This principle is believed by LUDANY[88] to be responsible for the motility of the intestinal villi. *Eledoisine*, isolated from salivary glands of octopodes by ERSPAMER[89] and synthetized by SANDRIN and BOISSONNAS[90] is an undecapeptide which has several actions in common with SP, especially the depressor effect on circulation and the strong stimulating effect on salivation.

9. *Conclusions.* SP is a basic polypeptide with a molecular weight of about 1600 containing 13 different amino acids. It is found in large amounts in the intestinal tract and in the nervous system of man and of all the vertebrates so far studied. Its distribution in the central nervous system shows close similarity with that of serotonin and the catecholamines which are probably playing an important part in the normal function of the central nervous system.

SP is the most active of the known naturally occurring substances stimulating intestinal smooth muscle and lowering the blood pressure. A physiological significance of SP for the intestinal motility seems probable. The topographic and subcellular distribution in the nervous system suggests a participation of SP in neuronal transmission processes. Its function as an actual transmittor substance as, e.g. in sensory neurones is, however, rather unlikely. The effects on central mechanisms of small doses of SP administered parenterally, as reported by various authors using crude extracts, could not be confirmed by us for highly purified material. It may well be that still unknown substances present in the crude extract are responsible for the contradictory effects reported. Actually, SP in much higher doses had a sedative effect in mice and dogs.

Recently three independant laboratories have succeeded in isolating SP in a rather pure form from horse intestine and bovine brain. Definitive conclusions concerning the physiological significance of SP will not be possible before synthetic SP is available for pharmacological purposes.

Zusammenfassung. Substanz P (SP) wurde als körpereigenes Polypeptid erstmals 1931 von v. EULER und GADDUM beschrieben und durch ihre glattmuskelstimulierende und hypotensive Wirkung charakterisiert. Sie kommt beim Menschen und bei den meisten untersuchten Tierspezies vor. Ihre Verteilung im Körper – Intestinaltrakt, peripheres und zentrales Nervensystem, vor allem dessen phylogenetisch ältere Teile – gleicht jener biologisch aktiver Amine und lässt somit an eine physiologische Bedeutung in der Regulierung der Darmmotilität und der Nervenaktivität denken. Verschiedene pharmakologische Wirkungen roher SP-Extrakte auf das Zentralnervensystem wurden beschrieben.

Kürzlich gelang drei Forschergruppen unabhängig voneinander die Isolierung von SP in ziemlich reiner Form aus Pferdedarm und Rinderhirn, und es ist wohl in absehbarer Zeit mit deren Aufklärung zu rechnen.

Es werden einige biologische Eigenschaften eines hochgereinigten SP-Präparates (50000 E/mg) aus Pferdedarm beschrieben. Das basische Polypeptid mit einem Molekulargewicht von ca. 1600 enthält 13 verschiedene Aminosäuren. Es ist die aktivste bisher bekannte Substanz mit stimulierender Wirkung auf die glatte Muskulatur. Ihre hypotensive Wirkung am Blutdruck des Kaninchens ist stärker als die von Acetylcholin. Bei intravenöser Verabreichung bewirkt SP beim Meerschweinchen eine Bronchokonstriktion. Was die Wirkung auf das Zentralnervensystem betrifft, waren zur Erzeugung eines leichten sedativen Effektes viel höhere Dosen des hochgereinigten Präparates nötig, als von verschiedenen Autoren für rohe Extrakte angegeben wurde. Die von verschiedenen Autoren postulierte Rolle von SP als Überträgerstoff im sensiblen System ist wenig wahrscheinlich. Ein eindeutiger Nachweis von Veränderungen des SP-Gehaltes im Gehirn durch zentral wirksame Pharmaka ist bisher nicht erbracht worden.

