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SHORT COMMUNICATIONS

The binding of salicylate to human serum

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IN 1908, JACOBY[1] showed that salicylate was bound *in vivo* to the protein or polypeptides in rabbit serum. Chabanier *et al.*[2] demonstrated a similar effect *in vitro* using dialysis through a semipermeable membrane. Albumin has been shown to be the component of serum predominantly responsible for binding salicylate in man[3] and in the cow.[4] Analysis of binding data by a Scatchard plot[5] has indicated more than one species of binding site on the albumin molecule,[4] and Davidson and Spitzer[6] suggested that there were two kinds of interaction, one with a few sites of high avidity, the other with more sites of weak avidity. They found that blocking free amino groups on the albumin molecule resulted in loss of binding avidity[7] which conforms with the suggestion of Lindenbaum and Schubert[8] that binding of salycylate results from electrostatic interaction with the cationic centres in albumin.

A consequence of this type of binding would be a decrease in binding with increase in serum pH. The only observation on pH dependence is by Smith *et al.*[9] who found no significant difference in binding when the pH was changed from 7·41 to 8·12. The magnitude of any change that does occur is important since it is customary to treat salicylate poisoning by introducing an alkalosis so that an alkaline urine will be passed. If the change in plasma pH resulted in release of bound salicylate from protein, the increase in free drug could affect the patient adversely since it is probably only the free drug that exerts any pharmacological effect.[3]

The capacity of human serum to bind salicylate and the effect of pH change have been investigated using an ultrafiltration apparatus that permits rapid ultrafiltration with close control of pH and temperature. The use of ^{14}C-labelled salicylate permits the accurate measurement of concentrations of salicylate down to 0·2 mg/100 ml.

METHODS

Two separate pooled sera were prepared (represented by ● and ▲ in the figures) each from four normal individuals. The albumin conservation of each pool was adjusted to 3·8 g/100 ml by dilution with 0·9% saline. Salicylate was added to both as the sodium salt in variable amounts up to 100 mg% together with a trace amount of sodium ^{14}C-salicylate equivalent to 0·2 mg/100 ml. Ultrafiltration was carried out at 37° using the apparatus of Walker.[10] 5 ml samples of serum were equilibrated with 5% CO_2 and ultrafiltered through Visking tubing at 4 atm pressure. 2·5 ml ultrafiltrate was collected in 30–45 min, the first 1·5 ml was discarded and the final 1 ml taken for analysis.

Total salicylate was estimated by the colorimetric method of Trinder.[11] The proportion bound was estimated by counting total and ultrafilterable salicylate in a Nuclear Chicago liquid scintillation counter using hyamine-OH to bring protein into solution. Colorimetric estimation of the salicylate concentration in selected ultrafiltrates gave values for the proportion bound which were identical with those derived from radioactivity estimations.

Albumin was estimated by an Autoanalyser method using bromocresol green.[12]

Serum pH was adjusted by adding the required amount of N/10 HCl or N $NaHCO_3$ before equilibrating with 5% CO_2.

RESULTS

All results are expressed in mg/100 ml sodium salicylate unless otherwise stated.

Adsorption to cellophane. A definite adsorptive loss was noted with aqueous standards and with serum. Only after the filtration of 1·0 ml did the salicylate concentration in the ultrafiltrate reach the initial value of an aqueous standard or a constant level in the case of serum.

TABLE

Initial concentration	Successive 0·5 ml aliquots			
	I	II	III	IV
36·5 aqueous	32·0	34·5	36·5	36·5
33·5 aqueous	31·5	32	33·5	33·5
30·0 serum	11·0	12·0	12·0	12·0
47·5 serum	19·5	24·0	25·0	25·0
57·0 serum	35·0	36·0	36·5	36·5

Salicylate concentration in aqueous and serum standards and in their ultrafiltrates. (mg sodium salicylate/100 ml).

Binding at pH 7·4. The relationship between bound (B) and free salicylate (F) is shown in Fig. 1. The same data has been set out using a Scatchard plot in Fig. 2. Here the abscissa is B/F.A. and the ordinate B/A, the concentrations of B, F and A being transformed into molar units. With a single species of binding site, the Scatchard plot gives a linear relationship between B/A and B/F.A. with

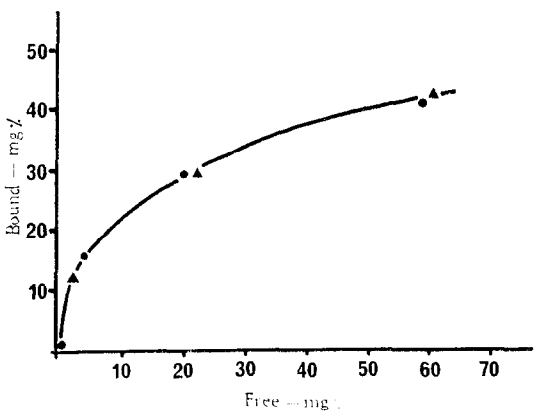

FIG. 1. Salicylate bound to normal serum.

