with a molecular weight of 25000 as determined by comparison with protein standards. The molecular weight of the enzyme was in good agreement with that reported by Genell *et al.*[4]

One mg of the purified elastase hydrolyzed 1.53 μmol of N-succinyl-L-alanyl-L-alanyl-L-alanine *p*-nitroanilide and solubilized 1.45 mg of elastin per min.

The immunological properties of the rat elastase were analyzed by the three radioimmunoassay methods for rat elastase, human elastase-1 and hog elastase. As shown in Table II, the rat elastase did not react with the antibodies against human and hog elastase, but the antibody against rat elastase showed slight cross-reactivity with human elastase-1.

Immunoreactive Elastase in Tissues

A representative standard curve for the radioimmunoassay of rat elastase is shown in Fig. 3, and indicates that 50% of the total ^{125}I-labeled elastase bound to the antibody can be replaced by 35 ng of elastase per ml.

Immunoreactive elastase was estimated in several tissues, and the results are shown in Fig. 3. The aorta and spleen homogenates contained a detectable amount of immunoreactive elastase, but the lung, kidney and liver did not. The immunoreactive elastase detected in the aorta, which was partly characterized by Miyake *et al.*,[13] and that in the spleen both require further characterization.

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Adsorption of Benzothiadiazines by Carbon Black from Aqueous Solution, and Related Phenomena[1,2]

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The adsorption of benzothiadiazines by carbon black from aqueous solution was investigated in detail. The adsorption isotherms obtained were well described by the Langmuir equation. It was shown that the adsorbability was positively related to the relative diuretic activity. The adsorption was found to be reversible with change of temperature in a perturbation experiment, suggesting a physical adsorption mechanism. The pH of the buffer solution had no clear effect on the adsorption of benzothiadiazines near the neutral pH region. It was shown qualitatively by thin-layer chromatography that the hydrolysis of benzothiadiazines was accelerated by carbon black.

Keywords——adsorption from aqueous solution; benzothiadiazine; carbon black; Langmuir equation; pH dependence; temperature dependence; hydrolysis

1) This paper forms part XXXIV of "Physico-chemical Approach to Biopharmaceutical Phenomena." The preceding paper, Part XXXIII: H. Ueda and T. Nagai, *Chem. Pharm. Bull.*, **28**, 1415 (1980).
2) A part of this work was presented at the 93rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1974.
3) Location: *Ebara 2-4-41, Shinagawa-Ku, Tokyo, 142, Japan.*

Benzothiadiazines are widely used as diuretic agents in clinical treatment, and the relationship between their physico-chemical properties and pharmacological activities has been studied by several investigators.[4-7] It was demonstrated in previous papers that drug adsorbability by carbon black is related to various biopharmaceutical phenomena.[8] The present study was carried out to investigate the adsorption of benzothiadiazines in relation to other physico-chemical properties and biological activities of the drugs.

Experimental

Meterial——Carbon black, marketed as "Seisei Shirasagi" by Takeda Chemical Ind., Ltd., was used after the treatment described in a previous paper.[9] All the benzothiadiazine derivatives used were recrystallized from dilute alcohol. Commercial chloraminophenamide (4-amino-6-chloro-*m*-benzenedisulfonamide) was used after recrystallization from dilute alcohol.

Batch Procedure for Determination of the Amount of Drug Adsorbed——Unless otherwise stated, the adsorption procedure was the same as that described in a previous paper,[9] except that 10 mg of carbon black (CB) was added to 100 ml of 1/30 M phosphate–citric acid buffer solution (pH 6.5) of each drug.

Quantitative Determination of Benzothiadiazine Derivatives——The concentrations of drugs were determined by the ultraviolet absorption method, using a Hitachi 323 spectrophotometer at the following wavelengths: chlorothiazide, 281.0 nm; hydrochlorothiazide, 272.5 nm; bendroflumethiazide, 273.5 nm; trichlormethiazide, 269.5 nm; cyclothiazide, 272.5 nm; methychlothiazide 269.5 nm; cyclopenthiazide, 273.0 nm; hydroflumethiazide, 274.0 nm; chloraminophenamide, 264.0 nm.

