possibility is suggested by the great reactivity of the ethylene groups adjacent to the —CN groups. With an oxidative mechanism, the substance produced would eventually be cyanacetic acid, through the formation of a keto-intermediate, which by addition of a molecule of water gives the unstable acetal. In both these postulated reactions there would be the formation of one mole of cyanide per mole of SDN. Thus this amount of —CN ought to be eliminated in the urine as —SCN. In fact, over 70 per cent of the residual radioactivity can be extracted with isoamyl alcohol after reaction with Fe^{3+} in the presence of relatively large amounts of inert carrier —SCN. It can be assumed that the reaction is not quantitative in excess of iron.

This hypothesis of an enzymatic reaction resulting in a liberation of cyanide is substantiated by the fact that the amount of thiocyanate increases considerably in animals treated with repeated doses of SDN, while at the same time the total of intermediate metabolites decreases by the same amount (Table 2).

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Biochemical properties of azapropazone and other anti-inflammatory drugs

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RECENTLY, many attempts have been made to establish relationships between the anti-inflammatory actions of drugs and their biochemical actions on organelles and macromolecules. Such attempts include the actions of the drugs in stabilizing lysosomes[1] and proteins[2] and in uncoupling oxidative phosphorylation.[3] It appears probable that uncoupling oxidative phosphorylation and protein stabilization may be of particular importance for non-steroidal drugs.

In this investigation we have examined the new anti inflammatory drug Azapropazone together with other established drugs for their actions on mitochondria and proteins.

Protein stabilization. A modification of the method of Mizushima and Nakagawa[4] was used. Bovine albumin (1% w/v) was dissolved in phosphate (0·1 M with respect to phosphate) buffered saline at either pH 5·8 or 7·4. The drugs were dissolved in alkaline saline (0·1 N NaOH in 0·9% w/v NaCl) and portions (0·1 ml) added to 2·7 ml of buffered albumin solution in stoppered test tubes. Portions (0·1 ml) of the alkaline saline alone was added to control tubes. The pH of each solution was checked. The absorbancies of the solution at 420 nm were determined and the tubes heated for 1 hr at 70° in a waterbath. After incubation the tubes were cooled in ice–water and the absorbancies again determined at 420 nm. Preliminary experiments had shown that 420 nm was the most sensitive wavelength for determining changes in turbidity due to protein denaturation.

Effect on mitochondrial respiration. Mitochondrial respiration was measured polarographically using a modified Clark oxygen electrode[5] coupled to a Honeywell Brown "Electronik" recorder.

Mitochondria were isolated from rat liver homogenate as the fraction sedimenting between 600 and 8500 *g* for 10 min in an isolation medium—0·25 M sucrose buffered at pH 7·4 with 5 mM Tris–HCl and 1 mM E.G.T.A. (ethylene glycol-bis-(amino ethyl ether)N,N′ tetra-acetic acid). The final suspensions contained the equivalent of 1 g liver to 2 ml of medium.

Portions (0·5 ml) of the mitochondrial suspension were added to 2·5 ml of a respiratory medium in the reaction vessel of the oxygen electrode which was maintained at 30° by a water jacket. The respiratory medium consisted of 0·25 M sucrose–12 mM $MgSO_4$–12 mM phosphate buffer (pH 7·4). Portions (10 μl) of sodium succinate (0·5 M) were added and respiration followed. Drugs were added

dissolved in 10 μl of DMSO (dimethyl sulphoxide). The chart was calibrated with respect to oxygen uptake by recording the stoichiometric oxidation of NADH by frozen and thawed mitochondria preparations. In uncoupling experiments ADP (0·05 M) was added (10 μl) after the addition of succinate. The ADP/O was determined (ADP/O = Micromoles ADP added/microatoms oxygen utilized).

Protein stabilization. The results (Table 1) show the effect of the drugs on the thermal denaturation of albumin. The differences between the final and initial absorbance values have been calculated as a percentage of the increase of the absorbance values of the controls. Values below 100 per cent indicate that the drugs have stabilized the protein.

Clearly azapropazone has stabilized the protein although its action at high concentration is less than that of phenylbutazone.

TABLE 1. THE EFFECT OF DRUGS ON BOVINE ALBUMIN STABILITY

Concentration	Azapropazone (pH 5·8)	Azapropazone (pH 7·4)	Phenylbutazone (pH 5·8)	Phenylbutazone (pH 7·4)
None (control)	100	100	100	100
10^{-3} M	39 ± 0·9	73·2 ± 0·8	29·1 ± 0·6	61 ± 0·3
10^{-4} M	72·1 ± 2·1	81·8 ± 1·1	56·1 ± 3·1	69·7 ± 0·8
10^{-5} M	86·2 ± 2·2	87·1 ± 1·7	91·2 ± 3·4	71·2 ± 1·4
10^{-6} M	87·3 ± 1·0	88·6 ± 0·8	94·0 ± 1·0	77·1 ± 0·3

Each result is the mean ± S.D. of four experiments.

