experiment by the condenser discharge so that the effect of the various procedures upon this form of electrical stimulation could be studied. Provision was made for 5-sec. periods of faradization of the muscle.

RESULTS

When DFP was added to the bath so that the final concentration was over about 0.75 mg. per ml., a spontaneous contraction resulted from which the muscle rarely relaxed. Higher concentrations of DFP killed the tissue as indicated by its lack of response to stimuli. With lower concentrations, DFP had little immediate effect upon the muscle

beyond a slight transient depression of the contraction elicited by the condenser discharge, but after the addition of DFP to the bath the height of the contractions was very gradually and progressively enhanced (Fig. 7).

The depression of the mechanical response to motor nerve stimuli in the cat's tibialis anterior muscle observed some time after intra-arterial administration of DFP (Brown, Burns, and Feldberg, 1948) had no counterpart in the experiments with the frog's rectus. The augmentation of the mechanical response to the condensor discharge usually attained maximal dimensions in 2 to 3 hours and was well maintained over a period of several further hours.

In none of the experiments was it possible to observe fasciculation of the muscle after the addition of DFP or acetylcholine to the bath. In a few experiments the action potentials of the rectus were recorded and evidence of repetitive contraction of the muscle fibres was found in the rectus subjected to DFP (Knox and Quilliam, unpublished observation).

Changes in the sensitivity to acetylcholine

DFP markedly sensitized the frog rectus to the action of acetylcholine. About 0.1 to 0.2 mg. DFP per ml. was the optimal bath concentration. The sensitization was partially developed in 30 min. but took at least one hour to become maximal (Fig. 7). In thirty-two experiments, the increase in sensitivity to acetylcholine was quantitatively assessed and the mean value was 5 ± 3 times. On four occasions there was a tenfold and on one a thirteenfold sensitization (Fig. 8).

The type of response to acetylcholine was altered considerably. The first dose of acetylcholine after DFP caused a marked and prolonged augmentation of the muscle twitch in response to the condenser discharge stimulus (Fig. 7) lasting from 10 to 60

