ACTIVE SITES IN STEREOSELECTIVE ADSORBENTS AS MODELS OF DRUG RECEPTORS AND ENZYME ACTIVE SITES*

BY A. H. BECKETT AND H. Z. YOUSSEF

From School of Pharmacy, Chelsea College of Science and Technology, Manresa Road, London, S.W.3

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The treatment of quinine-selective adsorbents by steam, heat, in presence of water and by treatment with acid and dry organic solvents destroyed their selectivity whereas the adsorptive power of control adsorbents was almost unaffected by these treatments. A progressive increase in pH of the adsorbate solutions from pH 1 to 3 increased the adsorptive power of the selective adsorbents more than that of the control adsorbents. Increasing concentrations of sodium chloride in the adsorbate solutions increased the quinine-uptake on the adsorbents. Stereoselectivity is probably due to the formation of "footprints" in the surface of selective adsorbents. The similarities between the behaviour of active sites in biological surfaces and those in these stereoselective adsorbents are described.

THE formation of adsorbents having stereoselective properties has already been described, e.g., for cinchona alkaloids (Beckett and Anderson, 1957). The use of stereoselective adsorbents in assigning configuration to morphine-type alkaloids has also been reported (Beckett and Anderson, 1959; 1960). Experiments are now outlined to ascertain how stereoselectivity is achieved; the data from these is used to indicate the similarities in behaviour of active sites in enzymes and drug receptors and the behaviour of those in stereoselective adsorbents.

EXPERIMENTAL

Preparation of the Adsorbents

This was carried out by the method of Beckett and Anderson (1960), with the modification of washing with distilled water after grinding the gel, to remove any residual sodium chloride before finally extracting with methanol.

Adsorption measurements. These were made by the method of Beckett and Anderson (1960) using quinine hydrochloride and 5-aminoacridine hydrochloride as adsorbates on control and quinine-selective adsorbents.

Treatment of Adsorbents

(*a*) *Treatment with Dry Organic Solvents*

An aliquot portion (10 g.) of the adsorbent (selective or control) was packed in a column provided with a dropping funnel and protected from moisture. Ether (2 ml.), acetone-ether 1 : 1 (10 ml.), ether (8 ml.) and ligroin b.p. 60–80° (10 ml.) were successively passed through the gel which was then transferred to a vacuum desiccator.

* Dedicated to Arne Tiselius in honour of his Sixtieth Birthday. This article was requested for *Perspectives in the Biochemistry of Large Molecules*, Academic Press, December, 1962, but failed to meet the dateline.

(*b*) *Heat Treatment*

Treatment by steam. Steam was passed for various periods of time through a flask containing an aliquot sample of the adsorbent. The adsorbent was filtered, extracted with methanol and dried at room temperature overnight.

Heating at 90°. The adsorbents (1 g. samples) were weighed in the flasks used to carry out the adsorption measurements and heated in an air oven for various periods of time.

Heating at 90° *in the presence of water.* The adsorbents were weighed as above, water (10 ml.) was added to the flasks which were shaken in an incubator at 90°, for various periods of time. The adsorbent was allowed to settle, as much as possible of the water was removed and the adsorption of quinine measured.

(*c*) *Acid Treatment*

Aliquot samples of the adsorbents were left in contact with 11·6N and 5·7N hydrochloric acid overnight, washed with distilled water till free from chloride, extracted with methanol and dried at room temperature overnight.

Other Treatments

Determination of "unextractable quinine" in selective adsorbents. The adsorbent (0·5 g.) was dissolved in 3N sodium hydroxide (5 ml.) and the solution was extracted with chloroform (10 g.). The quinine thus removed was then determined.

TABLE I

CONCENTRATION OF UNEXTRACTABLE QUININE AND THE ADSORPTIVE POWER OF SOME QUININE-SELECTIVE AND CONTROL ADSORBENTS

Adsorbent	Unextractable quinine expressed as moles × 10^4/kg. of adsorbent	Adsorption of quinine expressed as moles × 10^4/kg. of adsorbent
B_1	0·000	191
B_2	0·000	209
B_3	0·000	231
B_4	0·000	193
B_5	0·000	174
Q_1	232	328
Q_2	216	357
Q_3	154	324
Q_4	178	333
Q_5	314	320

Re-extraction of the stored adsorbents. The adsorbents, after storage at room temperature for the periods stated in Table II, were extracted with hot methanol for 5 hr. and the quinine uptake and "unextractable quinine" determined.