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The Influence of Diuretics on the Absorption of Salts, Glucose, and Water from the Isolated Small Intestine of the Rat[1]

By W. RUMMEL and H. F. STUPP*

The mucosal cells of the small intestine actively pump sodium ions from the lumen into the submucosal space (HEIDENHAIN[2], INGRAHAM and VISSCHER[3], BURNS and VISSCHER[4], VISSCHER and INGRAHAM[5,6], CURRAN and SOLOMON[7], RUMMEL and STUPP[8]). Like

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[1] A preliminary report was presented at the 25th Meeting of the German Pharmacological Society in September 1959, Basel.
[2] R. HEIDENHAIN, Pflüg. Arch. ges. Physiol. *56*, 579 (1894).
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the tubular epithelial cells of the kidney, the enteric epithelial cells possess the ability to concentrate sodium. Under certain conditions one can demonstrate even in the isolated intestine that the mucosa can produce on the serosal side a fluid containing twice as much sodium as does the intraluminal fluid, i.e. that it can transport sodium uphill (RUMMEL and STUPP[8]). The absorption of water is passive and depends mainly on the absorption of sodium (GOLDSCHMIDT and DAYTON[9], McDOUGALL and VERZÁR[10], CURRAN and SOLOMON[7], RUMMEL and STUPP[8], STUPP[11]). Poisoning of the metabolism inhibits not only the transport of sodium across the mucosa (HEIDENHAIN[2], CURRAN[12], BREYER[13]), but usually depresses absorption processes in general, e.g. the absorption of glucose (DARLINGTON and QUASTEL[14], ESCRIBANO and PONZ[15], SMITH and TAYLOR[16], RIKLIS and QUASTEL[17], RUMMEL, PFLEGER, and JACOBI[18]).

The present investigation was undertaken to see whether or not diuretics can produce a differential effect upon intestinal mucosa, as they do in the tubular epithelia of the kidney. Here, they inhibit the absorption of salts and thereby of water, but do not impair other active processes, e.g. absorption of glucose. To characterize the effect, chloride, sodium, potassium, calcium, water, and glucose were measured in the absorbed fluid. Mersalyl and hydrochlorothiazide, and—for comparison—another organic mercurial compound without diuretic activity, *p*-chloromercuribenzoate (KESSLER, LOZANO, and PITTS[19]) were used.

Methods. Loops of small intestine from male rats (200 g) were suspended in a glass apparatus of the type described by FISHER and PARSONS[20]. Four loops of 10 mm length were removed from each animal, starting at the duodeno-jejunal flexure. The dry weight was 213 ± 40 mg in 123 loops. Blood flow to the loop was interrupted only after the perfusion of the lumen had started. The segment was kept at 37° C in a glass vessel, into which a mixture of 95% O_2 and 5% CO_2 was led after it had been used to saturate the perfusion fluid. Thus, since temperature and humidity in the vessel were kept constant, condensation or concentration were avoided. Fluid penetrating through the wall of the gut was collected by a funnel in the bottom of the vessel. The perfusion fluid contained in g/l: 8.0 NaCl; 0.2 KCl; 0.2 $CaCl_2 \times 6H_2O$; 0.1 $MgCl_2 \times 6H_2O$; 1.0 $NaHCO_3$; 0.05 NaH_2PO_4; 3.0 glucose.

Glucose was determined according to the photometric method of Nelson as modified by FRANK and KIRBERGER[21]. Calcium, potassium, and sodium were analyzed by flame-photometer, chloride by a potentiometric method according to NORTHROP[22]. Following a suggestion by PFLEGER, we used the capillary slit of a stopstock cut as a bridge instead of agar. The pH of the solutions in the lumen and of the absorbed fluid was measured by glass electrode and was 6.8 and 7.2 respectively. As an additional control, the concentrations of Na, K, Ca, Cl, and glucose were determined in the perfusion fluid before and after each experiment. Increase of the potassium concentration in the intraluminal fluid is a sensitive indicator of damage to the mucosal cells and was regularly seen under the influence of e.g. KCN (10^{-2} to 10^{-3} *M*). In the experiments reported here no such increase of the potassium concentration was observed. Trial calculation of a large series showed that it was unnecessary to relate the values observed to the dry weight of the gut, if the length of the segment was always the same and the animal material used was uniform.

For *in vivo* experiments rats were anesthetized with urethane (2 g/kg s.c.). Their kidneys were tied off to eliminate the influence of general dehydration upon water absorption from the intestine in consequence of a diuretic effect of these agents. After injection of mersalyl (10 mg/kg i.v.) homologous loops, 10 cm long, were tied off at a point 5 cm distally from the flexura duodenojejunalis. The segments were filled with 2 ml of Tyrode solution, their blood supply was left intact. In experiments with hydrochlorothiazide this substance was added to the Tyrode solution (2×10^{-3} *M*).

The substances used were: mersalyl[23] as anhydrous salyrganic acid, MW 466; hydrochlorothiazid[23], MW 297; *p*-chloromercuribenzoate, MW 373; cysteine hydrochloride, MW 157.

