(4) Storage of intact brains of 5HTP-treated animals for 2 h in 0·1 *n* HCl at room temperature caused a significant 5HT increase (118 ± 11%) as compared to brains homogenized immediately after death (53 ± 10%; $p < 0{\cdot}01$). This 5HT rise was, however, significantly less than that in brains kept at room temperature but not in HCl (226 ± 22; $p < 0{\cdot}01$).

*Discussion.* The present results with rat brain frozen or homogenized immediately after decapitation confirm that 5HTP causes a significant 5HT rise which is markedly enhanced by pretreatment with iproniazid. The findings show, however, that storage of the brain at room temperature or at 37°C after death causes a further considerable 5HT increase. This rise cannot even be completely abolished by keeping the intact brain in 0·1 *n* HCl.

In earlier experiments this post mortem rise of 5HT might not have been considered. Thus the reported values for the 5HTP induced increase of 5HT in brain were possibly too high and did not reflect the true content *in vivo*. In order to get more reliable results it is necessary to homogenize the brains in HCl or to freeze the skulls immediately after death.

The 5HT accumulation after death is probably due to continuing decarboxylation of injected 5HTP penetrated into the brain. This agrees with the fact that 5HTP decarboxylase does not require oxygen[6–8]. Monoamine oxidase activity, however, which strongly depends on oxygen tension[9] is probably reduced markedly after decapitation so that the newly formed 5HT mostly accumulates. The slight decrease of 5HT in brain of untreated animals during the first half hour *post mortem* can possibly be attributed to a limited amount of oxygen still present which enables a small MAO activity. Other metabolic pathways for 5HT seem not to be involved since this small disappearance of 5HT is blocked by iproniazid.

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*Pharmazeutische Abteilung der F. Hoffmann-La Roche & Co. AG., Basel, May 27, 1960.*

*Zusammenfassung*

Ratten mit und ohne Iproniazid-Vorbehandlung erhielten 30 min vor Dekapitation 5-Hydroxytryptophan (5HTP) i. p. Das intakte Hirn wurde bis zu 4 h bei 37°C, Zimmertemperatur und in 0·1 *n* HCl aufbewahrt. In allen Fällen ergab sich eine starke postmortale 5-Hydroxytryptamin (5HT)-Vermehrung im Gehirn.

Bei unbehandelten Kontrolltieren zeigte sich postmortal ein leichter 5HT-Abfall im Gehirn, der durch Iproniazid-Vorbehandlung aufgehoben wurde.

## A New View on an Old Drug: Pilocarpine

Pilocarpine is one of the oldest parasympathomimetic drugs. Where the action upon the iris[1], the salivary glands, the sweat glands, and intestinal smooth muscle is concerned, pilocarpine shares the action of muscarine and acetylcholine[2]. There are, however, many reports[3] from which it appears that pilocarpine has a more complex mode of action. Especially its action upon the heart and the sacral parasympathetic nerve endings is contradictory to that of muscarine. Moreover, the effect of muscarine on the heart can be abolished by pilocarpine in larger doses[4].

It has been found that progressive elongation of the alkylchain in 2-alkyl-4-(trimethylammonium)methyl-1:3-dioxolane ($RFMe_3$) causes a gradual decrease in the intrinsic activity and consequently a gradual change from parasympathomimetics into parasympatholytics[5]. The intrinsic activity[6], being high (1) for a mimetic, low (0) for a lytic, and intermediate for a partial agonist[7], is a measure for the ratio of agonistic-competitive antagonistic actions. The transition substance $PrFMe_3$ has an intermediate intrinsic activity (0·5) and hence exerts both a parasympathomimetic and a parasympatholytic influence. $PrFMe_3$ is an example of a partial agonist or competitive dualist.

These findings led to the supposition that pilocarpine might be an example of a natural dualistic drug. This implies that pilocarpine would behave both as a parasympathomimetic and a parasympatholytic like $PrFMe_3$. Consequently pilocarpine has to act synergistically with low doses of acetylcholine, but competitive-antagonistically with high doses of ACh.

As a partial agonist, the maximal height of the dose-response curve of pilocarpine on the intestine should necessarily be a fraction of that of acetylcholine. This, as a matter of fact, appeared to be true, as may be seen from Figure 1. The maximal response of pilocarpine is actually about 70% of that of a pure parasympathomimetic such as acetylcholine or furmethonium (HFurf).

