STUDIES CONCERNING THE NATURE OF THE SECRETORY ACTIVITY OF THE ISOLATED RINGER-PERFUSED FROG LIVER

II. The Inhibitory and the Promoting Influence of Organic Electrolytes and Non-Electrolytes upon the Secretion of Dyestuffs*

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INTRODUCTION

As described in the preceding paper (Höber (1)), the isolated Ringer-perfused frog liver is characterized by its ability to collect from the circulating fluid a great number of dyestuffs to such a degree that they appear in the secretion many hundred times concentrated. Now, this activity of the surviving gland can be abolished temporarily in a very striking way by substituting in the perfusion fluid 1/8 to 1/10 of the NaCl with the isosmotic amount of certain organic non-electrolytes or electrolytes. After a short time the color of the secretion fades, often completely, but reappears after resumption of perfusion with Ringer (Höber (2), Valdecasas (3)). Non-electrolytes producing this inhibitory influence are chiefly the disaccharides, hexoses, pentoses, and hexahydric, pentahydric, and lower polyhydric alcohols as well as amino acids and succinamide. Electrolytes acting in the same way are the sodium salts of the lower fatty acids (acetate, propionate), of lactic acid, and of amino dicarboxylic acids. It appears that all these inhibitory substances have a strong hydropolarity and therefore lack surface activity. The opposite action, promotion of the secretory power of the liver, has been known for a long time to be exerted by salts of the bile acids. A similar effect has been observed with some anesthetics, with saponin, and with salts of some organic bases and of some higher fatty acids. In contrast to the group of inhibiting agents these substances are characterized by surface activity. Investigation of their influence pre-

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sented some difficulty, since they cause irreversible loss of activity, unless the concentrations are kept below a low, somewhat variable limit and unless the application is restricted to a short and likewise variable period. Further investigation of these phenomena seemed to be desirable in order to throw more light upon the unknown mechanism of one of the main features of glandular activity.

EXPERIMENTS

I. Inhibition of the Secretion of Different Dyestuffs

Since the earlier experiments were concerned with only a small number of dyestuffs, we decided to extend the work in order to determine whether any generalization could be made concerning the relationship of the physicochemical character of the dye to the aforementioned inhibitory effect. Table I summarizes four experiments with (1) a basic dye, (2) a lipoid-insoluble acid dye, (3) a lipoid-soluble acid dye, and (4) a mixture of one lipoid-soluble and one lipoid-insoluble acid dye. Each dye was injected into the liver through the abdominal vein at a concentration of 0.0005 per cent, as described in the previous paper (1).[1]

TABLE I

The Inhibitory Effect of Mannitol and of Sucrose

	I	II	III	IV	V	VI
1. Rhodamin 3B (+ 0.027 mol mannitol)	250	250	**125**	**60**	**80**	250
2. Eriocyanin A (+ 0.027 mol mannitol)	250	**180**	**60**	**0**	375	
3. Orange R (+ 0.027 mol sucrose)	4000	4000	**2000**	**90**	**60**	500
4. Orange R (+ 0.027 mol mannitol)	120	**0**	167			
Patent blue V (+ 0.027 mol mannitol)	120	**0**	81			

From these and a great number of analogous experiments it appears that neither lipoid solubility nor the acidic or the basic character of the dyestuff has a special bearing upon the result.

The inhibitory action of the aforementioned organic substances might be thought of as attributable to alterations in the circulatory conditions. As a matter of fact, the decrease in dyestuff concentration is usually associated with a fall in the amount of secretion. However, this decrease is by no means invariably connected with a diminished perfusion rate, but,

[1] The figures in Table I indicate the concentration ratios during the experimental periods I to VI, each of which extended over one-half to one hour. The numbers in bold faced type are the concentration ratios observed in the presence of the non-electrolytes indicated.

on the contrary, appears to be associated with a more rapid as well as a slower perfusion (Table II). A definite connection between circulation and dyestuff concentration in the secretion does exist as can be shown by simply changing the perfusion pressure without the addition of any of the organic substances. Raising the pressure raises the amount of secretion and diminishes the concentration ratios; reducing the pressure has the opposite effect (Table II). By these and many other data we are led to conclude that the inhibitory action of the organic substances applied must be looked upon as a direct influence upon the secretory structure and not as being brought about indirectly by alteration of the circulatory conditions.

