Biochemical Pharmacology, 1963, Vol. 12, pp. 1017–1021. Pergamon Press Ltd., Printed in Great Britain.

# THE INHIBITORY ACTION OF PHENYLBUTAZON ON THE SUCCINOXIDASE ACTIVITY OF RAT TISSUE HOMOGENATES

P. GÖRÖG and L. SZPORNY

Chemical Works Gedeon Richter Pharmacological Laboratory, Budapest, Hungary

(*Received* 9 *March* 1963; *accepted* 10 *May* 1963)

**Abstract**—Phenylbutazon inhibits the succinoxidase activity of rat heart and skeletal muscle in concentrations from $5 \times 10^{-5}$ to $5 \times 10^{-3}$ M. It fails to exert any effect on the enzyme activity of brain and liver. The inhibitory action is not competitive. It can be blocked entirely with rat serum and partly with human albumin. Therapeutical doses of phenylbutazon *in vivo* inhibits the succinoxidase activity of rat heart muscle to about 30 per cent. The possible correlations between the inhibitory and the antiinflammatory actions are discussed.

ONE of the best known and most effective antiflogistic compounds of non-steroid type is 3,5-dioxo-1,2-diphenyl-4-n-butyl-pyrazolidine (Phenylbutazon, Butazolidin® Richter). Though several communications have dealt with the effect on carbohydrate metabolism of this substance,[1–3] the significance of these effects in the pharmacological action has not been elucidated so far. In the course of our investigations, the purpose of which has been the analysis of correlation between glucose metabolism and anti-inflammatory action, we have studied the influence of phenylbutazon on the succinoxidase activity of rat tissues.

## MATERIAL AND METHODS

Phenylbutazon is a preparation of our factory. Albumin is a product of the 'Phylaxia' factory, a human serum albumin preparation, that proved to be homogeneous when tested by paper electrophoresis.

Enzyme preparation was obtained from a 10% (w/v) homogenate of the tissue to be examined in phosphate buffer (M/15, pH 7·4). The homogenate was centrifugated ($2000 \times g$ for 20 min) and the supernatant used as an enzyme preparation. The enzymic activity was estimated according to the method of Neufeld *et al.*[4] The velocity of reduction of dichlorphenolindophenol between 1·0 and 0·6 extinctions was measured in a Hilger 'UVISPEK' spectrophotometer at 660 mμ. The cell contained: $2 \times 10^{-5}$ M dichlorophenolindophenol, $3 \times 10^{-3}$ M KCN, $3 \times 10^{-2}$ M succinate, and enzyme preparation (0·1 ml) in M/15 phosphate buffer (pH 7·4) give a final volume of 4·0 ml. The enzyme had been preincubated with the inhibitor for different periods at 38° before addition of substrate.

In experiments *in vivo*, the experimental animals were given 150 mg/kg phenylbutazon intraperitoneally. The enzyme activity was measured 2 hr later. The animals were sacrificed by decapitation and the enzyme activity of the heart muscle was measured after homogenization.

## RESULTS AND DISCUSSION

Phenylbutazon, as evident from Fig. 1, possesses a marked inhibitory effect in a final concentration range from $5 \times 10^{-5}$ to $5 \times 10^{-3}$ M as investigated on heart muscle homogenate. As to the course of the reaction with time, in the case of enzyme not inhibited, the reaction speed is constant while it deviates more and more from

