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FURTHER STUDIES ON COMPOUNDS REDUCING SYNOVIAL MEMBRANE PERMEABILITY

BY

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It has been shown (Bianchi, 1953) that benzoylcarbinol acetate decreases synovial permeability in the talo-crural articulation of the rabbit, but not the permeability of periarticular subcutaneous tissue, capillaries, or renal tubules. The results obtained with other compounds related to benzoylcarbinol acetate are reported in this paper with a view to increasing our knowledge of compounds which could be used in human arthritic processes.

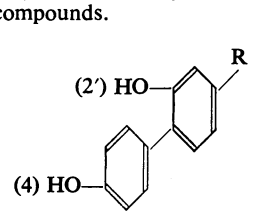
The method used is that first described by Seifter, Baeder, and Begany (1949). These authors consider it useful for the evaluation of new anti-arthritic compounds (Seifter, Baeder, Begany, Rosenkranz, Djerassi, Pataki, and Kaufmann, 1950), but this opinion is not accepted by others (Paul, Hodges, Knouse, and Wright, 1952; Hidalgo, McClure, Henderson, Whitehead, and Smyth, 1952). Human synovial membrane permeability is also known to be modified by drugs (Fitch, Warter, Seifter, Jallo, and Corn, 1950). Seifter's test only gives information on synovial membrane permeability, and not on the total and more complex anti-rheumatic effect of a drug. Only a study of the activity of new drugs in human arthritis, as compared with their effects on the permeability of synovial membrane of laboratory animals, could establish the value of Seifter's test.

METHODS

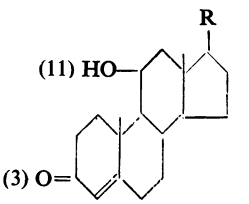
Male rabbits weighing about 2 kg. were treated for four days with a 2% (w/v) concentration of the drugs suspended in a 10% (w/v) gum acacia solution. The test products were usually injected intraperitoneally (75 mg./kg. per day) for four days. About 1 hr. after the last injection, the rabbits were anaesthetized intravenously with 40 mg./kg. 5-5'*iso*amylethylbarbituric acid ("Ethamyl," Zambeletti). A soft rubber catheter was inserted through the urethra into the bladder. In the first group of experiments 0.25 ml. of a 0.5% (w/v) phenol red solution was injected into the right talo-crural articulation. In the second group of experiments the same quantity of phenol red was injected into the periarticular connective tissue. In the third group of experiments 5 ml. of a 0.025% (w/v) phenol red solution was injected intravenously. The recovery of phenol red in the urine was assessed 1, 2, and 3 hr. after the intra-articular, periarticular, or intravenous injection. Details of the methods used are given in a previous paper (Bianchi, 1953).

The chemical properties of the compounds tested are described elsewhere (Logemann and Giraldi, 1951; Logemann, 1952; Logemann and Giraldi, 1953). The compounds are divisible into four groups: (1) Those with a keto-alcoholic side chain. (2) Those derived from dioxyacetone. (3) Those derived from 2': 4-dioxydiphenyl. (4) Miscellaneous compounds.

The compounds of group 1 have the same side chain as DOCA. Those of group 2 have the same side chain as cortisone. Those of group 3 have two hydroxy groups in positions analogous to the 3: 11 positions of steroid compounds.



Diphenyl structure



Steroid structure

In group 4 are derivatives of acetophenone, one cyclic keto-alcohol (inosose), the therapeutic agent phenylbutazone ("butazolidin"), and sodium benzoate. The last-named compound was tested because Bernini (1953) proved that nearly 50% of benzoylcarbinol is excreted as hippuric acid. It is known that sodium benzoate is excreted as hippuric acid. It was interesting to ascertain whether benzoic acid, presumably produced in the metabolism of benzoylcarbinol, had any action on synovial permeability.

RESULTS

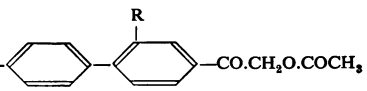
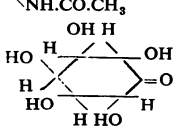
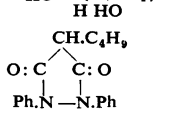
Renal excretion of phenol red 1 and 3 hr. after intra-articular injection is shown in Table I.

The most active of the compounds tested may be arranged, in descending order of their activity in reducing the permeability of the synovial

TABLE I

RENAL EXCRETION OF PHENOL RED INJECTED INTRA-ARTICULARLY. MALE RABBITS TREATED INTRAPERITONEALLY FOR 4 DAYS (75 MG./KG. PER DAY) WITH THE COMPOUNDS INDICATED

(For amount of phenol red injected, see text. The amount excreted is the % of that injected±S.E.)