Results.

Influence of mersalyl upon absorption. It is known that differences exist between the intestinal segments with respect to their absorption activity and the com-

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position of the absorbed fluid[24]. In favour of a greater number of experimental values, a certain levelling caused by the calculation of the mean values from four segments could of course not be avoided. The comparison of homologue segments, however, did not lead to essentially different conclusions. The evaluation of the quantitative difference in the intestinal segments will be the subject of a further publication. In order to obtain a representative average of the normal values, controls of former experiments were included. The results are also statistically significant, when only the control values obtained simultaneously with the mersalyl and hydrochlorothiazide experiments were compared.

The absorption of water by an isolated segment of small intestine was reduced by 27% when mersalyl (5×10^{-4} *M*) was added to the perfusion fluid. This correlated well with the inhibition of the absorption of sodium (Figure 1). The absorbed fluid was isotonic according to the measured concentrations of the substances which provide the major portion of osmotic activity and according to the depression of the freezing point checked in 5 experiments by thermoelectric cryoscopy. This agrees with the findings in goldhamsters (WILSON[25]). The absorption of potassium was also reduced, but this is osmotically unimportant because of the small absolute quantities involved (see Table I). The decreased absorption of water, therefore, seems to be caused by the inhibition of the absorption of sodium.

Cysteine. Cysteine (5×10^{-3} *M*) prevented the inhibition of water absorption by mersalyl (5×10^{-4} *M*). The amount of fluid absorbed in 2 h from an isolated segment of gut was in controls 1.97 ± 0.47 ml ($n = 146$); with mersalyl it was 1.43 ± 0.21 ml ($n = 12$); and with mersalyl + cysteine it was 2.15 ± 0.33 ml ($n = 8$).

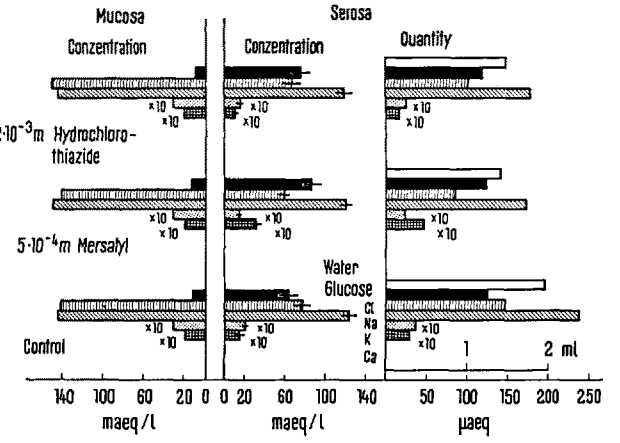

Fig. 1. The influence of mersalyl and hydrochlorothiazide upon absorption from small intestine. Isolated segment of small intestine from rats. Temperature 37°C; duration of the experiment 2 h. The columns labelled 'mucosal side' represent the concentrations in the fluid in the lumen at the end of the experiment. The columns labelled 'serosal side' represent concentration and amount of the fluid collected from the serosal side (see methods). ×10 indicates that the column is magnified 10 times. The small horizontal bars in the columns indicate the standard deviation.

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Tab. I. Influence of mersalyl, hydrochlorothiazide, and *p*-chloromercuribenzoate upon the enteric absorption of water, potassium, calcium, and glucose

