and chondroitin sulphate B[3] to be separated, in addition to hyaluronic acid, keratosulphate and chondroitin sulphates A and C (ref. 2).

The only fraction which contained a polysaccharide was that eluted with 0·3 M magnesium chloride. This was shown[3] to cause separation of the cetylpyridinium–heparitin sulphate complex from the other aortic mucopolysaccharides. Using cellulose acetate electrophoresis and a 0·1 M phosphate buffer, at a pH of 7·2, this fraction migrated as a single spot with a mobility similar to that of heparitin sulphate isolated from aorta, and intermediate between hyaluronic acid and the chondroitin sulphates. The compound from egg-white contained equimolar amounts of hexosamine[4] and uronic acid, determined by the orcinol procedure[5]. The hexosamines were shown by paper chromatography[6] and on a Dowex ion-exchange column[7] to consist largely of glucosamine (about 75 per cent of the total) and galactosamine. Paper chromatographic analysis on Whatman No. 1, using a descending front of isopropanol–water (160 : 40 by volume) showed that the uronic acid part corresponds to glucuronic acid; the spots were detected by naphthoresorcinol reagent[8]. There were 0·9 sulphate groups[9] per disaccharide unit. The failure to liberate N-acetylhexosamine[10] showed that the compound was not degraded by testicular hyaluronidase.

These results indicate that the acid mucopolysaccharide isolated from egg-white is heparitin sulphate or a closely related substance. The sulphate content is lower than that of heparin, and this is supported by the staining of samples dried on filter paper by toluidine blue. The degree of metachromasia is less intense than with heparin but similar to that of a chondroitin sulphate. Hyaluronic acid does not show metachromasia by this method. The sulphate content is, however, greater than that of the aortic heparitin sulphate isolated by Antonopoulos *et al.*[3], which has approximately one group for each two disaccharide units. The presence of galactosamine may be caused either by its partial replacement of glucosamine in the polysaccharide chain or by contamination with a chondroitin sulphate or a glycopeptide. The absence of any of the chondroitin sulphates in the appropriate fractions, however, and the use of 1 per cent cetylpyridinium chloride as a solvent to remove peptides makes such contamination unlikely.

Schiller[11] isolated heparitin sulphate from the oestrogen-stimulated chick oviduct, but hyaluronic acid and chondroitin sulphates A and B were also shown to be present. The hen oviduct also contains several sulphotransferases, of which heparitin sulphotransferase is the most abundant and the most stable[12].

This work was supported by grants from the University of London Central Research Fund and the Medical Research Council.

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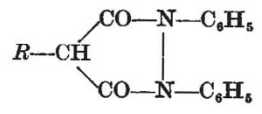
Activation of Fibrinolysis by Derivatives of Diphenyldioxopyrazolidine

The mechanism of the anti-inflammatory action of non-steroid anti-phlogistics has not yet been fully explained, and further information is required. In our studies of the biochemical properties of derivatives of 1,2-diphenyl-3,5-dioxopyrazolidine which possess anti-inflammatory activity and have an oxoalkyl group in position 4 of the pyrazolidine nucleus[1], we have investigated their influence on fibrinolysis, using the method of von Kaulla[2]. We compared the following three derivatives of diphenyldioxopyrazolidine, which have been used clinically, with indomethacin and salicylic acid.



1, R = $C_6H_5COCH_2CH_2$—
2, R = $CH_3CH_2CH_2CH_2$–
3, R = $CH_3COCH_2CH_2$—

In the conditions of our experiment, increased fibrinolysis was observed, causing the dissolution of recalcified human plasma clots incubated in a buffered solution of the examined substance for up to 24 h. The dissolution only occurred with certain concentrations of the examined substances, and both higher and lower concentrations showed no influence on the fibrinolysis. The order of activities expressed both in molar and p.p.m. concentrations of the substances examined is presented in Table 1. Salicylic acid was inactive in this test.

Table 1. Activity of Substances Examined

Substance	Molecular weight	Complete fibrinolysis		Partial fibrinolysis	
		Molarity	Wt. per cent	Molarity	Wt. per cent
1. Benzopyrazone	384·42	0·006	230·6	0·004–0·007	153·7–269·0
Indomethacin	357·9	0·007	249·9	0·006–0·009	214·7–322·1
2. Phenylbutazone	308·37	0·008	246·6	0·009–0·01	277·5–308·3
3. Ketophenyl butazone	322·37	0·04–0·07	1,289·0–2,256·0	—	—

The substances examined showed fibrinolytic activity *in vitro* at concentrations about 10 times greater than those found in the blood after the usual therapeutic dose of the drugs, which lie between 100 and 200 p.p.m. when derivatives of diphenyldioxopyrazolidine are being administered.

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Catechol-o-methyl Transferase Activity in Skeletal Muscle

It has been reported that catechol-*o*-methyl transferase (COMT) is found in most organs of the rat except skeletal muscle[1]. In the course of studies on the isolation of this enzyme from various tissues, we obtained results indicating that muscle shows a COMT activity approaching that found in the lungs and heart.

The various tissues were homogenized in a 1·15 per cent solution of potassium chloride (two parts of tissue to five parts of solution) at 4° C. The COMT activity was determined in the supernatant obtained after centrifuging the homogenate at 80,000g for 30 min.

To determine the activity of COMT, we used tritiated *S*-adenosyl-L-methionine. 50 μl. of the soluble fraction (5 μl. for hepatic tissue) was incubated for 60 min at 37° C in a mixture containing 10 μmoles of 0·5 molar potassium phosphate buffer, pH 7·6; 10 μmoles of molar magnesium chloride; 100 mμmoles of tritiated *S*-adenosyl-L-methionine methyl (1·74 μ) ('Tracerlab'; specific activity 274 m/mmole). The mixture was made up to a final volume of 150 μl. Control solutions were made by boiling the supernatant in advance for 5 min at 100° C or by replacing it with water.