Effect of pH on selectivity. The pH was varied by carrying out the adsorption measurements in solutions of acetic acid of different concentrations.

Effect of strong electrolytes on adsorption. Solutions of various concentrations of sodium chloride in 0·5 per cent acetic acid were used.

Adsorption of quinine by control and selective adsorbents from these solutions was determined.

Determination of the loss of water of the adsorbents on heating. The adsorbent (1 g.) was heated at 200° to constant weight.

TABLE II

EFFECT OF RE-EXTRACTION WITH METHANOL OF STORED ADSORBENTS ON THE AMOUNT OF UNEXTRACTABLE QUININE AND ON THEIR ADSORPTIVE POWER

Adsorbent	Time of storage in months	Unextractable quinine expressed as moles × 10^5/kg. of adsorbent		Adsorption of quinine expressed as moles × 10^5/kg. of adsorbent	
		Before re-extraction	After re-extraction	Before re-extraction	After re-extraction
B_5	3	0·000	0·000	174	174
B_6	4	0·000	0·000	202	204
Q_5	4	314	202	320	343
Q_6	14	276	213	307	333
Q_7	10	264	173	333	345

RESULTS

Some representative results from many batches of adsorbents are presented herein. Batch to batch variation occurred in the absolute figures of adsorptive power, but the relative figures and the patterns described below did not. Subsequent unpublished work has shown that batch to batch variation may be almost eliminated by rigid temperature control during the drying of the gel to form the adsorbent.

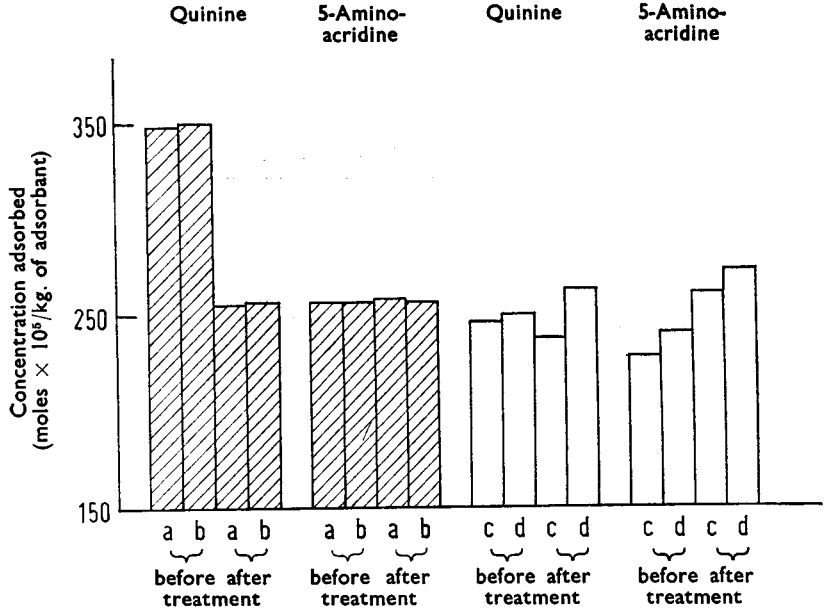


FIG. 1. Adsorption of quinine and 5-amino-acridine on quinine-selective and control adsorbents before and after treatment with dry organic solvents. ▨ Quinine-selective adsorbent; □ Control adsorbent. The letters indicate various batches.

The initial concentration for the quinine adsorption experiments was 35×10^{-5}M except for the measurements in the presence of sodium chloride in which the initial concentration was 50×10^{-5}M; for the 5-aminoacridine experiments, 40×10^{-5}M was used. All results quoted below are calculated with reference to the adsorbent dried to constant weight at 200°. Control adsorbents are indicated in the Tables by the letter B and quinine-selective adsorbents by Q; the other figures indicate various batches.