Compd. No.	Chemical Name	Structural Formula	Phenol Red Excreted After		No. of Animals Used
			1 hr.	3 hr.	
	Compounds of General Formula: R—CO—CH_2OH				
	Controls		53·4±1·4	76·7±1·5	22
1	Benzoylcarbinol	$C_6H_5CO.CH_2OH$	32·3±2·3	61·6±2·3	8
2	*N*-β-Naphthylglycylcarbinol	β-$C_{10}H_7NH.CO.CH_2OH$	37·5±4·6	70·6±4·7	6
3	*N*-α-Naphthylglycylcarbinol	α-$C_{10}H_7NH.CO.CH_2OH$	45·7±5·4	69·2±6·2	4
4	Benzoylcarbinol acetate	$C_6H_5CO.CH_2O.CO.CH_3$	38·2±3·6	62·2±2·2	7
5	Benzoylcarbinol salicylate	$C_6H_5CO.CH_2O.CO.C_6H_4OH$	35·8±2·7	59·8±5·6	5
6	Benzoylcarbinol propionate	$C_6H_5CO.CH_2O.CO.C_2H_5$	46·4±9·4	69·3±10·4	4
7	4-Acetoxybenzoylcarbinol acetate	4-$AcC_6H_4CH_2O.CO.CH_3$	21·9±4·4	50·5±3·4	6
8	4-Styrylbenzoylcarbinol acetate	4-$C_6H_5CH:CH.C_6H_4CO.CH_2O.CO.CH_3$	37·5±5·1	59·2±6·7	7
9	4-Phenylbenzoylcarbinol acetate	4-$C_6H_5C_6H_4CO.CH_2O.CO.CH_3$	34·0±3·9	65·6±2·6	4
10	1(4′-Diphenylyl)-3-acetoxyacetone	4-$C_6H_5C_6H_4CH_2CO.CH_2O.CO.CH_3$	49·3±3·5	79·1±1·2	4
11	4-Methoxybenzoylcarbinol acetate	4-$MeO.C_6H_4CO.CH_2O.CO.CH_3$	43·2	79·1	2
12	β-6-Methoxynaphthoylcarbinol acetate	β-6-$MeO.C_{10}H_6CO.CH_2O.CO.CH_3$	56·5±3·5	76·9±5·9	3
13	β-6-Acetoxynaphthoylcarbinol acetate	β-6-$AcO.C_{10}H_6CO.CH_2O.CO.CH_3$	41·7±3·0	68·9±3·6	7
	Compounds of General Formula: R—C(OR″)(R′)—CO—CH_2—OH				
14	*O*-Acetylmandeloylcarbinol	$C_6H_5CH(OAc)CO.CH_2OH$	30·0±5·2	62·9±4·5	5
15	1(4′-Diphenylyl)-1-methyldioxyacetone-1-methylcarbonate	4′-$C_6H_5C_6H_4C(O.CO_2Me)(Me)$—$CO.CH_2OH$	45·2±6·8	73·4±5·8	4
16	1(4′-Diphenylyl)dioxyacetone-1-methylcarbonate	4′-$C_6H_5C_6H_4CH(O.CO_2Me)$—$CO.CH_2OH$	40·7±5·5	68·2±4·3	4
	Compounds of General Formula: 				
17	4: 2′-Dimethoxy-4′-acetoxyacetonyldiphenyl	4-$MeOC_6H_4C_6H_3$(OMe(2′))(CH_2COCH_2OAc(4′))	47·7±6·0	75·7±3·7	4
18	4(4′-Methoxyphenyl)-3-methoxybenzoylcarbinol acetate	4(4′-$MeOC_6H_4$)C_6H_3(OMe(3))($CO.CH_2OAc$(1))	51·6±2·9	78·7±2·6	4
19	4(4′-Acetoxyphenyl)-3-acetoxybenzoylcarbinol acetate	4(4′-$AcOC_6H_4$)C_6H_3(OAc(3))($CO.CH_2OAc$(1))	53·0±6·3	78·4±2·7	4
	Miscellaneous Compounds				
20	Acetophenone	$C_6H_5CO.CH_3$	43·5±3·1	63·5±1·5	5
21	ω-Amino-acetophenone	$C_6H_5CO.CH_2NH_2$	46·5±6·1	74·1±2·0	4
22	*N*-Acetyl-4-nitro-ω-amino-acetophenone	4-$NO_2C_6H_4CO.CH_2NH.CO.CH_3$	47·7±5·2	74·9±2·9	4
23	2-Acetamino-3-hydroxy-propiophenone	$C_6H_5CO.CH(CH_2OH)(NH.CO.CH_3)$	44·5±1·9	77·7±1·5	4
24	Inosose		52·8±7·8	85·7±5·2	5
25	4-Butyl-1-2-diphenyl-3-5-diketopyrazolidine (Phenylbutazone, " butazolidin ")		39·3±2·5	66·3±2·6	6
26	Sodium benzoate	$C_6H_5CO_2Na$	42·8±5·0	68·7±3·7	7

membrane (as measured by the elimination of phenol red 1 hr. after its injection), as follows: 4-acetoxybenzoylcarbinol acetate (No. 7); *O*-acetylmandeloylcarbinol (No. 14); benzoylcarbinol (No. 1); 4-phenylbenzoylcarbinol acetate (No. 9); benzoylcarbinol salicylate (No. 5); 4-styrylbenzoylcarbinol acetate (No. 8); *N*-β-naphthylglycylcarbinol (No. 2); benzoylcarbinol acetate (No. 4); phenylbutazone (No. 25); 1-diphenylyldioxyacetone-1-methylcarbonate (No. 16).