TABLE II

Perfusion Rate and Secretory Activity

		Perfusion	Secretion	Concentration ratio	Dye secreted mg./hr. × 10^4
		cc./hr.	*mg./hr.*		
Eriocyanin A	—	200	3	198	30
"	+ 0.027 mol adonitol	80	**1**	**60**	3
"	—	120	4	500	100
Ponceau 2R	—	65	10	240	120
"	+ 0.027 mol mannitol	75	**8**	**62**	25
"	—	52	12	660	400
Orange R	—	60	5.7	5350	1525
"	+ 0.009 mol succinate	60	**2**	**13**	1.3
"	—	70	5	1080	270
Eriocyanin A	at 10 cm. H_2O pressure	48	2.5	800	100
"	20 "	198	**9**	**40**	**18**
"	10 "	92	2.5	200	25

Attention had been turned by previous work (Höber and Titajew (4), Valdecasas (3)) to the fact that those organic electrolytes and non-electrolytes which appear to inhibit the liver activity are characterized by common chemical and physicochemical features, which may be essential for their action; namely, the possession of polar groups, —COOH, —OH, and —NH_2. For this reason, the molecules are strongly hydropolar, surface-inactive, and lipoid-insoluble and they exhibit anti-dispersing, consolidating properties toward hydrophilic colloids. In recent experiments we have extended our study to the Na salts of some markedly hydropolar acids, to malonate, succinate, glutarate, gluconate. They appeared

to exhibit vigorous but reversible effects (for the effect of succinate see Table II), whereas glycolate displayed a considerably weaker inhibiting influence.

II. Organic Substances Promoting Liver Secretion

The salts of the bile acids are the only substances which it is generally agreed show a definite choleretic power. Their essential function in the intestine seems to rest upon their hydrotropic properties (Neuberg (5)), *i.e.* the capacity to bring into aqueous solution substances which otherwise

TABLE III

Effect of Benzene Sulfonate at Various Concentrations

Mol benzene sulfonate	Rate of secretion	Concentration ratio	Dye secreted mg./hr. × 10⁴
	mg./hr.		
—	1.25	400	25
0.22×10^{-2}	2	250	25
"	4	500	100
"	4	750	150
—	5	600	150
—	1	500	25
0.45×10^{-2}	2	500	50
"	7	300	110
"	4	500	100
—	6	500	150
—	1	500	25
0.9×10^{-2}	2	125	13
"	2	120	12
"	3	40	6
—	4	250	50
—	2	600	60
3.6×10^{-2}	3	300	45
"	5	80	20
—	6	30	9

are scarcely soluble, like higher fatty acids and sterols. This is brought about by their polar-non-polar structure, the organophilic portion of the molecule being strongly attached to the undissolved substance, the hydrophilic portion pulling the substance towards the water. This concept was an incentive to testing other hydrotropic substances for possible choleretic power. In this respect, we have studied the Na salts of fatty acids with carbon chains longer than C_5, of benzoic and oxybenzoic and of aromatic sulfonic acids. Because of their surface activity and lipoid-solvent proper-

ties, we also have investigated the effects of some anesthetics and alkaloids, of saponin and digitonin, and other related substances. All of these, above certain concentrations and exposure periods, produce or at least promote cytolysis, by favoring disintegration of the surface structure of cells. For this reason, it was very difficult to secure unequivocal and uniform results and to avoid irreversible damage.