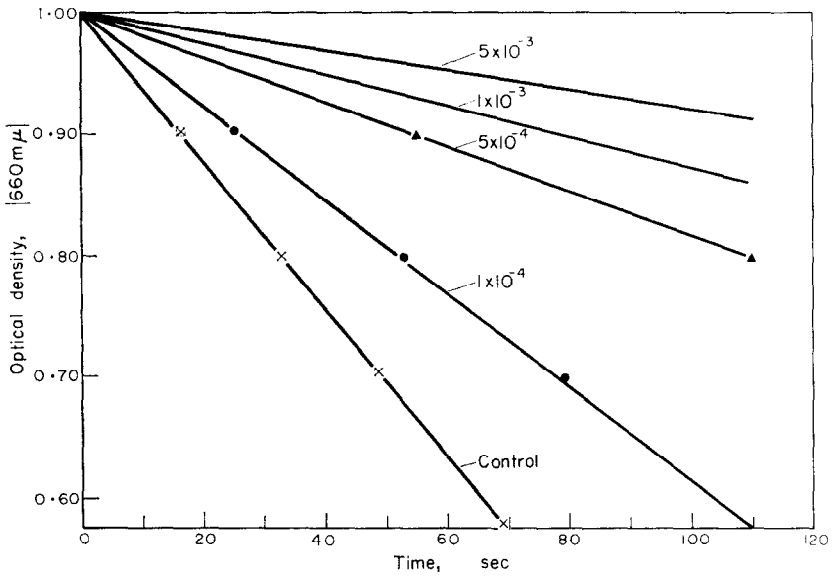


FIG. 1. Succinoxidase inhibitory action of phenylbutazon.
After preincubation of the drug for 10 min at 38° with the complete system minus substrate, activity was measured. Details of the reaction system see in the text.

linearity with increasing inhibition. As it can be seen from Fig. 2, inhibition does not depend on substrate concentration; thus it is not competitive. Table 1 shows succinoxidase activity of various tissues in the presence of phenylbutazon. It appears that significant inhibition occurs merely in the case of heart and skeletal muscle. As it is known that the linkage of enzyme and inhibitor, in the case of succinoxidase is not instantaneous, we incubated the inhibitor together with the enzyme for different times in the same concentration, before adding the substrate. As it appears from Fig. 3, the intensity of inhibiting action depends on the duration of pre-incubation, while the activity of enzyme incubated under similar conditions but without inhibitor for the same time, has not changed.

The inhibitory effect observed in experiments *in vivo* was investigated. *In vivo* 29·2 $\pm$ 3·1 per cent inhibition is obtained with the therapeutical doses on heart muscle homogenate. From the results obtained, both *in vivo* and *in vitro*, the above mentioned effect must be reckoned with when using this compound in therapy. Under therapeutical conditions, the inhibitory effect might be more elective and of much greater extent, since phenylbutazon accumulates in inflamed tissues. Wallenfels and Sund[5] suppose a conjunction between phenylbutazon and albumin and explain the accumulation of the compound in inflamed regions by this hypothesis. Therefore we examined

the influence of rat serum and human albumin added to the medium on the enzyme inhibitory action of phenylbutazon. As it is clear from Table 2, 0·9 ml normal rat serum interrupts completely the inhibitory action.

The inhibitory action can be blocked with albumin to a maximum extent of 60 per cent. This level may be obtained already with 3 mg albumin, but further protective

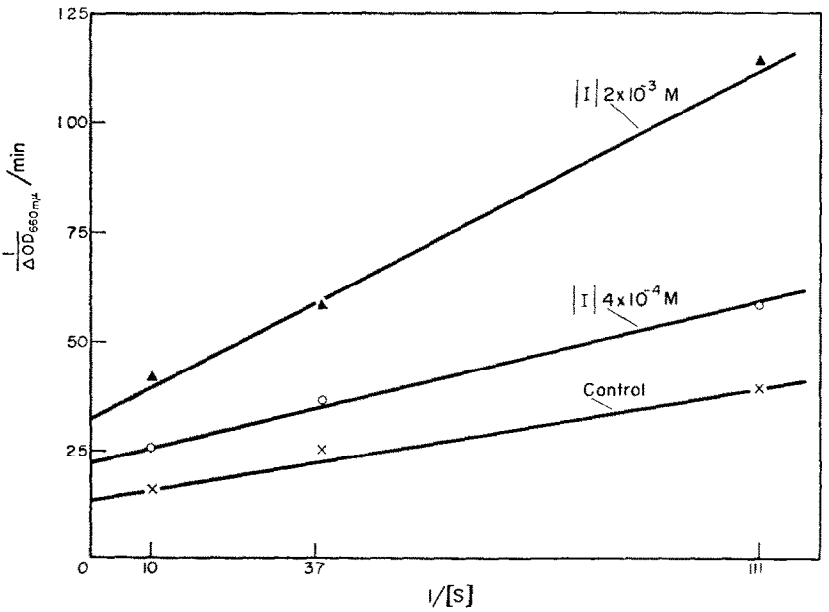


FIG. 2. Investigation of type of inhibition.
Relation between 1/V and 1/S in the presence and absence of inhibitor |I| is plotted according to Lineweaver–Burk.[7]

TABLE 1. EFFECT OF PHENYLBUTAZON ON SUCCINOXIDASE ACTIVITY OF DIFFERENT RAT TISSUES

| Phenylbutazon M × $10^{-4}$ | % Inhibition | | | |
|---|---|---|---|---|
| | Heart | Brain | Muscle | Liver |
| 2·5 | 19 | 5 | 39 | 12 |
| 5·0 | 38 | 6 | 53 | 23 |
| 10·0 | 47 | 8 | 70 | 17 |
| 20·0 | 58 | 5 | 77 | 10 |

effect can not be brought about even with 9 mg albumin. The case of pseudo-irreversible inhibition is known from the investigation of Reif and Potter[6]. The tissue specificity of the succinoxidase inhibitory action of the compound and its increased accumulation in inflamed tissues can be interpreted on these grounds.