When the selective adsorbents were washed with dry organic solvents to eliminate free water (Trueblood and Malmberg, 1949), the adsorptive power for quinine was reduced to values comparable with those of the control adsorbents. This treatment did not release any of the unextractable quinine nor did it affect the mean adsorptive power of selective or control adsorbents for 5-aminoacridine (Fig. 1).

When steam was passed through the selective adsorbents their adsorptive power decreased markedly in less than 1 hr., while the same treatment increased, though to a lesser extent, the adsorptive power of the control, to give values approaching those of the selective adsorbents (Fig. 2).

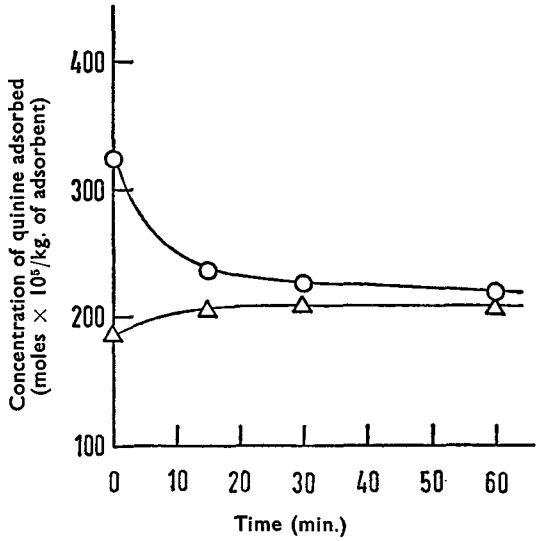


FIG. 2. Effect of steam treatment on selectivity. ○ Quinine-selective adsorbent. △ Control adsorbent.

Heating the adsorbents at 90° in aqueous suspension produced the same effect as steam treatment but at a slower rate, whereas dry heating at the same temperature for comparable times gave virtually no change in the adsorptive power of selective and control adsorbents (Fig. 3).

Acid treatment with 11·6N hydrochloric acid greatly reduced the adsorptive power of the selective adsorbent and *slightly* reduced that of

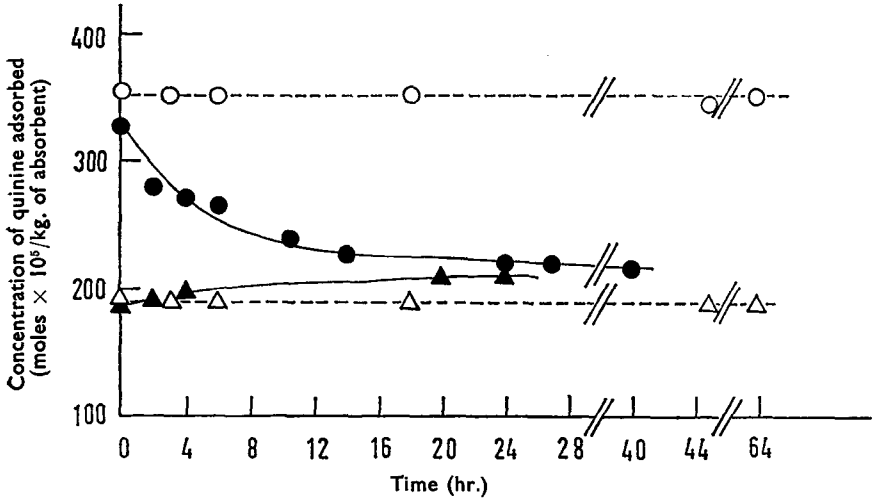


FIG. 3. Effect of heating at 90° on the adsorption on quinine-selective and control adsorbents. ● selective adsorbent heated in presence of water. ▲ control adsorbent heated in presence of water. ○ selective adsorbent air heated. △ control adsorbent air heated.

the control so that both values became not too dissimilar; under comparable conditions 5·7N hydrochloric acid only partially reduced the adsorptive power of the selective adsorbent (Fig. 4).