	Amount and composition of the fluid absorbed during 2 h						
	ml	Concentration in mEq/l			Calculated total amount in µEq		
	H_2O	K	Ca	Glucose	K	Ca	Glucose
Controls	1.97 $\sigma = \pm 0.47$ $n = 146$	2.01 $\sigma = \pm 0.31$ $n = 128$	1.49 $\sigma = \pm 0.59$ $n = 115$	63.7 $\sigma = \pm 12.4$ $n = 144$	3.93	2.94	122.4
Hydrochlorothiazide 2×10^{-3} *M*	1.49 $\sigma = \pm 0.186$ $n = 23$	1.77 $\sigma = \pm 0.22$ $n = 23$	1.15 $\sigma = \pm 0.12$ $n = 23$	78.9 $\sigma = \pm 11.5$ $n = 18$	2.67	1.73	119.2
Mersalyl 5×10^{-4} *M*	1.43 $\sigma = \pm 0.209$ $n = 12$	1.59 $\sigma = \pm 0.21$ $n = 12$	3.2 $\sigma = \pm 0.47$ $n = 12$	86.2 $\sigma = \pm 9.0$ $n = 12$	2.28	4.54	122.8
Mersalyl 5×10^{-3} *M*	0.376 $\sigma = \pm 0.099$ $n = 8$	4.26 $\sigma = \pm 0.97$ $n = 8$	4.45 $\sigma = \pm 0.5$ $n = 8$	24.0 $\sigma = \pm 5.98$ $n = 8$	1.55	1.68	9.0
p-Chloromercuribenzoate 5×10^{-5} *M*	1.19 $\sigma = \pm 0.21$ $n = 8$	2.23 $\sigma = \pm 0.16$ $n = 8$	1.95 $\sigma = \pm 0.37$ $n = 8$	60.6 $\sigma = \pm 10.3$ $n = 8$	2.61	2.34	73.3
p-Chloromercuribenzoate 5×10^{-4} *M*	0.315 $\sigma = \pm 0.095$ $n = 4$	2.23 $\sigma = \pm 1.6$ $n = 4$	3.0 $\sigma = \pm 0.45$ $n = 4$	29.9 $\sigma = \pm 4.1$ $n = 4$	0.69	0.92	9.4

Glucose and Calcium. The absorption of glucose and of calcium was not inhibited by mersalyl (5×10^{-4} *M*). Thus the concentration of glucose and calcium in the absorbed fluid, i.e. in the fluid dropping down from the serosa, was higher than in the controls (Figure 1). Mersalyl did not diminish the glucose consumption. The consumption within 2 h amounts to 66 μM ($n = 33$) in the experiments with, and to 68 μM ($n = 16$), $p > 0.8$ in the experiments without mersalyl.

The amount of calcium absorbed under mersalyl was not only decreased but rather increased to $1^1/_2$ times normal. Under the influence of 5×10^{-3} *M* mersalyl (Table I) the concentration of calcium in the absorbed fluid was 2 times that in the intraluminal fluid (2 mEq Ca/l). Therefore, under these conditions a concentration gradient arises, although mersalyl in this concentration reduced absorption in general (Table I). Calcium seems to be transported uphill. This observation is in agreement with similar results observed under other conditions (SCHACHTER et al.[26,27], WASSERMAN[28]).

Hydrochlorothiazide. Hydrochlorothiazide acted like mersalyl except for the absorption of calcium (Figure 1). Like mersalyl it inhibited the absorption of salts, and therefore water, without affecting the transport of glucose. Its potency (per unit weight) was less than that of mersalyl; the concentration required for an equal effect was 4 times higher. In contrast to mersalyl, hydrochlorothiazide did not increase the absorption of calcium but inhibited it (Table I).

Of special interest is the effect of these diuretics upon the absorption of Cl and Na. The concentration of chloride in the absorbed fluid under the influence of mersalyl and hydrochlorothiazide was lower than in the controls, while the concentration of sodium was unchanged (Table II). With mersalyl (5×10^{-4} *M*), for example, the amount of Cl^- transported across the mucosa was reduced by 42%, the amount of sodium by 27% only. The ratio of Cl/Na in the absorbed fluid decreased. Therefore, the absorption of chloride is more sensitive to the action of the diuretics than that of sodium.

Inhibition of absorption in vivo. The inhibition of water absorption by mersalyl and by hydrochlorothiazide was also seen in anesthetized rats (see methods). The weight of intestinal loops after $1^1/_2$ h was in the control rats 1.14 ± 0.38 g ($n = 10$); in rats treated with mersalyl it was 1.78 ± 0.39 g ($n = 11$); and in rats treated with hydrochlorothiazide it was $1.99 \pm \pm 0.46$ g ($n = 10$). The differences between the control animals and the treated rats was significant, the value of p was in both cases < 0.01.

p-Chloromercuribenzoate. This organic mercurial compound without diuretic activity (KESSLER, LOZANO, and PITTS[19]) acted upon the enteric absorption of salts, water, and glucose in a different way than did mersalyl. In a concentration of 5×10^{-5} *M* it reduced water absorption about as much as mersalyl $5 \times 10^{-4} M$, by 40% and 30% respectively (Table I). However,

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Tab. II. Chloride and sodium absorption under hydrochlorothiazide, mersalyl, and *p*-chloromercuribenzoate