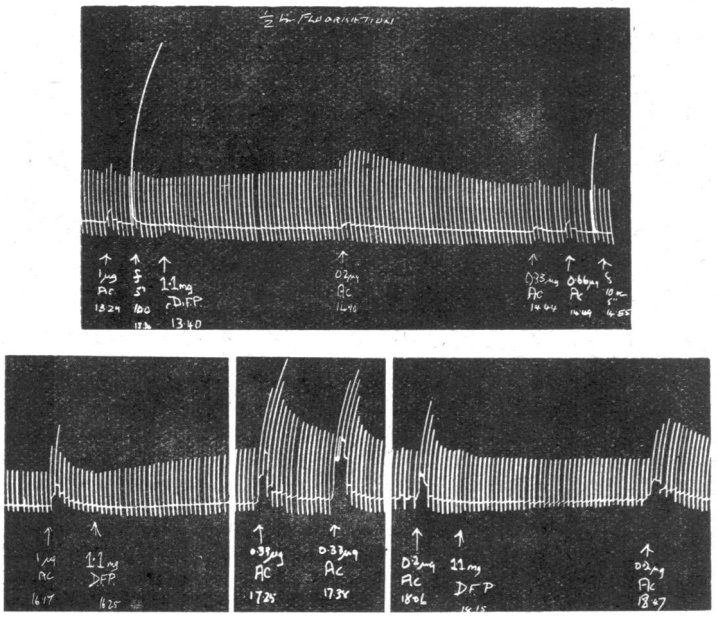

FIG. 7.—In the upper record of the isotonic contraction of the frog's rectus abdominis muscle, the effect of a reference dose of 1 μg. of acetylcholine (Ac) at 13.29 and of a 5 sec. tetanus (f. 5") at 13.34 will be seen. A slight transient depression followed by a gradual augmentation of the contraction occurred after the addition of 1.1 mg. DFP to the 10-ml. bath at 13.40. After 30 min. 0.2 μg. of acetylcholine at 14.10 demonstrates the prolonged augmentation of the contraction after the acetylcholine had been allowed to act upon the rectus for 60 sec. and then washed off three times. At 14.44 and 14.49, attempts to measure the sensitivity of the preparation showed that 0.66 μg. of acetylcholine at 14.49 just failed to give a response as great as that to 1 μg. at 13.29. At 14.55, the effects of the same short tetanus as was given at 13.34 will be seen. The post-tetanic augmentation of the contraction is seen in both instances. In a new preparation (lower tracing) exposure to DFP for 60 min. was required to develop the fivefold increase in sensitivity to acetylcholine illustrated. A second application of 1.1 mg. DFP at 18.15 caused no further increase in sensitivity. The form of the post-acetylcholine augmentation may be compared at 17.25, 17.38, 18.06, and at 18.47. The muscle was stimulated by the condenser discharge every 50 sec. The tracings are to be read from left to right.

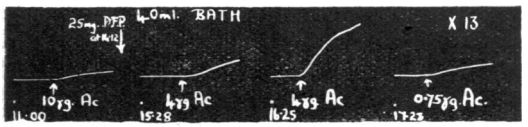

FIG. 8.—Frog's rectus abdominis. Method of Chang and Gaddum (1933); 40-ml. bath; a thirteenfold sensitization of a frog's rectus will be seen after treatment with 25 mg. DFP for 76 min.

minutes or more, even after several washings. This post-acetylcholine augmentation was seen after subsequent doses of acetylcholine but only to a smaller degree. After prolonged exposure to DFP the augmentation after the first dose of acetylcholine was less marked.

The sensitivity to acetylcholine in control muscles showed a slight decline with time and there was also a similar slight falling off in the sensitivities of DFP-treated muscles of about the same order. The sensitization with which DFP endowed the muscle appeared to last as long as 6–8 hours without any but the slight reduction comparable with that seen in control muscles.

Changes in response to faradic stimulation

In the normal rectus, a 5-sec. period of faradization resulted in a powerful, brief contraction, and several succeeding muscle twitches in response to the condenser discharge were larger than normal as found by Brown and von Euler (1938) in mammalian muscle (Fig. 7). After a period of exposure to DFP, the muscle responded to faradic stimulation by a contracture lasting for some minutes, upon which the muscle twitches in response to the condenser discharge were superimposed (Fig. 9). Feng (1936) observed a similar contracture in the toad's sartorius muscle on faradic stimulation of the nerve after eserine.

Action of eserine after DFP

In view of the " atropine-like " action that eserine can exert in the rabbit's heart after full sensitization with DFP, it was of interest to see whether a similar action could be demonstrated in the frog's rectus muscle. Fig. 9 illustrates a typical experiment: a rectus abdominis muscle, already fully sensitized with DFP, was exposed to eserine (1/100,000) and