Progressively increasing the pH of the solutions for the adsorption measurements (from pH 1 to 3), increased the adsorptive power of the

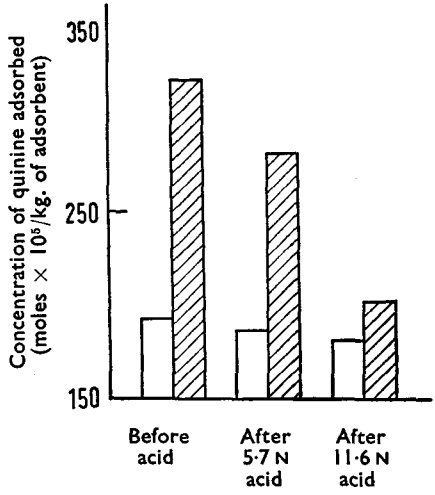


FIG. 4. Effect of treatment with 11·6N and 5·7N hydrochloric acid on selectivity. ▨ Quinine-selective adsorbent. □ Control adsorbent.

selective adsorbent and to a lesser extent that of the control so that selectivity was greater at the higher pH values (Fig. 5).

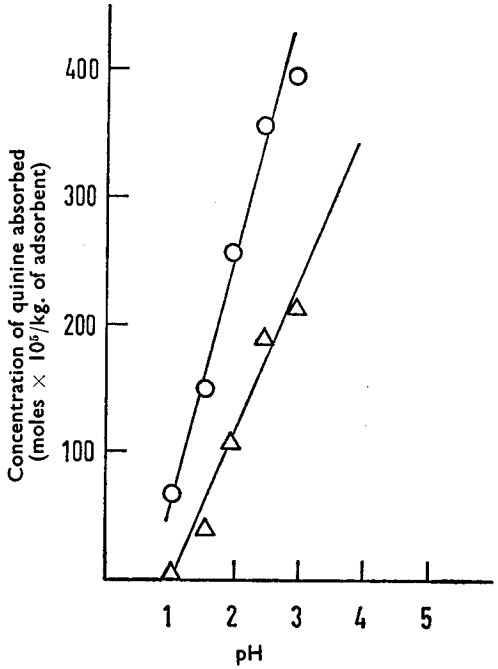


FIG. 5. Effect of pH on the adsorption of quinine on a selective and control adsorbent. ○ Quinine-selective adsorbent. △ control adsorbent.

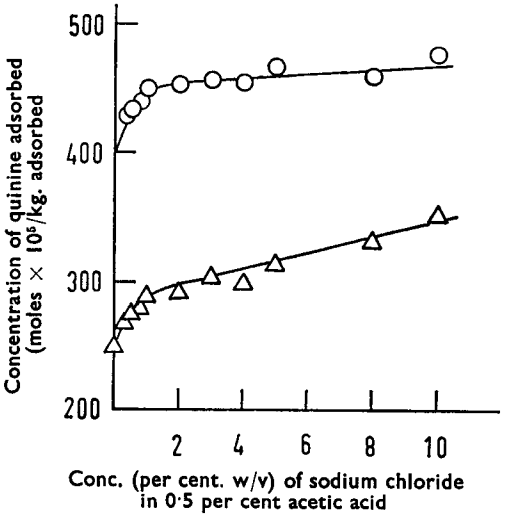


FIG. 6. Effect of sodium chloride on the adsorptive power of a quinine-selective and control adsorbent. ○ Quinine-selective adsorbent. △ Control adsorbent.

Increasing concentrations of sodium chloride up to 1 per cent in the solutions for adsorption measurements gave a significant increase in the adsorptive power of the selective adsorbent and to a slightly lesser extent of the control; beyond this concentration the increase of adsorptive power with concentration was less pronounced (Fig. 6).

The quantities of "unextractable quinine" in various batches of selective adsorbents are shown in Table I, together with the adsorptive power of the respective adsorbents.

Quinine was not liberated from the selective adsorbents when they were shaken with chloroform in the absence of sodium hydroxide.

Re-extraction of stored selective adsorbents reduced the amount of unextractable quinine and increased the adsorptive power of the adsorbent, while similar treatment of stored control adsorbents did not alter their adsorptive power (Table II).

The loss of water from some adsorbents on heating at 200° to constant weight is shown in Table III.