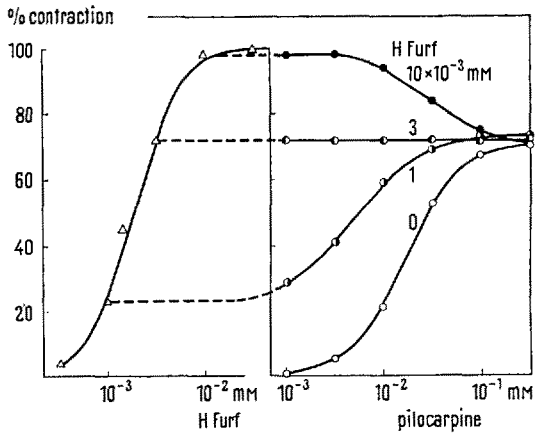


Fig. 1. a) Dose-response curve for the parasympathomimetic furmethonium (HFurf). b) Dose-response curves for the partial agonist pilocarpine in the presence of various doses of HFurf on the isolated gut of the rat.

Note the parasympathomimetic action of pilocarpine in the absence of furmethonium and the competitive antagonistic action when the intestine is brought into contraction by high doses of furmethonium. When a contraction is produced by the agonist equal to the maximal contraction possible with pilocarpine, the latter drug only displaces furmethonium from the receptors without altering the degree of contraction.

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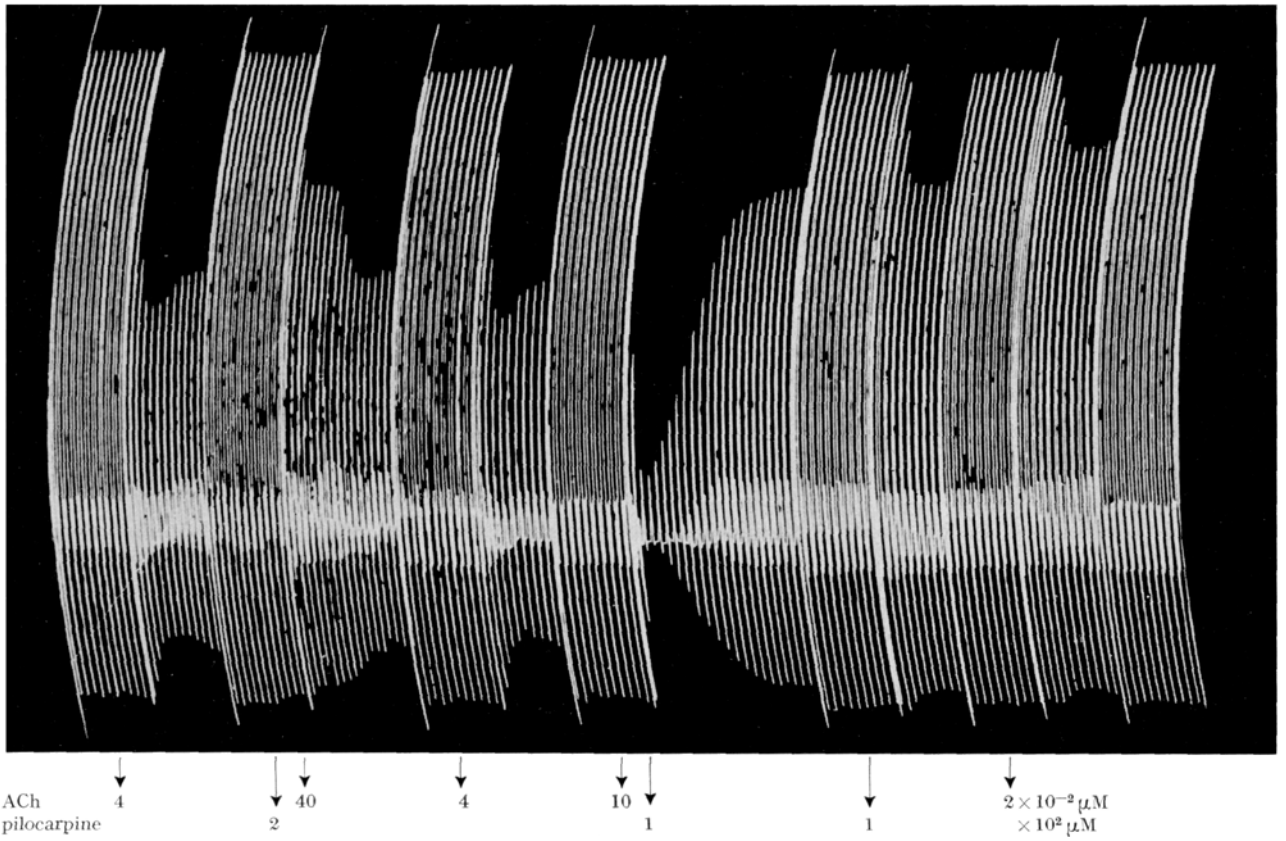