In a series of several hundred experiments, substances of this group were applied in varying concentrations and for varying periods of time until

TABLE IV

Effect of Caprylate at Various Concentrations

Mol caprylate	Rate of secretion	Concentration ratio	Dye secreted mg./hr. × 10^3
	mg./hr.		
—	15.2	930	71
3×10^{-5}	14.1	1120	78
—	10.2	1310	67
—	11.0	390	21
—	5.5	3200	89
3×10^{-5}	6.6	1090	36
—	8.0	7000	280
—	7.2	2270	81
—	8.6	320	14
6×10^{-5}	11.6	260	15
—	8.4	330	13
—	11.4	2520	140
3×10^{-4}	5.0	900	22
—	2.4	120	14
—	3.85	140	2.7
6×10^{-4}	3.25	100	1.6
—	3.15	50	0.8

conditions were found in which the stimulating action was preponderant over the toxic. For some substances such conditions were never found. Frequently even with supposedly optimum concentration and duration of experiment toxicity was evident, especially during warm weather and following starvation attendant upon hibernation. For this reason and bearing in mind the fact that the isolated Ringer-perfused liver is, in any case, a dying organ, we felt justified in discarding all experiments in which the liver was evidently moribund as indicated by the progressive loss of secretory power.

The variability in the course of our "good" experiments is illustrated by Tables III, IV, and V.

In Table III the results of four experiments are reported, in which sodium benzenesulfonate in various concentrations was added to the Ringer solution. 0.9×10^{-2} mol and 3.6×10^{-2} mol cause a definite diminution of the secretory power, while 0.45×10^{-2} mol and 0.22×10^{-2} mol produce a promoting effect. This is particularly demonstrated by the data regarding the absolute amounts of dyestuff secreted per hour.

The series of six experiments described by Table IV displays the more complicated behavior of the liver when subjected to the influence of Na caprylate. The poisoning effect of the higher concentrations (3×10^{-4} and 6×10^{-4} mol) is evident. However, with the markedly lower concentration of 3×10^{-5} mol a promoting effect is produced, which is characterized by its appearance only after a certain lapse of time.

TABLE V

Substances Expected to Have a Promoting Influence upon Liver Secretion

	Optimal concentration	Concentration ratio	Dye secretion/hr.
Caprylate	3.10^{-5} to 6.10^{-5}	4+, 2−	4+, 1−
Heptylate	1.10^{-5}	1+, 2−	3+
Oleate	5.10^{-6}	4+, 2−	4+, 2−
Benzoate	$0.5.10^{-2}$	3+	2+
Salicylate	7.10^{-5}	5+, 2−	6+, 1−
Benzenesulfonate	$2.2.10^{-3}$ to $4.5.10^{-3}$	2+	2+
p-Toluenesulfonate	6.10^{-4}	3+, 1−	3+, 1−
Glycocholate	3.10^{-5} to 20.10^{-5}	3+, 2−	5+
Taurocholate	3.10^{-5}	5+, 1−	6+
Propylcarbamate	$0.6.10^{-2}$ to 5.10^{-2}	4+, 1−	4+, 1−
Codein hydrochloride	3.10^{-4} to 15.10^{-4}	4+	4+
Veratrin	5.10^{-6}	1+, 1−	2+
Digitonin	$0.5.10^{-4}$ to 1.10^{-4}	3+, 1−	2+, 2−

Table V is a survey of a series of experiments with substances expected to show a promoting influence on the basis of their polar-non-polar structure, their surface activity, and their lipoid solubility. In more than 70 per cent of the experiments the substances tested in concentrations which seemed suitable were found to stimulate the secretory capacity.[2]

On the other hand, with substances likewise exhibiting the aforemen-

[2] In Table V a plus sign means presence, a minus sign, absence of a promoting action reflected by the concentration ratios or the absolute amount of dyestuff secretion or by both of them.

tioned physicochemical properties, we have failed as yet to secure a promoting action. Those substances were nonylate, laurate, cinnamate, and β-naphthalenesulfonate.

III. Antagonistic Effect of Two Substances of Opposite Action upon the Dyestuff Secretion

The failure of the experiments dealt with in the preceding section, to yield clear cut results was believed to be due to the fact that there is a gradual transition in the influence of the organic substances from a mere loosening to a definitely disintegrating effect. This interpretation seems to be supported by observations dealing with the neutralization of the vigorous inhibitory influence of one organic substance by the addition of a

TABLE VI

Antagonistic Effect of Inhibiting and Promoting Substances

	Concentration ratio	Secreted dye mg./hr. × 10^4
Ringer	99	10
" +⅛ isotonic sucrose	0	0
" " +8.10^{-6} mol taurocholate	364	130
Ringer	500	100
" +⅛ isotonic sucrose	0	0
" " +$2.5.10^{-3}$ mol diethylurea	165	8
Ringer	148	19
" +⅛ isotonic sucrose	>0	>0
" " +8.10^{-6} mol oleinate	250	25

second one. This behavior is demonstrated by experiments given in Tables VI and VII.