It seems that greater inhibiting action is associated with the structural group R—CO—CH_2OH. Substitution of the alcoholic group with H or NH_2 decreases activity. The compounds most active in decreasing the renal excretion of phenol red injected intra-articularly were also tested for their action on subcutaneous tissue. The results obtained are shown in Table II. Renal excretion of phenol red injected subcutaneously was decreased by benzoylcarbinol salicylate and by phenylbutazone.

TABLE II

RENAL EXCRETION OF PHENOL RED INJECTED SUBCUTANEOUSLY. MALE RABBITS TREATED INTRAPERITONEALLY FOR 4 DAYS (75 MG./KG. PER DAY) WITH THE COMPOUNDS INDICATED

(For amount of phenol red injected, see text. The amount excreted is the % of that injected ± S.E.)

Compd. No.	Chemical Name	Phenol Red Excreted After		No. of Animals Used
		1 hr.	3 hr.	
	Controls	55·4±3·5	80·7±3·1	15
1	Benzoylcarbinol ..	52·9±2·8	86·8±1·7	5
4	Benzoylcarbinol acetate	58·3±4·6	84·8±3·8	4
5	Benzoylcarbinol salicylate	37·7±4·4	78·7±1·7	8
7	4-Acetoxybenzoylcarbinol acetate ..	53·1±5·1	84·8±3·1	4
25	Phenylbutazone ..	36·6±5·7	69·2±5·4	5

TABLE III

RENAL EXCRETION OF PHENOL RED INJECTED INTRAVENOUSLY. MALE RABBITS TREATED INTRAPERITONEALLY FOR 4 DAYS (75 MG./KG. PER DAY) WITH THE COMPOUNDS INDICATED

(For amount of phenol red injected, see text. The amount excreted is the % of that injected ± S.E.)

Compd. No.	Chemical Name	Phenol Red Excreted After		No. of Animals Used
		1 hr.	3 hr.	
	Controls	73·6±2·0	89·0±2·0	20
5	Benzoylcarbinol salicylate	58·7±3·4	80·3±2·4	6
25	Phenylbutazone ..	63·4±5·0	82·1±3·8	6

This test does not exclude the possibility of some renal impairment as a factor in the results.

The results on renal excretion are shown in Table III. Benzoylcarbinol salicylate alone slightly decreases renal excretion of intravenously injected phenol red; phenylbutazone does not.

DISCUSSION

The results cited in this paper call attention to studies on synovial permeability, because it is evident that there are some simple chemical compounds which have a specific "antipermeability" action on synovial membranes. Clinical trials on compounds which are active on the rabbit synovial membrane might indicate the value of this method as an anti-arthritic screening test.

The results already published give some indication as to the mode of action of these compounds. Bianchi and Meli (1952) showed that, when injected into the synovial cavity, 4-acetoxybenzoylcarbinol acetate is devoid of activity on synovial membrane permeability. Capraro and Meli (1951) and Bianchi and Meli (1952) reported on the anti-hyaluronidase action of these compounds. Cresseri and Meli (1953) observed that benzoylcarbinol and benzoylcarbinol acetate do not increase the survival of bilaterally adrenalectomized rats; do not protect mice against the toxic effects of KCl; do not affect liver glycogen deposition in bilaterally adrenalectomized rats, or the adrenals and body weight of normal rats. Benzoylcarbinol on the contrary has, like cortisone, a strong inhibitory action on the granulomatous tissue. Linnell and Roushdi (1941) observed that benzoylcarbinol acetate has very little activity compared to that of DOCA on the survival of bilaterally adrenalectomized rats (1/2,500). De Caro and Cappelli (1953) observed that the adrenal weight of unilaterally adrenalectomized rats increased significantly after treatment with benzoylcarbinol, benzoylcarbinol acetate, and 4-phenylbenzoylcarbinol acetate.

Many of the reported data are in agreement with a direct action of these compounds. But the absence of an inhibitory action of 4-acetoxybenzoylcarbinol acetate injected intra-articularly, and the data of De Caro *et al.* on unilaterally adrenalectomized rats, give the impression that these substances also act by some intermediate mechanism, presumably through stimulation of the hypophysis-adrenal system.

SUMMARY

1. Many compounds related to benzoylcarbinol decrease synovial permeability in the talo-crural articulation of the rabbit but not the permeability of periarticular subcutaneous tissue or excretion by the renal tubules.

2. 4-Acetoxybenzoylcarbinol acetate has a greater action than benzoylcarbinol.

3. Phenylbutazone decreases the permeability of the synovial membrane and the periarticular tissue, but does not impair renal function.

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