Fig. 2. Registrogram of the spontaneous heart beats of the isolated frog heart (*Rana temporaria*) and the effects of ACh, pilocarpine, and their combinations. Pilocarpine abolishes the depression of the heart beat by ACh, while pilocarpine as such also produces a depression and is able to protect the heart against large concentrations of ACh. Note a slight agonistic and mainly antagonistic action of pilocarpine.

The intrinsic activity (i.a.) is expressed as the ratio between the maximal response of a drug and that of acetylcholine, while the affinity[8] is expressed as a $pD_2$ value, being the negative logarithm of the molar concentration which produces 50% effect in the dose-response curve. Intrinsic activities and $pD_2$ values are collected in Table I.

Since the intrinsic activity and the affinity are dependent on the molecular properties of both the drug and the specific receptors for different biological objects, a variation in both parameters is possible. It is logical that the dependence of the intrinsic activity on various objects becomes especially evident as regards partial agonists[9]. In fact it appeared from studies on the relationship between structure and activity in the $RFMe_3$ series that $PrFMe_3$ is a partial agonist in the rat intestine, but a competitive antagonist on the frog heart, whereas on the blood pressure of the cat it is a pure agonist[9].

It might therefore be expected that the intrinsic activity of pilocarpine on the frog heart is lower than on the intestine. This also was found to be the case; consequently pilocarpine on the frog heart behaves mainly like atropine. Pilocarpine prevents the depression of the heart beat as caused by ACh (antagonistic effect), whereas as such it depresses the heart action to a slight degree (agonistic effect) (see Fig. 2). Larger doses produce only the same slight depression which implies that the intrinsic activity on the frog heart is only about 0·2. Moreover pilocarpine abolished the effect of high concentrations of ACh in a competitive way. As may be expected, the recovery under pilocarpine is not, however, complete (see Fig. 2).

Also a nicotinic action has been ascribed to pilocarpine[10]. Since the rat intestine is not sensitive to nicotinic actions,

Table I. Effect in isolated organs

| Drug | Rat Intestine | | | Frog Heart | | |
|---|---|---|---|---|---|---|
| | i. a. | $pD_2$[a] | $pA_2$ | i. a. | $pD_2$ | $pA_2$ |
| ACh | 1 | 7·0 | | 1 | 7·1 | |
| HFurf | 1 | 5·9 | | 1 | 5·3 | |
| Pilocarpine | 0·6–0·8 | 5·0 | | 0·1–0·2 | | 3·8 |
| Atropine | 0 | | 8·7 | 0 | | 7·4 |

[a] $pD_2$ and $pA_2$ are mean values with a confidence range of 0·1 above and below the number given

Table II. Effects on the blood pressure of the cat

| Drug | Parasympathomimetic action | | Ganglionic action | | Ratio ps/g activity |
|---|---|---|---|---|---|
| | i. a. | log aff[a] | i. a. | log aff[a] | |
| ACh | 1 | 9·8 | 1 | 5·8 | $10^4$ |
| HFurf | 1 | 8·9 | 1 | 4·5 | $3{\cdot}10^4$ |
| Pilocarpine | 1 | 7·3 | — | $< 4$ | $>2{\cdot}10^3$ |
| Nicotine | — | — | 1 | 6·3 | $<1$ |

[a] Negative logarithm of equiactive molar concentrations

[8] E. J. ARIENS and J. M. VAN ROSSUM, Arch. int. Pharmacodyn. *110*, 275 (1957).

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the parasympathetic actions on the rat intestine, however, are not disturbed by possible ganglionic actions. Ganglionic actions may be observed in objects which are not sensitive to parasympathetic actions, as for instance the blood pressure of the atropinized cat. Parasympathomimetic and ganglionic activities were studied on the blood pressure of the cat; the results are collected in Table II. From this Table, it may be seen that the ganglionic activity of pilocarpine is actually extremely low.

The mode of action of pilocarpine, however, is still more complex since in high doses ($>1$ *mM*) papaverine-like actions become apparent in the intestine. On the same object, papaverine is active in concentrations of $10^{-2}$ *mM*. This implies that pilocarpine behaves both as an agonist and as a competitive antagonist in low doses, and as a non-competitive antagonist in high doses.