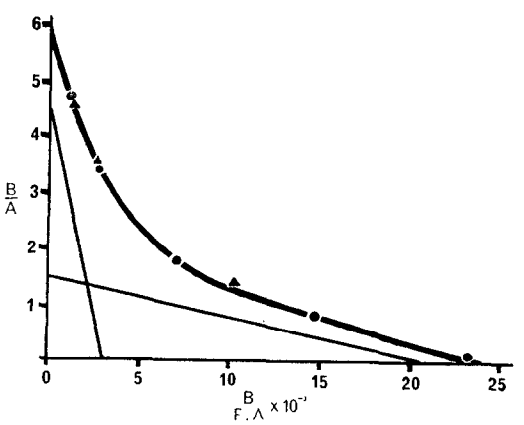

FIG. 2.

slope -k (k = dissociation constant) and intercept on the B/A axis equal to n, the number of binding sites per molecule of albumin. The present data clearly does not conform to one species of binding site. The curved line in Fig. 2 corresponds to the summation of the two straight lines representing $n = 1{\cdot}5, k = 0{\cdot}7 \times 10^{-4}$; $n = 4{\cdot}5, k = 15 \times 10^{-4}$. It can be seen that the experimental points do not deviate significantly from the curved line. The present data confirms the conclusion of Davidson and Spitzer[6] that there are two species of binding sites: one with low capacity (about 12·5 mg salicylate/100 ml in a normal plasma) and with high avidity; the other with three times this capacity but with low avidity.

pH *effect.* The effect of change in pH at a total serum salicylate concentration of 50 mg/100 ml is shown in Fig. 3. There is a small fall in binding as the pH increases through the physiological range with a more pronounced fall above pH 8·0.

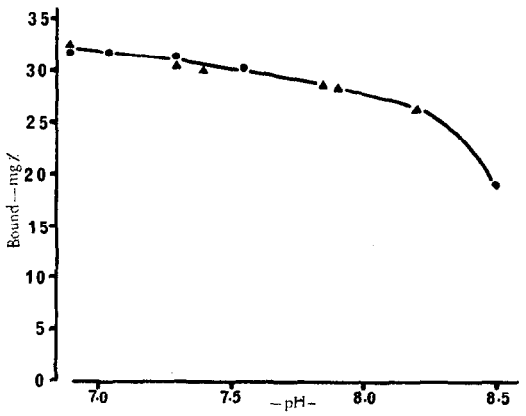

FIG. 3. Relationship between pH and binding.

DISCUSSION AND CONCLUSIONS

Contrary to the findings of Lester[13] an appreciable adsorption loss of salicylate onto cellophane was noted with aqueous and plasma samples. This may be due to the relatively much greater surface area exposed to the solution in the present apparatus.

The presence of two species of binding sites is confirmed with three times as many weak as there are strong sites. The binding capacity is continuously variable with pH so that an exact integral number of sites would not be expected when calculated by Scatchard's procedure. Some alubumin molecules have fewer binding sites available than others, either because of inter-molecular interaction or because of a pH dependent ionisation of binding sites. The observed effect of pH is consistent with ionic binding to a reactive group having a pK of 8·5–9·0.

Over the physiological range of blood pH the magnitude of the change in binding is very small and can have no relevance to the management of salicylate poisoning.

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The influence of cyclic 3′, 5′-AMP and theophylline on oxygen consumption of rats

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THERE is much evidence that glycogenolytic and lipolytic actions of catecholamines are mediated by the formation of cyclic 3′, 5′-AMP (adenosine-3′, 5′-monophosphate).[1–4] Calorigenic action of catecholamines, on the other hand, is probably the consequence of catecholamine induced glycogenolysis and lipolysis.[5–8] Cyclic 3′, 5′-AMP, therefore, should be expected to possess calorigenic action too. However, the influence of cyclic 3′, 5′-AMP on metabolic rate has not been investigated till now.

Cyclic 3′, 5′-AMP is inactivated by a phosphodiesterase.[9] Methylxanthines such as caffeine, theophylline or aminophylline, inhibit phosphodiesterase,[10,11] and thereby may cause accumulation of endogenous 3′, 5′-AMP.[12] Methylxanthines thus also should be able to increase metabolic rate. In fact, caffeine and aminophylline are said to augment oxygen consumption.[13–16] But these investigations were performed only in awake animals and men. Therefore it is unknown, whether the calorigenic responses in those experiments were due to a real increase of basal metabolism or to increased motility because of the centrally stimulating actions of methylxanthines.

Thus, it was necessary to investigate the influence of cyclic 3′, 5′-AMP (Sigma Chemical Company) and theophylline (Theophyllinum, DAB 6) on the oxygen consumption of rats which had been anesthetized with urethane (1·2 mg/kg). Anesthesia by urethane excludes motility, but does not diminish calorigenic action of catecholamines in rats.[17]

Adult male wistar rats with a weight from 260 to 340 g were used. Oxygen consumption and respiratory quotient (RQ) were measured with NOYON's Diaferometer (Kipp and Zonen, Delft). After three determinations of basal metabolic rate within 30 minutes the drugs were injected, and oxygen consumption and RQ were recorded continuously throughout 120 min. The increase of oxygen consumption over basal metabolic rate was integrated for each animal and served (expressed as ml O_2 per 100 g body wt.) as an index of calorigenic response. Ambient temperature throughout the experiments was 28°. Rectal temperature of the rats lay between 36° and 37°.

The results of our experiments with regard to oxygen consumption are compiled in Table 1. Basal values of oxygen consumption ranged between 1·9 and 2·1 ml/min/100 g. Due to physiologic variations of metabolic rate, there was a small integrated increase of oxygen consumption in control animals injected with saline, but mean oxygen consumption did not exceed more than 8 per cent over basal values. In preliminary experiments 3′, 5′-AMP, injected intraperitoneally (100 mg/kg), did not produce a calorigenic response. The same was true for the intravenous application of 3′, 5′-AMP (Table 1): Integrated augmentation of oxygen consumption did not differ significantly from control values, though the doses of 3′, 5′-AMP were high (50 and 100 mg/kg).

The failure of 3′, 5′-AMP to increase oxygen consumption could have been the consequence of a rapid destruction by phosphodiesterase. Therefore, in another series of experiments theophylline (6·6 mg/kg i.p.) was given 30 min before cyclic 3′, 5′-AMP. Yet there was no increase of oxygen consumption in this group either (Table 1).