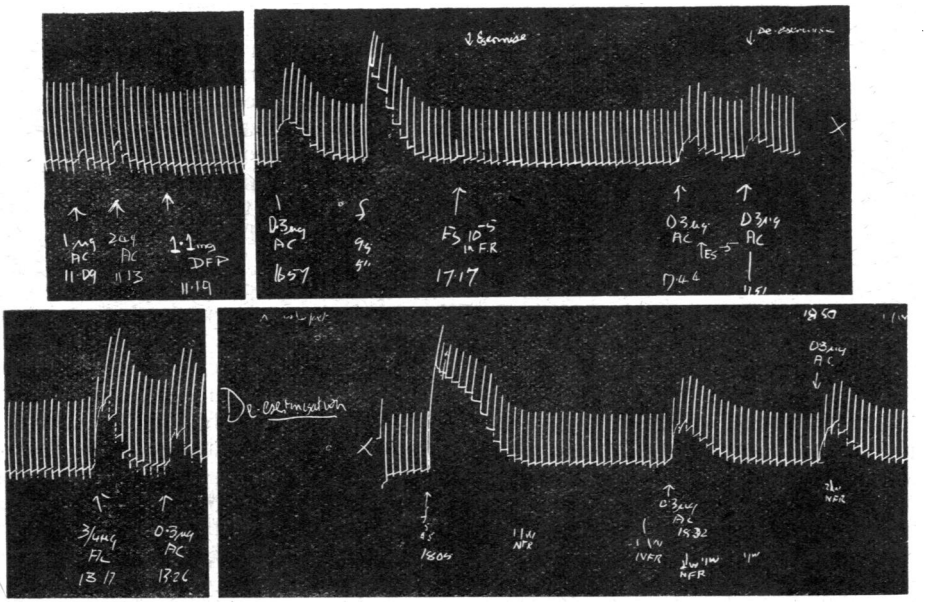

FIG. 9.—The upper left tracing shows the sensitivity of a rectus to 1 μg. and 2 μg. of acetylcholine (Ac) at 11.09 and 11.13 respectively. At 11.19 1.1 mg. DFP was added to the 10-ml. bath, and after 118 min. the increase in sensitivity was over sevenfold as indicated by 0.3 μg. of acetylcholine at 13.26 in the lower left tracing. In the upper right tracing 3½ hours later, the sensitivity of the rectus is well maintained as the action of 0.3 μg. of acetylcholine at 16.57 indicates. Next at f, the contracture following a standard period of tetanization is seen. At 17.17 eserine (1/100,000) was added to the bath and the sensitivity to the test dose, 0.3 μg. acetylcholine, progressively diminished at 17.44 and at 17.51. In the lower right tracing washing of the preparation was commenced at 17.54 and at 18.05 the effect of a standard tetanization is seen, and at 18.32 and 18.50 the sensitivity of the preparation to the test dose of acetylcholine has returned.

27 minutes later a test dose of acetylcholine produced a smaller effect than it had done before eserine. This action of eserine can be removed by washing the preparation repeatedly with normal frog Ringer fluid. Brown (1937) showed that atropine injected intra-arterially into a frog's gastrocnemius muscle depressed the response of the muscle to acetylcholine similarly injected.

Action of DFP on the muscle after eserine

The addition of DFP to the bath when the rectus had been maximally sensitized with eserine resulted in no further increase in the sensitivity of the preparation to acetylcholine.

The actions of curare and atropine

If a sufficiently high concentration of either of these two substances is added to the bath it is possible to block the action of acetylcholine upon the rectus either completely or nearly so. The response of the muscle to the condenser discharge stimulus is unaltered. The blocking action of curare can be removed by soaking the muscle in Ringer solution containing DFP for a long time. DFP can also reverse to some extent the effects of atropine upon the frog rectus.

The action of propylene glycol upon the rectus abdominis muscle

One muscle of a pair of recti was set up in a series of experiments as a control and received the same quantity of propylene glycol as that given in a dose of DFP to the test preparation. With the quantities of propylene glycol used in our experiments with DFP, no changes in sensitivity of the rectus were seen.

As propylene glycol is often used as a solvent for drugs, it was of interest to observe the effect of larger doses upon the rectus. When 1.5 ml. or more were added to the 40-ml. bath for 40 minutes there was a slight increase in sensitivity of the preparation to acetylcholine. In addition three other phenomena were seen. A series of large spontaneous contractions might develop which were sometimes rhythmical in nature and which might give place to small spontaneous twitches. Both these reactions could be precipitated by washing but often occurred without. The large rhythmical contractions usually lasted for a few minutes, but the twitching lasted from one half to one hour; repeated washing of the preparation appeared to accelerate the disappearance of both phenomena. The third change seen was an exaggerated stretch response. Normally an isolated frog rectus does not respond to a slight stretch, but in a preparation which has received an excess of propylene glycol a slight mechanical stretch results in one or more large spontaneous contractions. This exaggerated stretch response was associated with the period during which the twitches were seen and was, like the slight increase in sensitivity to acetylcholine, removed by repeated washings. Smaller quantities of ethylene glycol produced similar changes.

Discussion

The sensitization to acetylcholine with which DFP endows the isolated rabbit's heart differs from that seen after eserine or prostigmine in the following manner: a single injection of 0.25 mg. of DFP can produce a permanent one-hundredfold sensitization within two minutes. Eserine or prostigmine must be added to the perfusion fluid for at least 10 to 20 minutes in order to sensitize the heart to acetylcholine, and they are both easily removed by continued perfusion with normal Ringer-Locke fluid.