	Concentration, amount, and ratio of Cl^- and Na^+ in the fluid absorbed during 2 h				
	mEq/l		Ratio	Calculated total amount in μEq	
	Cl	Na	Cl/Na	Cl	Na
Controls	77.2 $\sigma = \pm 13.4$ $n = 104$	123.3 $\sigma = \pm 8.3$ $n = 130$	0.63	145.6	239.4
Hydrochlorothiazide $2 \times 10^{-3} M$	68.0[a] $\sigma = \pm 10.4$ $n = 23$	120.0 $\sigma = \pm 5.5$ $n = 8$	0.56	102.3	180.4
Mersalyl $5 \times 10^{-4} M$	59.2[a] $\sigma = \pm 5.5$ $n = 12$	121.1 $\sigma = \pm 5.3$ $n = 12$	0.49	84.1	172.9
Mersalyl $5 \times 10^{-3} M$	114.7 $\sigma = \pm 16.9$ $n = 8$	154.7 $\sigma = \pm 6.6$ $n = 7$	0.74	42.9	55.1
p-Chloromercuribenzoate $5 \times 10^{-5} M$	88.3 $\sigma = \pm 8.0$ $n = 8$	134.6 $\sigma = \pm 5.5$ $n = 8$	0.66	105.0	160.2
p-Chloromercuribenzoate $5 \times 10^{-4} M$	115.4 $\sigma = \pm 8.2$ $n = 4$	140.6 $\sigma = \pm 12.5$ $n = 4$	0.82	35.9	43.5

[a] Compared with controls, $p < 0.01$.

under *p*-chloromercuribenzoate the chloride concentration and the ratio Cl/Na in the absorbed fluid were unchanged (Table II), but the absorption of glucose, which had been entirely unaffected by the concentration of mersalyl used, was depressed by 40% (Table I).

Influence of changes in pH and chloride concentration. In view of the special sensitivity of the chloride absorption, it seemed desirable to examine the relation between the degree of inhibition of water absorption and the concentration of chloride. Variation of the chloride concentration leads to changes in pH. To test for possible interference of pH changes, the effect of pH changes upon the action of mersalyl was also studied.

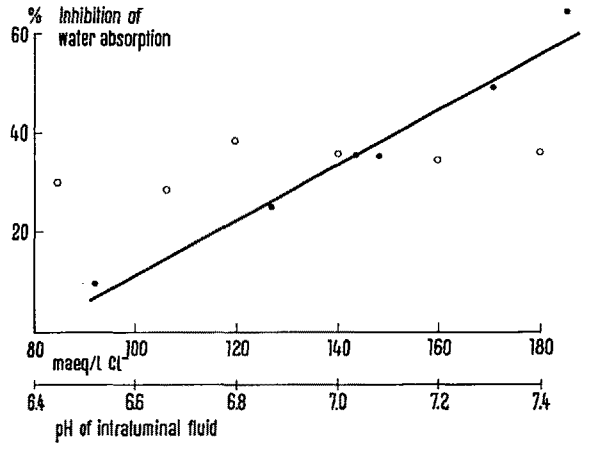

Fig. 2. Inhibition of water absorption by mersalyl at different concentrations of Cl^- ions and H^+ ions. All values given are percent of the respective control values (see text). Mersalyl $5 \times 10^{-4} M$. Solid circles refer to the different concentrations of Cl^- (upper scale on the abscissa). Open circles refer to different pH values of the intraluminal fluid (lower scale on the abscissa), at constant Cl^- concentration. All points give mean values from 8 experiments.