TABLE III

DETERMINATION OF THE LOSS OF WATER FROM QUININE-SELECTIVE AND CONTROL ADSORBENTS ON HEATING AT 200° TO CONSTANT WEIGHT

Control adsorbent	Per cent of loss of water (w/w)	Selective adsorbent	Per cent of loss of water (w/w)
B_1	15·5	Q_3	17·9
B_2	15·3	Q_4	19·4
B_3	18·0	Q_5	18·2
B_7	17·0	Q_9	19·9
B_8	16·9	Q_{10}	17·5
B_9	16·3	Q_{11}	20·2

DISCUSSION

Formation of Silica Gel

The formation of silica gel involves the elimination of water from orthosilicic acid to form spherical primary particles or micelles. As the gelation proceeds these micelles grow in size and condense together to give a very open but continuous structure extending throughout the medium and interpenetrated by the liquid phase. During drying, the

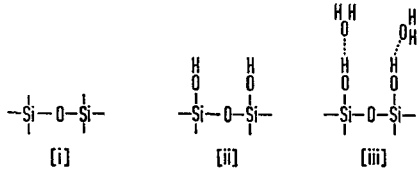


FIG. 7. Different types of chemical groups at the surface of silica gel. (i) siloxan group. (ii) silanol group. (iii) hydrated silanol group.

gel shrinks owing to the pressure exerted upon the structure by the surface tension of the liquid around the boundary of the gel. The packing density increases and the pore diameter diminishes; as certain particle to particle bonds break, portions of the network thus released come into contact with other members and new contacts and bonds are

formed. The shrinkage, essentially an irreversible process, proceeds until the mechanical strength of the gel can withstand the pressure of the surface tension (Iler, 1955). The rigid xerogel thus obtained consists of three dimensional silicon-oxygen lattice work (see Fig. 8). This aggregate structure accounts for the following characteristic features of silica gel; firstly it is highly porous due to the high specific surface area which can reach values of 1,000 $m.^2/g.$ (Klein, 1961). Most of this area is present as internal surface in the form of pores of variable shapes and diameters. Secondly the surface-energy is heterogeneous owing to different types of chemical groups and their local geometry on the amorphous surface (Klein, 1962). Under normal conditions it is possible to distinguish siloxane groups (Fig. 7, i), silanol group (Fig. 7, ii) and hydrated silanol groups with physically bound or free water (Fig. 7, iii).

Theory of the Formation of "Active-site" Footprints

In selective adsorbents, the foreign organic molecules dissolved in the liquid phase will be attracted to the silanol surface as the structure of the gel is built up. Some of the molecules will be mechanically trapped within the primary particles as they grow in size: others will be persorbed in the fine pores and acted upon by two or more neighbouring surfaces at the same time. The remaining molecules will be adsorbed to the surface while the gel structure is still flexible. On drying and subsequent extraction with methanol, the adsorbed and some of the persorbed molecules will be removed leaving on the gel surface their footprints and partial footprints to become part of the rigid structure of the adsorbent. The molecules trapped within the silica framework will not be extracted by this treatment.

We conclude for reasons given below that stereoselectivity is due to the formation of these "active-site" footprints in the selective adsorbents. These footprints will preferentially adsorb the molecules used to make them, rather than other molecules. Alternative compounds of not too dissimilar structure, having "like" configuration will fit into these footprints better than will their isomers, although not quite so well as the reference molecule itself (cf. Beckett and Anderson, 1957, 1959, 1960).

Evidence Against Stereoselectivity Being Due to Foreign Molecules Trapped Inside the Selective Adsorbent

After the methanol extraction of the gel formed in the presence of quinine, there remains some "unextractable" quinine in the product. Instead of footprints accounting for selectivity, it has been suggested that these trapped molecules may attract the adsorbate molecules and may account for differences in the adsorptive powers of selective and control adsorbents (Morrison, Worsley, Shaw and Hodgson, 1959). Our case against this explanation of selectivity and against any explanation based on differences in surface area per g. of selective and control adsorbent rests on the following evidence:

1. The selective adsorbents are stereoselective to enantiomorphs and diastereoisomers (Beckett and Anderson, 1959 and 1960). Furthermore,