Pilocarpine is an example of a parasympathetic drug with a dualism in action. Its intrinsic activity varies for different organs and species. Hence pilocarpine is more muscarinic in its effect on certain organs but more atropinic on others.

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*Pharmacological Institute, R. C. University of Nijmegen (the Netherlands), January 13, 1960.*

*Zusammenfassung*

Pilocarpin ist ein geeignetes Beispiel einer parasympathischen Substanz mit dualistischer Wirkung. Auf Grund der Variationen in der Eigenaktivität (intrinsic activity) des Pilocarpins an verschiedenen Organen und Arten tritt entweder die parasympathomimetische oder die parasympatholytische Wirkung in den Vordergrund.

## Serum Glutamic Oxaloacetic Transaminase after Hepatic Artery Ligature

Serum glutamic oxaloacetic transaminase (SGOT) is a very sensitive index of acute necrosis of many tissues, including the heart, liver, kidneys, bowel, and lungs (LIONEL *et al.*[1]).

Experimental destruction of liver tissue by carbon tetrachloride was investigated by WROBLEWSKI and LA DUE[2]. The level of SGOT was found by these authors to be a highly specific index of hepatocellular injury: the height and duration of enzymatic activity was proportional to the amount of carbon tetrachloride, as well as to the extent of liver cell damage.

Ligation of the hepatic artery, which was introduced by RHEINHOFF[3], is performed in Egypt in the treatment of advanced cases of hepatosplenomegaly with ascites. In view of the fact that the results are controversial and the mortality rate is as high as 20% (SHALABY[4]; KHAIRY[5]), the present work was undertaken to study the effect of this operation on the SGOT level in an effort to assess the extent of hepatocellular damage which accompanies such a procedure.

The operation was performed on six dogs weighing between 9 and 10 kg. The hepatic artery was ligated distal to the right gastric branch. The animals were divided into two groups of three.

Animals of group I received no antibiotics and animals of group II received 0·5 g of Achromycin daily, 2 days prior to the operation and through the post-operative period.

In a third group of animals (3 dogs), laparatomy was performed using the same technique applied to group I and II but without ligating the hepatic artery (control experiment). SGOT was estimated according to the method of DUBACH[6].

As regards group III, no rise in enzyme level was detected.

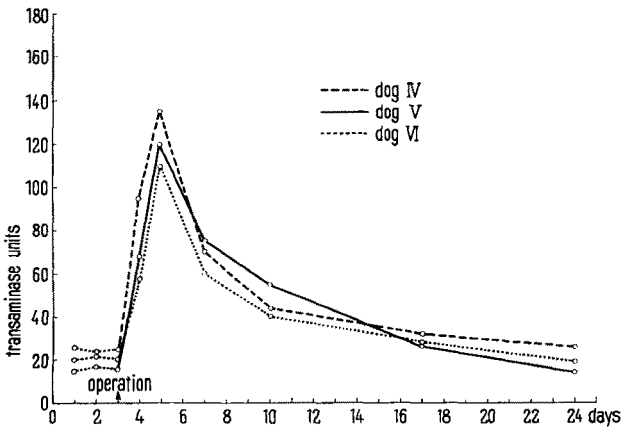
A review of these results indicates that hepatic artery ligature caused a rapid liberation of the enzyme from the anoxic liver cells into the blood stream. The enzyme level in the serum reached a maximum after 24 or 72 h. These results are in agreement with data obtained with other tissues like heart, kidneys, and lungs after ligation of their arterial supply (LIONEL *et al.*[7]).

*Table I.* Animals of group I, SGOT units per ml

| | h after operation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 6 | 12 | 24 | 48 | 72 | 96 |
| Dog I | 25 | 28 | 42 | 65 | 125 | 132 | Died |
| Dog II | 10 | 10 | 35 | 50 | 160 | Died | |
| Dog III | 15 | 17 | 28 | 55 | 115 | 145 | Died |

*Table II.* Animals of group II, SGOT units per ml

| | Days before operation | | Operation Day | Days after operation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 1 | | 1 | 2 | 3 | 4 | 7 | 14 | 21 |
| Dog IV | 26 | 24 | 25 | 95 | 135 | 100 | 70 | 44 | 32 | 26 |
| Dog V | 15 | 17 | 16 | 68 | 120 | 95 | 75 | 55 | 26 | 14 |
| Dog VI | 20 | 22 | 20 | 58 | 110 | 80 | 60 | 40 | 28 | 19 |

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