Succinoxidase inhibition leads to energy deficit, via diminution of the whole citric acid cycle. It seems that phenylbutazon acts not only on one but almost on three

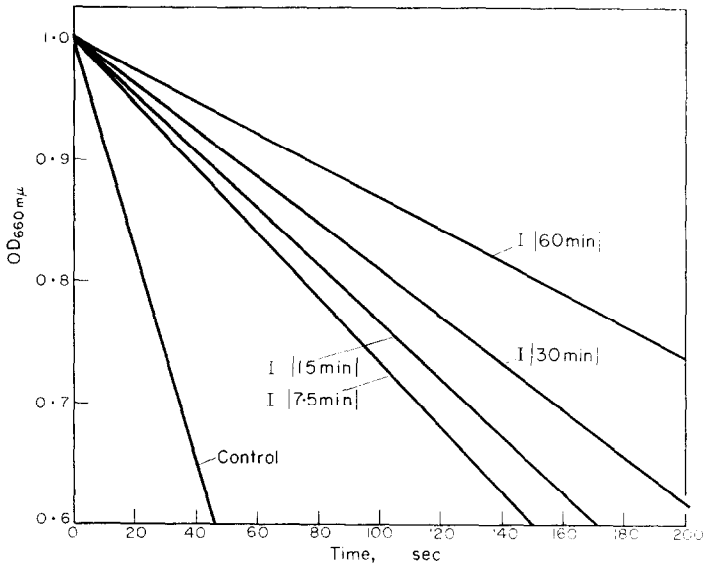


FIG. 3. Effect of duration of enzyme-inhibitor simultaneous preincubation on the extent of inhibitory action.

I (inhibitor) = $1 \times 10^{-3}$ M phenylbutazon. Minutes in bracket indicates the duration of enzyme-inhibitor preincubations before adding the substrate.

TABLE 2. PHENYLBUTAZON INHIBITION BLOCKING EFFECT OF RAT SERUM AND OF HUMAN ALBUMIN

| Rat serum ml | Human albumin mg | $\Delta$V sec | % Blocking of phenylbutazon inhibition |
|---|---|---|---|
| — | — | 52 | 0 |
| 0·15 | — | 43 | 17 |
| 0·30 | — | 32 | 39 |
| 0·90 | — | 1 | 98 |
| — | 0·75 | 50 | 4 |
| — | 1·5 | 40 | 23 |
| — | 3·0 | 22 | 58 |
| — | 6·0 | 21 | 60 |
| — | 9·0 | 24 | 54 |

Serum or albumin preincubated for 10 min with the inhibitor ($1 \times 10^{-3}$ M) and enzyme in a 3·0 ml volume at 38°, before adding the substrate heart muscle homogenate, 10% (w/v) used as enzyme. (0·1 ml).

$\Delta$V = $\Delta$ reaction time between the inhibited and non-inhibited reaction

All of other condition was the same.

steps of the citric acid cycle.[1, 2] It is not simple to decide whether the energy deficiency is connected with the antianabolic property of the drug in biosynthesis of mucopolysaccharides, or with the depression of histamine release, caused by the compound. As for all the antiphlogistic compounds, having in common an inhibiting action on citric acid cycle, a correlation between the above mentioned and antiinflammatory action of the drug is likely to exist.

## REFERENCES

1. K. H. Bässler and K. Lang, *Arch. exp. Path. Pharmak.* **229**, 568 (1956).
2. K. H. Bässler and K. Lang, *Arch. exp. Path. Pharmak.* **231**, 254 (1957).
3. B. Exer, *Experientia* **12**, 294 (1956).
4. H. A. Neufeld, C. R. Scott and E. Stotz, *J. Biol. Chem.* **210**, 869 (1954).
5. K. Wallenfels and H. Sund, *Arzneim. Forsch.* **9**, 81 (1959).
6. A. E. Reif and V. R. Potter, *J. Biol. Chem.* **205**, 279 (1953).
7. H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.* **56**, 658 (1934).