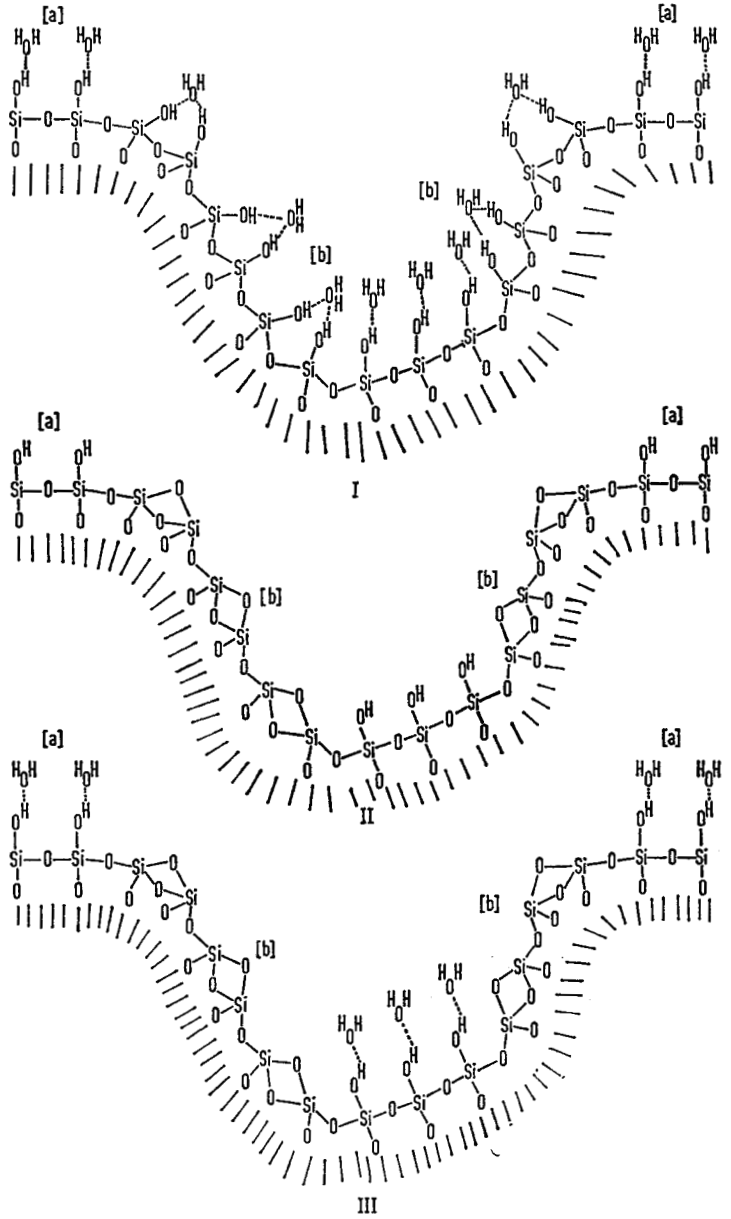


FIG. 8. Diagrammatic representation of a "footprint" and its deactivation. Position (a) represents the normal arrangement of silanol groups on the surface of silica gel. Position (b) represents the special arrangement of silanol and siloxan groups in the footprint.

I, active site. II, dehydrated surface. III, deactivated site.

if the trapped molecule in the adsorbent is an optical isomer, and if this isomer forms a racemic compound with its enantiomorph, then the attraction of the trapped L-isomer would be expected to be greater for its corresponding D- rather than for its L-adsorbate; that the reverse obtains implies footprints rather than attraction from the trapped molecules in the selective adsorbent.

2. If the trapped molecules led to selective attraction forces, it would be expected that an increase in the concentration of trapped molecules would give increased selectivity relative to the control. That there is no such correlation between the concentration of unextractable quinine and the adsorptive power of various quinine-selective adsorbents is seen from Table I.

3. Dry organic solvents passed through the selective adsorbents to remove water, abolished the selectivity relative to the similarly treated control, although the trapped quinine molecules in the selective adsorbents were not extracted by this treatment (Fig. 1). That changes in the surface area per g. of adsorbent were not an explanation of the loss of selectivity is indicated by the fact that both selective and control adsorbents before and after treatment adsorbed 5-aminoacridine, a molecule completely dissimilar to quinine, to the same extent (see Fig. 1).

4. Storage for some months of the selective adsorbents produced changes so that some of the previously "unextractable" quinine could then be extracted with methanol. This reduction in the concentration of trapped quinine *increases* rather than decreases the adsorptive power of the adsorbent for quinine while the adsorptive power of the similarly treated control did not change (Table II).