Determination of the Degradation Products of Trichlormethiazide by Thin-Layer Chromatography (TLC)——The filtrate of the mixture of trichlormethiazide and CB shaken in the adsorption experiment was evaporated to dryness under reduced pressure, and the residue was extracted with a small amount of ethanol. The extract was concentrated under reduced pressure, spotted on a plate precoated with 0.25 mm of silica gel G_{254} and developed to a height of 15 cm from the origin in toluene/xylene/dioxane/isopropanol/ammonium hydroxide (25%) (10: 10: 30: 30: 20, v/v).[10] The plate was air-dried and examined using a Hitachi 323 spectrophotometer with a scanning device at a wavelength of 270 nm.

Results and Discussion

The adsorption isotherms obtained for all the benzothiadiazines tested were well described by the Langmuir equation under all experimental conditions, as shown in Fig. 1. The solubility of benzothiadiazines depended sensitively on pH. Benzothiadiazines other than the four drugs shown in Fig. 1 were only slightly soluble under these experimental conditions. Therefore, experiments should be carried out in buffer solution containing 5% ethanol. The effect of a third component on the adsorption has already been reported in detail.[11] Consequently, the adsorption from buffer solution containing 5% ethanol was a useful indicator of relative adsorbability among benzothiadiazines.

It has been reported that a correlation exists between the partition coefficients (ether/water) and biological activities (natriuretic activity) of four benzothiadiazines,[5] and also between the partition coefficients (octanol/pH 6.5 buffer) of forty-one benzothiadiazines and their activities.[4] However, the correlation in the latter case was not good, especially as regards the relative diuretic activity in man (oral administration). On the other hand, as shown in Fig. 2, a good correlation (correlation coefficient $r=0.768$) was found between adsorbability

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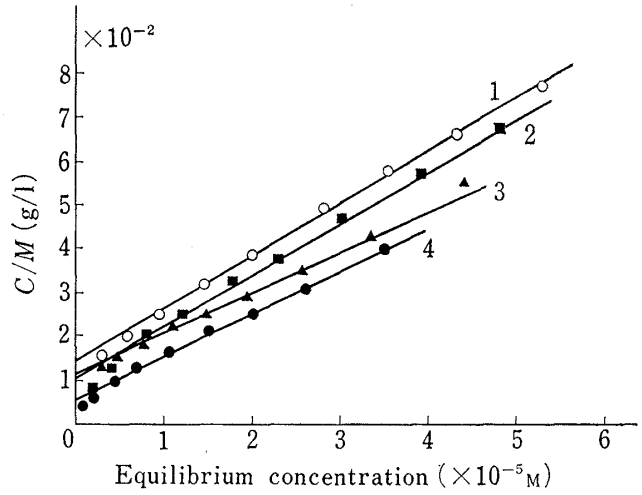

Fig. 1. Langmuir Plots of the Adsorption of Benzothiadiazines by Carbon Black from 1/30 M Phosphate-citric Acid Buffer Solution (pH 6.5) at 35°

C: equilibrium concentration (M). M: amount adsorbed (mol/g).
1: chlorothiazide (○). 2: hydrochlorothiazide (■).
3: hydroflumethiazide (▲). 4: trichlormethiazide (●).

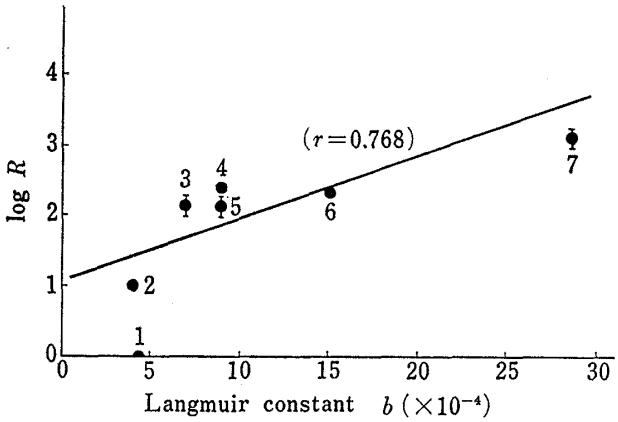

Fig. 2. Relationship between the Langmuir Constant b and the Relative Diuretic Activity R

R: Relative diuretic activity in man (oral).[4]
1: chlorothiazide. 2: hydrochlorothiazide.
3: trichlormethiazide. 4: cyclothiazide.
5: bendroflumethiazide. 6: methychlothiazide.
7: cyclopenthiazide.

and relative diuretic activity[4] for seven benzothiadiazines in this study. This correlation was improved to $r=0.853$ by excluding the data for chlorothiazide, the structure and pK_a of which are somewhat different from those of the other compounds. In addition, a good relationship was observed between the adsorbability and relative diuretic activity(in the dog, *i.v.*).[5] These results suggest that measurements of adsorption by carbon black could give useful information regarding the biopharmaceutical behavior of drugs.