Table VI surveys three experiments in which substitution of 1/8 of the NaCl in the Ringer solution with 1/8 isotonic sucrose was succeeded by the entire or practically entire disappearance of the dyestuff from the secretion. Following addition of one of the polar-non-polar substances in a concentration found previously to be promoting the dyestuff secretion was resumed. The same antagonistic influence has been observed with the following compounds: Na glycocholate 9×10^{-6} mol, phenylurea 1×10^{-3} mol, propyl alcohol 1×10^{-2} mol, and caprylic acid 7×10^{-4} mol. When substances showing neither polar structure nor a distinct surface activity nor lipoid solubility were tested, it was found that glucose, on one hand, is unable to restore the secretory activity which has been abolished by lactose,

but that urea, acetamide, and ethylene glycol can definitely do so (Table VII). In general, these and other experiments have demonstrated that the inhibitory influence of disaccharides and hexoses is not diminished by other disaccharides, hexoses, and hexahydric alcohols, but is by urea, thiourea, methylurea, acetamide, propionamide, ethylene glycol and to some degree and more or less irregularly by glycerol and arabinose.

TABLE VII

Antagonistic Effect of Substances with Higher and Lower Hydropolarity

	Concentration ratio	Secreted dye mg./hr. × 10^4
Ringer	165	25
" +⅛ isotonic glucose	11	4.5
" " +⅛ isotonic urea	250	50
Ringer	400	100
" +⅛ isotonic sucrose	15	0.35
" " +⅛ isotonic acetamide	1000	42
Ringer	500	25
" +⅛ isotonic lactose	15	0.75
" " +⅛ isotonic glucose	3.7	0.25
Ringer	375	75
" +⅛ isotonic maltose	12	0.75
" " +⅛ isotonic ethylene glycol	185	9

IV. Physicochemical Properties of the Inhibiting and the Promoting Compounds

It has already been mentioned that those organic substances which show a marked inhibitory effect, exhibit a great affinity to water, which prevents their entering a lipoid phase, opposes their anchorage on the interface between water and organic matter, causes them to compete with the water molecules attached on hydrophilic colloids, and confers upon them in this way a shrinking influence. The promoting substances, in general, display opposite properties. Inasmuch as they show a polar-non-polar molecular configuration, the hydrophobic portion of their molecule can adhere to organic substances, the hydrophilic portion pulling towards the water and exerting in this way a loosening and swelling effect. However, there are also present in this group of promoting substances more or less apolar compounds which, because of their organophilic character as a whole, penetrate a lipoidal surface structure, change the intermolecular adhesive forces, and counteract the close molecular packing so that the final result is disintegration. Between these two contrasting groups are intermediate sub-

stances which either show only a slight hydroaffinity, like ethylene glycol and glycerol, or a slight organotropy, like acetamide and urea.

This interpretation of the influence of the two chief groups of organic compounds is supported by the following experimental facts: Katz (6) has investigated the effect of numerous organic substances upon the swelling and shrinking of starch. Of particular interest in connection with the present work was his observation of a shrinking effect with disaccharides and monosaccharides and with polyhydric alcohols in the order: mannitol, erythritol, glycerol, ethylene glycol. In contrast, some swelling is brought about by acetamide and urea and a stronger swelling by propyl and butyl carbamate. In his work with electrolytes, he observed marked shrinking with oxalate, malonate, succinate, glutarate, and citrate. A smaller effect was obtained with lactate and glycolate and with salts of the lower fatty acids (C_2 to C_4), whereas those of the higher members of this series (C_5 to C_9) produce swelling. This is true also with benzoate and still more with salicylate and with the salts of aromatic sulfonic acids.