5. The change in selectivity when steam is forced through the adsorbent (Fig. 2) and the differences in the results of "dry" and "wet" heating of the adsorbent (Fig. 3) may be better explained by changes in surface footprints consequent on alteration of silanol groups (see later) rather than any explanation involving attraction forces from trapped molecules.

The Nature of the Footprints (Active Sites) and Modified Footprints

Implicit in the conclusion that footprints on the adsorbent surface and pores are responsible for the selectivity, is the acceptance that the arrangement of the silanol and hydrated silanol groups at these active sites differs from that of these groups in the control adsorbent surface or the ordinary surfaces of the selective adsorbents.

A diagrammatic representation of a hypothetical footprint resulting from removal of the foreign organic molecule from the surface of the silica gel is presented in Fig. 8, I; the differences in the relative orientation of some of the hydrated silanol groups in the footprint and general surface is depicted.

To account for the results presented herein, it is postulated that removal of water by treating the selective adsorbent with organic solvents, under the conditions specified, removes not only the free water but, *at the*

active sites also removes structural water molecules by converting some of the silanol groups (Fig. 8, I,b) to siloxane groups (Fig. 8, II,b). This can take place, we suggest, because of their relative orientation and because these groups probably have a higher energy than the normal silanol groups (see Fig. 8, I,a). When these "dehydrated" adsorbents are treated with water again, as occurs in the adsorption experiments, the silanol *but not the siloxane groups* will be rehydrated (see Fig. 8, III). On the other hand, the control adsorbents do not possess these abnormally orientated high energy hydrated silanol groups (cf. footprint and general surface of Fig. 8, I of the selective adsorbent), and when dehydrated with organic solvents and then rehydrated, the silanol groups will be regenerated mainly as in the original gel. Thus the adsorptive power of the control will be unchanged by treatment with the organic solvents whereas that of the selective adsorbent will be decreased to a level similar to that of the control.

The above argument implies that upon drying, a selective adsorbent should show a greater loss of water than a control adsorbent; the results in Table III agree with this deduction.

It also follows that the silanol-bound water plays an important role in the preservation of the high energy silanol groups in the footprint. The difference in the rate at which dry heat and heating the adsorbent in water causes loss of selectivity (Fig. 3) may be explained as follows: Water is bound firmly to silanol groups and is therefore slowly removed and in consequence the "dehydration" of silanol to siloxane groups at the active site upon dry heating at 90° is also slow. Although bombardment of the adsorbed water at the active site with water molecules permits hydration of the silanol groups, it involves interchange of water molecules and removes the protection of the adsorbed water at the high energy silanol bonds with the resultant effect of converting these to siloxane bonds with attendant loss of selectivity. A similar explanation accounts for the rapid loss of selectivity upon passing steam through the adsorbent (see Fig. 2).

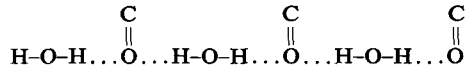
The gradual loss of selectivity on storage (Beckett and Anderson, 1959) may also be explained by a slow conversion of some silanol to siloxane groups. During storage, it is to be expected that, as in the initial formation of the gel, there will be some slight breaking and making of bonds. This will result in some of the trapped molecules becoming less firmly enclosed in the silica lattice and so re-extraction of stored selective adsorbent will remove some previously trapped molecules with the formation of new active sites (see Table III).

Active Sites in Selective Adsorbents as Model Drug Receptors

Since suitable biologically-active reference molecules may be used to make the footprints in the surface of the adsorbent, and physically bound water on the silica surface plays an important role in the selectivity, these footprints may be regarded as simple models of drug receptors and enzyme active sites. Because the adsorption of some cinchona alkaloids to certain selective adsorbents is reversible (Beckett and Anderson, 1957),

then water molecules bound to the high energy silanol bonds must be retained during the adsorption of these alkaloids to their respective footprints. Consequently, binding of these molecules to active sites in selective adsorbents involves substantial binding to oriented hydroxy groups of adsorbed water molecules rather than a simple binding to a surface containing silanol groups; approximation to a biological surface is therefore closer than is first apparent.