In order to check the effect of the pH of the buffer solution on the amount adsorbed, the adsorption of hydrochlorothiazide by carbon black was determined at various pH values. As shown in Table I, pH had no distinct effect on the adsorption in the range of pH 6.5 to 9.0. However, a decrease of amount adsorbed and a change in the ultraviolet (UV) spectrum of the filtrate were recognized in the high pH region, possibly due to decomposition of the drugs themselves. This might also be explained by considering that pK_{a1} and pK_{a2} of hydrochlorothiazide are about 9.0 and 10.3, respectively, and thus that the hydrophobic and hydrophilic balance of the molecule changes under the present experimental conditions. In other words,

Table I. Effect of pH on the Adsorption of Hydrochlorothiazide from Aqueous Solution at a Constant Equilibrium Concentration 2×10^{-5} M by Carbon Black at 35°

pH	Amount adsorbed ($\times10^4$ mol/g)
6.5	5.9
7.5	5.3
8.0	5.9
8.5	5.7
9.0	5.0
10.0	2.5
11.0	1.5

pH 6.5 : McIlvaine buffer.
pH 7.5—9.0: Tris-HCL buffer.
pH 10.0—11.0: $NaHCO_3$-Na_2CO_3 buffer.

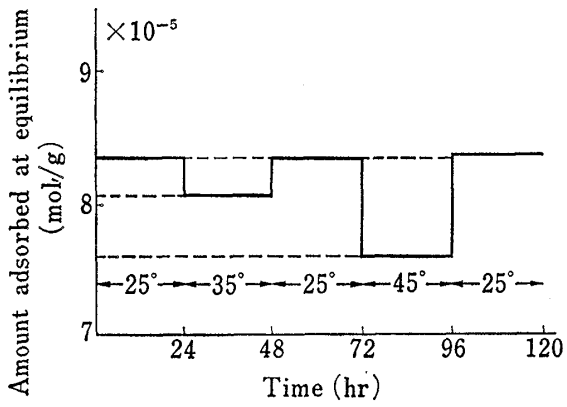

Fig. 3. Perturbation Experiment on the Adsorption of Hydrochlorothiazide from Aqueous Solution initially Containing 1.21×10^{-4} M

the increase in the amount of dissociated molecules with increase of pH may result in a distinct decrease in the hydrophobicity of the molecule, thus causing a decrease in adsorption.

Next, in order to investigate the mechanism of adsorption of benzothiadiazines by carbon black, a perturbation experiment was carried out at various temperatures. It was found that the amount adsorbed decreased with temperature. Upon change of temperature in the perturbation experiment, the adsorption was found to be reversible, as shown in Fig. 3.

In view of the results obtained above, it was considered that the adsorption of benzothiadiazines by carbon black occurred by a physical adsorption mechanism.[12] Therefore, calculating the thermodynamic functions in the manner described in the previous paper,[8a] the results obtained from the data at 25°, 35°, and 45° were $\Delta F = -6.98$ kcal/mol, $\Delta H = -4.02$ kcal/mol, and $\Delta S = 9.60$ e.u. at 35°. The positive entropy change suggested that structural change of the "iceberg" region around the adsorbate molecules took place during the adsorption process, as in the case of barbituric acid derivatives[8a] and sulfonamides.[12] The above results also support the view that adsorption proceeds by hydrophobic interaction between the hydrophobic moiety of the hydrochlorothiazide molecule and the carbon black surface.

The UV absorptions of trichlormethiazide, cyclothiazide, bendroflumethiazide and cyclopenthiazide in the filtrate after the adsorption experiment were shifted to shorter wavelengths (about 3—4 nm). According to the results on the stability of benzothiadiazines reported by Yamana *et al.*[13] and the hydrolysis study of hydrochlorothiazide reported by Mollica *et al.*,[14] these phenomena were presumed to be due to the formation of chloraminophenamide or analogous compounds by the hydrolysis of benzothiadiazines. Therefore, the filtrate of trichlormethiazide was analyzed by TLC.