However, model experiments with starch can hardly be comparable to the colloidal processes taking place on the surface of cells. For this reason, we have investigated the influence of the organic substances on lecithin, which seemed to provide a more appropriate model for cellular processes. A 0.2 per cent suspension of egg yolk lecithin in water or in 1 per cent NaCl was prepared. The pH was usually controlled with 0.04 mol phosphate buffer.[3] The increase (+) or decrease (−) in transparency following the addition of organic substances was measured by a photocell. An increase indicates a dispersing, a decrease an aggregating effect (Table VIII).

It is obvious that the results summarized in Table VIII are again confirmatory of the assumption that the dispersing and the antidispersing properties of the organic non-electrolytes and electrolytes are more or less correlated with their physiological effects.

Quite in line with our results are the observations of von Kuthy (7). Gelatin, like starch and lecithin, undergoes swelling in presence of hydrotropic substances like Na benzoate, benzenesulfonate, phenylacetate. The same substances facilitate the penetration of gelatin by dyestuffs.

Finally, with regard to the experiments on dyestuff secretion by the liver, Valdecasas (3) made the pertinent observation that solutions of a number of organic substances in the same concentrations in which they are found to increase the secretory activity, increased the rate of filtration through the hog's bladder.

[3] In order to avoid the influence of hydrolysis, the effect of the salts of weaker acids was, in general, studied at a higher pH (about 7.5 to 8) than that employed with the other substances.

In concluding, it should be emphasized that, although it can hardly be questioned that colloidal processes are involved in the physiological events, the importance of this statement must not be overestimated. Even such

TABLE VIII

Influence of Organic Substances on the Transparency of Lecithin Suspensions

	Effect	Transparency	pH
Sucrose	−	Minimum at 0.1 m.	4 to 4.6
Lactose	−	" 0.25 m.	4 to 4.4
Glucose	−	" 0.5 m.	4.2
Mannitol	−	" 0.1 to 0.4 m.	5
Erythritol	−	Flat minimum at 0.5 m.	4.2
Glycerol	−	Weak effect at 0.2 to 1.0 m.	4.2 to 4.4
Ethylene glycol	+	Increase of transparency above 0.1 m.	4 to 4.2
Glycine	−	Decrease " 0.1 to 1.0 m.	5.6 to 5.8
Amino butyric acid	−	" " 0.05 to 1.0 m.	7.2
Oxalate	−	" " 0.01 to 0.2 m.	4.2 to 4.4
Succinate	−	Strong decrease of transparency 0.02 to 0.2 m.	4 to 4.4
Glutarate	−	Minimum at 0.3 m.	5
Glycolate	−	" 0.5 m.	4.2
Malate	−	Rather strong decrease 0.01 to 0.08 m.	4
Capronate	+	Little effect up to 0.4, above 0.4 m. small increase	8 to 8.2
Heptylate	+	Increase 0.05 to 1.0 m.	7 to 8
Caprylate	+	" 0.005 to 0.04 m.	7.4 to 7
Pelargonate	+	Strong increase 0.01 to 0.8 m.	8.5
Oleate	+	Increase 0.01 to 0.1 m.	8 to 8.4
Benzoate	+	Strong increase 0.1 to 1.0 m.	7.8 to 8
Salicylate	+	" " 0.1 to 1.0 m.	4.5 and 8
Glycocholate	+	" " up to 0.01 m.	6
Taurocholate	+	" " up to 0.01 m.	6
p-Toluenesulfonate	+	Increase 0.02 to 0.8 m.	5
β-Naphthalenesulfonate	+	" 0.02 to 0.15 m.	4.5
Propylcarbamate	+	Small increase 0.03 to 0.4 m.	4
i-Amylcarbamate	0	No effect 0.0002 to 0.0012 m.	4.4
n-Butyl alcohol	+	Increase 0.18 to 0.65 m.	4
i-Amyl alcohol	+	Very small effect 0.005 to 0.08 m.	4.2
n-Heptyl alcohol	>0	" " 0.0005 to 0.007 m.	4.6
Veratrin	+	Strong increase 0.01 to 0.08 m.	7

striking results as those regarding the antagonistic effect of pairs of organic compounds, which belong to one and to the other of our main groups, fail to give us an insight into the secretory mechanism beyond the conclusion that a certain consistency and stability of the active cellular structure is indispensable.