As with selective adsorbents, adsorbed water is intimately involved in the structure and stabilisation of biological surfaces. For instance, X-ray diffraction and infra-red measurements on collagen (Harrington and Von Hippel, 1961) show that crystalline portions of the structure are probably stabilised by doubly hydrogen-bonded water bridges giving rise to continuous chains of structurally incorporated water along the fibre axis in the diffracting regions (see I).



(I)

Fraser and Macrae (1959) concluded that the bound water molecules are primary singly bonded to the carbonyl groups which project radially outward from the collagen molecules.

Further similarities between the characteristics of active sites of selective adsorbents and those of biologically active sites are as follows:

1. Enzymes and apparently many drug receptors differentiate between enantiomorphic forms of compounds (Beckett, 1959) as do footprints in selective adsorbents when asymmetric molecules are used to make footprints (Beckett and Anderson, 1959).

2. Changes in certain groups in enantiomorphic pairs of compounds may alter the affinity to biological receptors but the relative affinity in the stereochemical sense is unaltered; the same applies with stereoselective adsorbents (see Table IV) (Anderson, 1961).

3. Enzyme active sites may be deactivated by heating, treatment with organic solvents and contact with strong acids (Dixon and Webb, 1960) and these effects are irreversible because of alteration of the surface characteristics; similarly, active sites in selective adsorbents may be altered by these treatments by altering the organisation at the surface and thus the hydration and binding forces (see Fig. 2, 3 and 4).

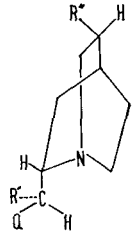
4. The interaction of enzymes and their substrates may be reversibly altered by changing the pH of the medium in which they interact (Dixon and Webb, 1960); similarly, the changes in interaction of quinine with a "quinine-footprint" is different from that of quinine with the control adsorbent upon changing the pH of the medium as indicated in Fig. 5 and these effects are reversible.

5. The degree of binding and the interaction of organic molecules with biological surfaces may be altered by the presence of salts. For

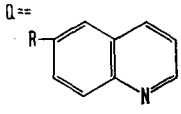
example the interaction of hexylresorcinol with the cytoplasmic membrane of *Escherichia coli* is increased by introducing sodium chloride into the biophase (Beckett, Patki and Robinson, 1959). Because the increase in the amount of quinine bound to a quinine-selective adsorbent is greater than that observed with the control up to concentrations of sodium chloride of 1 per cent, the interaction of quinine with active sites must have been increased (see Fig. 6). Further progressive increases in sodium chloride concentrations would be expected to increase the binding to both selective and control adsorbent by reducing the solubility of quinine in the aqueous medium; thus the contribution of the active site selective binding to the total binding will be reduced, so differences in selective and control will diminish as shown in Fig. 6.

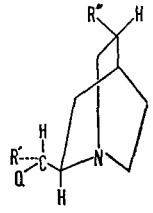
TABLE IV

EFFECT OF CHANGES OF CERTAIN GROUPS IN THE ADSORBATE MOLECULES ON THE ADSORPTION (Anderson 1961)



Configuration of
quinine (R = OMe)
cinchonidine (R = H)





Configuration of
quinidine (R = OMe)
cinchonine (R = H)

Control adsorbent

R′	R″	Adsorption of quinine type configuration	Adsorption of quinidine type configuration
OH	$CH = CH_2$	146	142
H	$CH = CH_2$	212	210
H	COOH	134	134

Quinine-selective adsorbent

R′	R″	Adsorption of quinine type configuration	Adsorption of quinidine type configuration
OH	$CH = CH_2$	394	232
H	$CH = CH_2$	480	410
H	COOH	240	220

All adsorption figures are calculated at an equilibrium concentration of 20×10^{-5} M/kg. of adsorbent.

We conclude, therefore, that active sites in stereoselective adsorbents may be used as rough models of active sites in enzymes and of drug receptors in addition to the proven use of these adsorbents in the determination of configuration of organic molecules. The probability that the stereoselective adsorbents may also act as catalysts (cf. oriented bonding of molecules to massive catalysts in hydrogenation) because of

the oriented binding in the active sites, is also receiving attention as is their use in separation techniques.

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The paper was presented by MR. YOUSSEF.