The resistance to the action of DFP that the heart, fully sensitized with eserine or prostigmine, exhibits is probably due to saturation of the cholinesterase of the tissue with eserine or prostigmine; when the eserine or prostigmine is removed—e.g., by perfusion with normal fluid—the cholinesterase is free to combine irreversibly with DFP and the tissue becomes permanently sensitized to acetylcholine.

It is of interest to note that a patient who has been receiving prostigmine regularly in the course of treatment for myasthenia gravis does not respond so satisfactorily to DFP as an untreated patient. This is a clinical counterpart to the experimental observation that prostigmine can protect tissues against DFP. Koster (1946) has shown that eserine can exert some protective action in cats against lethal doses of DFP.

The blocking effect that eserine exerted on the actions of acetylcholine, pilocarpine, and arecoline in a heart already fully sensitized with DFP was unexpected and, so far as could be determined, was "atropine-like" in nature; the acetylcholine sensitivity of the fully sensitized frog's rectus could also be reduced with eserine. These actions of eserine are quite distinct from its anticholinesterase activity and are only unmasked when the cholinesterase activity of the tissue has been fully inhibited. In each preparation the action was abolished by continued washing of the preparation in such a way as to remove eserine.

In the frog's rectus abdominis muscle DFP was found to be a more reliable and more effective sensitizing agent to acetylcholine than eserine; it had a permanent action but required at least one

hour for its effect to be fully exerted. Eserine, however, can sensitize the rectus in about 20 min., and a dose of eserine about one-tenth that of DFP was required.

Miquel (1946) stated that eserine increased the acetylcholine sensitivity of the frog's rectus after DFP. In our work we found that the quantities of DFP used by Miquel were insufficient to sensitize the rectus fully. In these circumstances a dose of another anticholinesterase drug would be expected to enhance the sensitivity of the tissue still further. But when the tissue is fully sensitized with DFP and exposure to more DFP does not alter the sensitivity, the administration of eserine results in a decrease in sensitivity and not an increase as found by Miquel.

Bacq's (1947) findings were also different from those of Miquel; he found that eserine did not change the acetylcholine sensitivity of the frog's rectus after DFP. In our experience, it is only by a study of the mechanical response of the rectus to electrical stimulation that the depressant action of eserine after full sensitization with DFP can be demonstrated.

Finerty (1947) showed that some potentiation of the acetylcholine response of the isolated rectus can be obtained with phosphoric acid, and he considered that the acidic properties of a solution of DFP that has deteriorated are in part responsible for the sensitization seen after this drug. However, the phosphoric acid sensitization illustrated was of the same order as that which we found after excessive doses of propylene glycol and was probably non-specific in nature. In addition, the periods of exposure to DFP used by Finerty seem extremely brief.

One of the most striking features of the action of DFP upon the rectus is the change in form of the response to the test dose of acetylcholine. The augmentation of the mechanical response to the condenser discharge stimulus was maintained for as long as sixty minutes even after three washings. More repeated washing encouraged the phenomenon to disappear. Brown (1937) considered that "in general, the reactions of normally innervated frog (*gastrocnemius*) muscle closely resembled those of mammalian muscle after denervation" rather than those of normally innervated mammalian muscle. Brown, Burns, and Feldberg (1948) found that in the cat's tibialis anterior muscle, whether denervated or not, the action of acetylcholine after DFP was to depress the mechanical response to stimulation. We saw no counterpart of this reaction in the rectus, but Coppée and Bacq (1947), using the frog's sciatic nerve-gastrocnemius preparation, illustrate such a depression both after a period of brief tetanic stimulation and after acetylcholine in the normally innervated frog's muscle after exposure to DFP.

Summary

1. The actions of DFP upon the perfused isolated rabbit's heart and the isolated frog's rectus abdominis muscle preparations are described.