V. Some Remarks about the Mechanism of the Dyestuff Secretion

In the following discussion the rate of secretion and the concentration of dyestuff will be considered separately. It has been pointed out earlier that, in general, on the one hand, a reversible decrease of the concentrating capacity of the liver, brought about by the addition of one group of organic substances, is accompanied by a decrease in the rate of secretion (Table II) and that, on the other hand, a reversible increase of concentration produced by the other group is often (although not regularly) associated with an increase in the rate (Tables III and IV). An irreversible increase of the rate of secretion is ordinarily observed after an overdose of a promoting substance has been given (Table III and also Valdecasas (3)).

The observations concerning the behavior of the rates might be interpreted as indicating some consolidating or shrinking effect of one group, some dispersing or loosening effect of the other group of substances. This would result in a greater or lesser permeability of the liver cells to water, in other words, in a narrowing or widening of their porous structure. Such a concept would be supported by recently published potentiometric measurements of Höber, Andersch, Höber, and Nebel (8) on muscle and nerve membranes. The membrane potentials appeared to be acted upon by the two groups of organic substances in opposite directions, indicating either an increase or a decrease in porosity or, respectively, a decrease or an increase of selective ion permeability.

With respect to the concentration of the dyestuffs, it is suggestive for several reasons, to regard their entrance into the liver as a matter of adsorbability. Two points may be emphasized here. (1) Apart from dyestuffs no substance has been definitely shown to be concentrated in the liver secretion. It is true that the passage of solutes through the liver has not, as yet, been thoroughly investigated. However, except for the dyestuffs, the substances which, up to the present, have been shown to pass the liver, pass it as they would a passive filter, *i.e.* without a marked change in concentration, and are characterized by their lack of adsorbability (Haywood and Höber (9)). (2) Apart from colloidal dyestuffs, which have been mentioned earlier (Höber (1)) as being unable to penetrate the liver, and apart from lipoid-soluble dyestuffs, which could be retained in liver fat, only a few diffusible and lipoid-insoluble dyes fail to find a pathway through the liver. From a series of about 30 azo-dyestuffs only those were not secreted, which contain in their molecule 4 to 5 sulfonate groups, azofuchsin V, Ponceau 6R, and a benzene-azo-naphthalene-pentasulfonate of the I. G. Farbenindustrie A. G. This may be explained by taking into account the great hydroaffinity of the sulfonate groups, which resist the

adhesive forces between the cells and the organophilic portion of the dye molecule. On the basis of such a concept one might infer that under the influence of the organic compounds the efficiency of the factors concerned in establishing the high concentration ratios can be varied, in one or the other direction, either by improving or impairing the accessibility to the dyestuffs of some cellular carriers, where the adsorption takes place, or by increasing or diminishing the rate of the carrier transport (Brinkman and von Szent-Györgyi (10) and Rosenthal (11)).

SUMMARY

The ability of the isolated Ringer-perfused frog liver, to concentrate dyestuffs in its secretion several hundred times, can be abolished entirely and reversibly by replacing in the Ringer solution about 1/8 of the NaCl by the isosmotic amount of a surface-inactive non-electrolyte (disaccharide, hexose, pentose, polyhydric alcohol, amino acid, acid amide) or electrolyte (salts of lower fatty acids, hydroxyl carboxylic, and dicarboxylic acids). This effect is not dependent upon changes in the perfusion rate.

The opposite effect, promotion of secretory activity, can be brought about by polar-non-polar electrolytes (salts of higher fatty acids, bile acids, and other aromatic carboxylic acids, aromatic sulfonic acids) and surface-active non-electrolytes (anesthetics, alkaloids, digitonin). However, reversibility of this effect cannot be regularly observed, since cytolysis is frequently the end result.

Suitable concentrations of inhibitory and promoting substances, simultaneously applied, counteract each other.

Inhibitory and promoting substances, in general, exhibit opposite effects upon the dispersion of colloids (starch, lecithin, gelatin).

The correlation between the physicochemical and the physiological action of the organic compounds is discussed.

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