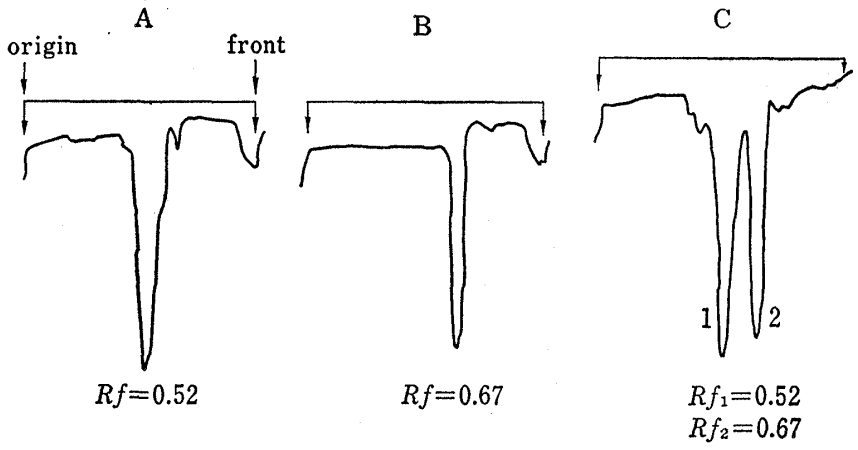

Fig. 4. Densitograms of Thin Layer Chromatograms

A: trichlormethiazide.
B: chloraminophenamide.
C: trichlormethiazide after adsorption experiment.

As shown in Fig. 4, the *Rf* values on TLC densitograms suggested that the degration product was identical with chloraminophenamide, as described above. These phenomena did not occur in the absence of carbon black during the experiment. Therefore, it was considered that the carbon black acted as a catalyst of hydrolysis.

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The adsorbability of chloraminophenamide [$a=6.45\times10^{-4}$ (mol/g), $b=4.19\times10^{4}$ (l/mol)] was similar to that of hydrochlorothiazide. Thus, it seemed likely that the adsorbability of trichlormethiazide, *etc.*, was larger than the values shown in Fig. 2. In conclusion, the present results supported the previous finding that there is a relationship between adsorbability and relative diuretic activity.

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Photolysis and Thermolysis of 3-Diazothiochroman-4-one and Related Compounds

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The photochemical and thermal reactions of 3-diazothiochroman-4-one (**2a**), 3-diazochroman-4-one (**2b**), 3-diazo-1-methyl-1,2,3,4-tetrahydroquinolin-4-one (**2c**), and 2-diazo-1-tetralone (**2d**) were investigated. Wolff rearrangement was observed with **2a**, **2c**, and **2d** only under photolytic conditions. In the case of **2a**, migration of the thioether group competed with the above reaction.

Keywords——tosyl azide; α-diazoketone; episulfonium ylide; carbene; Wolff rearrangement; participation of thioether; dimerization

Wolff rearrangement of cyclic α-diazoketones has been utilized as a method for ring contraction (Chart 1).[2] In view of the known susceptibility of diazo compounds substituted at the β-position with sulfur to undergo carbenic rearrangement of the thioether group,[3] it seemed of interest to investigate the photochemical and thermal behavior of cyclic α-diazoketones having a sulfur atom at the γ-position. One such molecule is 3-diazothiochroman-4-one (**2a**), which could undergo either phenyl migration (Wolff rearangement) or rearrangement of the thioether group, or both. We present the results of a study of the photochemical and thermal reactions of **2a**. For comparison, the behavior of the related compounds **2b**—**d** is also described.

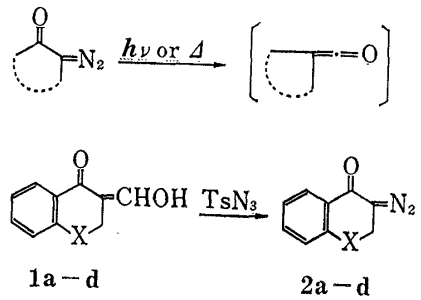

Chart 1

The starting α-diazoketones **2a**—**d** were synthesized by treatment of the known α-hydroxymethyleneketones **1a**—**d** with tosyl azide in ether in the presence of diethylamine.[4] All the compounds showed two strong infrared (IR) absorption bands at 2070—2080 cm^{-1} (N_2) and 1620—1630 cm^{-1} (C=O).

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