Effect on mitochondria. The results (Tables 2 and 3) showed that azapropazone and salicylate stimulated respiration whereas phenylbutazone and oxyphenbutazone inhibited respiration. However, phenylbutazone stimulated respiration at a lower concentration and the inhibitory effect at high concentration may have been due to drug induced damage of the organelles. The increase in respiration appears to be linked to the uncoupling of oxidative phosphorylation since the ADP/O ratios decreased at drug concentration where respiration was stimulated. Control ADP/O ratios varied between 1·2 and 1·7 with succinate as substrate.

TABLE 2. EFFECT OF DRUGS ON RESPIRATION RATE OF MITOCHONDRIA

Concentration of drug	Azaprop-azone	Oxyphen-butazone	Phenyl-butazone	Sodium salicylate
$3·3 \times 10^{-3}$ M	121·8 ± 3	20·5 ± 3	28·6 ± 4	110·6 ± 2
$2·6 \times 10^{-3}$ M	158·9 ± 5	32 ± 2	46·5 ± 2	130·5 ± 4
$2·0 \times 10^{-3}$ M	171·4 ± 5	44 ± 3	59·8 ± 2	132·8 ± 3
$1·3 \times 10^{-3}$ M	163·0 ± 7	53 ± 2	78·9 ± 1	153·4 ± 3
$6·6 \times 10^{-4}$ M	144·6 ± 4	84·3 ± 1·3	139·1 ± 2	159·2 ± 1

Respiration rates compared to control values of 100 per cent.
Each result represents mean value ± S.D. of four experiments.

Azapropazone has stabilized albumin and uncoupled oxidative phosphorylation. Its uncoupling action is less than that of either sodium salicylate or phenylbutazone.

Similarly, its stabilizing action on albumin is slightly less than phenylbutazone at pH 5·8 and 7·4. However, azapropazone has a stabilizing action *in vitro*[6] on rabbit liver lysosomes of a similar order to oxyphenylbutazone, phenylbutazone and prednisolone over a drug concentration range of 10^{-3}–10^{-9} M and a greater stabilizing action on rat ileum lysosomes *in vitro* than phenylbutazone over a drug concentration range of 10^{-3}–10^{-6} M.

TABLE 3. EFFECT OF DRUGS ON ADP/O RATIOS

Concentration of drug	Azapropazone	Phenylbutazone	Sodium salicylate
2×10^{-3} M	65·6 ± 3		
$1{\cdot}3 \times 10^{-3}$ M	73·0 ± 4		
$6{\cdot}6 \times 10^{-4}$ M	82·8 ± 2		71·0 ± 3
$3{\cdot}3 \times 10^{-4}$ M			82·0 ± 2
$1{\cdot}6 \times 10^{-4}$ M		70·0 ± 3	
$6{\cdot}6 \times 10^{-5}$ M		85·9 ± 1	

Results expressed as percentage of control values adjusted to 100 per cent.
Each result represents mean value ± S.D. of four experiments.

The biochemistry of azapropazone is similar to the two established anti-inflammatory drugs phenylbutazone and sodium salicylate in that it has stabilised albumin and uncoupled oxidative phosphorylation.

A large number of acidic non-steroidal drugs possess these properties. Both these properties have a theoretical importance in that they may reduce inflammation. However, further work is needed to link biochemical properties *in vitro* with pharmacological action *in vivo*.

Clearly, non-steroidal anti-inflammatory drugs possess a common set of biochemical properties which individually, or collectively, may be responsible for their anti-inflammatory action. Azapropazone possesses at least three properties of potential anti-inflammatory value. The relevance of these properties to the situation *in vivo* has yet to be determined.

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Further differentiation of cholinergic receptors in leech muscle

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THE CHOLINERGIC receptors of leech (*Hirudo medicinalis*) dorsal muscle, while formally classifiable as nicotinic,[1,2] appear to possess a number of features that distinguish them from the nicotinic receptors at vertebrate skeletal neuromuscular junctions. Thus, Flacke and Yeoh[2,3] argued for different receptors in the leech dorsal muscle for monoquaternary (acetylcholine, carbachol and nicotine) and bisquaternary (succinyl-choline, decamethonium) agonist ligands on the basis of the relative