2. A single injection of 0.25 mg. DFP will endow the isolated heart with a permanent hundredfold sensitization to acetylcholine which is fully developed within two minutes.

3. Whereas an injection of DFP does not alter the acetylcholine sensitivity of a heart already fully sensitized with eserine or prostigmine, the addition of eserine or prostigmine to the perfusion fluid of a heart fully sensitized to acetylcholine by DFP results in a *reduction* in its sensitivity; restoration of the normal perfusion fluid for a period causes the sensitivity of the heart to return. This "atropine-like" action of eserine is also seen with pilocarpine and arecoline in hearts that have previously received DFP.

4. After soaking the frog's rectus in a solution of DFP for at least one hour, its acetylcholine sensitivity may be enhanced permanently 5 ± 3 times. The response of such a sensitized rectus to maximal condenser discharge stimuli after acetylcholine is markedly augmented, often for quite a considerable period. The application of DFP to the rectus is not followed by depression of its response to electrical stimuli at any phase.

5. Administration of eserine to a rectus, already fully sensitized to acetylcholine with DFP, leads to a reduction in its sensitivity which can be restored to normal by repeated washing with normal frog Ringer solution.

6. In the amounts used in experiments with DFP, the solvent propylene glycol exerts no effect upon the acetylcholine sensitivity of the isolated rabbit's heart or the frog's rectus. When large quantities of propylene glycol are added to the bath containing a frog's rectus the following changes may occur: (*a*) a slight increase in the acetylcholine sensitivity, (*b*) large spontaneous twitches which may be rhythmical, (*c*) small spontaneous non-rhythmical twitches, and (*d*) an exaggerated stretch response. All these changes are abolished by repeated washing.

7. These pharmacological actions of DFP are discussed with special reference to eserine and prostigmine. Certain differences in the response of

the rectus to DFP when compared with mammalian and amphibian nerve muscle preparations are indicated.

It is a pleasure to thank Prof. R. J. S. MacDowall and Dr. D. B. Taylor for advice.

REFERENCES

Adrian, E. D., Feldberg, W., and Kilby, B. A. (1946). *Nature*, **158**, 625.
Adrian, E. D., Feldberg, W., and Kilby, B. A. (1947). *Brit. J. Pharmacol.*, **2**, 56.
Bacq, Z. M. (1947). *C.R. Soc. Biol., Paris*, **141**, 857.
Brown, G. L. (1937). *J. Physiol.*, **89**, 438.
Brown, G. L., Burns, B. D., and Feldberg, W. (1948). *J. Physiol.*, **107**, 346.
Brown, G. L., and von Euler, U. S. (1938). *J. Physiol.*, **93**, 39.
Chang, H. C., and Gaddum, J. H. (1933). *J. Physiol.*, **79**, 255.
Coppée, G., and Bacq, Z. M. (1947). *C. R. Soc. Biol., Paris*, **141**, 859.
Feng, T. P. (1936). *Chin. J. Physiol.*, **10**, 513.
Finerty, J. C. (1947). *Amer. J. Physiol.*, **151**, 107.
Grob, D., Lilienthal, J. L., Jr., and Harvey, A. M. (1947). *Johns Hopk. Hosp. Bull.*, **81**, 245.
Knox, J. A. C., Quilliam, J. P., and Strong, F. G. (1949). *J. Physiol.*, **108**, 3*P*.
Koster, R. (1946). *J. Pharmacol.*, **88**, 39.
Miquel, O. (1946). *J. Pharmacol.*, **88**, 67.
Quilliam, J. P. (1947). *Post-grad. med. J.*, **23**, 280.
Quilliam, J. P. (1949). *J. Physiol.*, **108**, 5*P*.
Quilliam, J. P., and Quilliam, T. A. (1947). *Med. Pr.*, **218**, 378.
Quilliam, J. P., and Strong, F. G. (1947a). *J. Physiol.*, **106**, 23*P*.
Quilliam, J. P., and Strong, F. G. (1947b). *Abs. 17th Internat. Physiol. Congress*, 163.
Quilliam, J. P., and Strong, F. G. (1949). *J. Physiol.*, **108**, 10*P*.