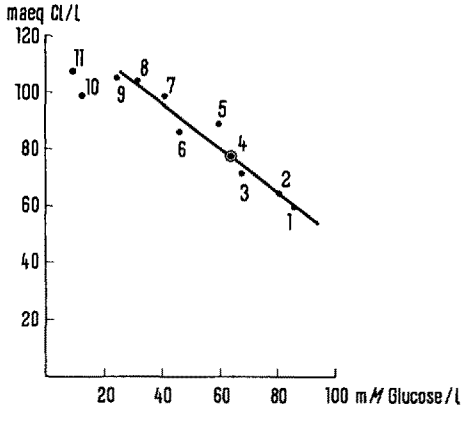

Fig. 3. Relation between the concentration of chloride and glucose in the absorbed fluid. Some of the values were taken from experiments not reported here. The numbers indicate: (1) Mersalyl $5 \times 10^{-4} M$; (2) Hydrochlorothiazide $2 \times 10^{-3} M$; (3) Tyrode solution with 56.5 mEq Ca/l and 99.6 mEq Na/l (RUMMEL and STUPP[8]); (4) Controls; (5) *p*-Chloromercuribenzoate $5 \times 10^{-5} M$; (6) Desoxycholat $10^{-4} M$ (RUMMEL and STUPP[30]); (7) Tyrode solution with 63.4 mEq K/l and 88.5 mEq Na/l (RUMMEL and STUPP[8]); (8) *p*-Chloromercuribenzoate $5 \times 10^{-4} M$; (9) Mersalyl $5 \times 10^{-3} M$; (10) KCN 10^{-3} (BREYER[13]); (11) Dinitrophenol $10^{-4} M$ (RUMMEL and STUPP[30]). All points give means of at least 4 experiments. The number of experiments for (1), (2), (4), (5), (8), (9), are given in Tables I and II.

The concentration of Cl^- was reduced by replacing it with HCO_3^- and increased by replacing NaCl with $CaCl_2$ or $MgCl_2$ in equimolar amounts. This alteration of the ionic composition of the solution influenced its absorption. The water and salt absorption, for instance, is diminished 20–25% by Ca 100 mEq/l (RUMMEL and STUPP[8]). The effect of mersalyl was therefore compared with control experiments with solutions of identical ionic composition and expressed as percent of these controls. Hydrogen ion concentration was varied by altering the concentration of CO_2 in the gas mixture or by addition of HCl and changing the concentration of HCO_3^-. The concentration of Cl^- was kept constant. The pH of the solutions was measured at the beginning and the end of each experiment.

A plot of the percentage inhibition of water absorption (as defined above) against the concentration of chloride present in the lumen of the intestinal loop (Figure 2) shows that the effect of mersalyl increases with increasing concentration of chloride (solid circles in Figure 2). The result is not due to the simultaneous change in pH since a change in pH at constant chloride concentration did not produce the effect (open circles, Figure 2). The degree of the relative inhibitory activity of mersalyl depends therefore upon the concentration of Cl^- ions not H^+ ions.

Relative effects on chloride and on glucose. The special position of mersalyl and hydrochlorothiazide can also be demonstrated by relating the concentration of chloride to that of glucose in the absorbed fluid (Figure 3). For mersalyl and hydrochlorothiazide in concentrations which inhibited only the absorption of chloride and therefore of sodium and water without affecting the transport of glucose, the points lie below the controls (point 4 in Figure 3), i.e. the chloride concentration was smaller and that of glucose higher than in the control experiments. On the other hand, if absorption was depressed by *p*-chloromercuribenzoate, by dinitrophenol, KCN, desoxycholate, KCl, and also under the influence of a 10 times higher concentration of mersalyl, the situation was reversed and the points lie above the control point. Here, the concentration of chloride in the absorbed fluid was increased and that of glucose decreased. In general we can state a reciprocal proportionality between the concentration of chloride and glucose in the fluid absorbed.

Discussion. Mersalyl and hydrochlorothiazide were useful tools to affect processes of enteric absorption in a more specific way. While the usual inhibitors of metabolism interfere with the absorption of salts and water generally, these diuretics in lower concentrations inhibit only the absorption of chloride, sodium and water without affecting the transport of glucose. The

absorption of water is passive and mainly a consequence of the absorption of chloride and sodium. This conclusion, already drawn by former investigators (HEIDENHAIN[2], GOLDSCHMIDT and DAYTON[9], INGRAHAM and VISSCHER[3], BURNS and VISSCHER[4], MCDOUGALL and VERZÁR[10], VISSCHER and INGRAHAM[5,6]) has in recent times been firmly established, both *in vivo* (CURRAN and SOLOMON[7]) and *in vitro* (CURRAN[12], RUMMEL and STUPP[8], STUPP[11]). The findings presented are additional evidence for this point of view.

The question arises whether the reduced absorption of chloride or that of sodium is primarily responsible for the reduction of water absorption. Two arguments can be advanced for a primary role of chloride: (1) The inhibition of water absorption by mersalyl depends upon the concentration of Cl^- in the fluid to be absorbed. It was about 60% at a Cl^- concentration of 185 mEq/l and only 10% at 90 mEq/l Cl^- (Figure 2). (2) The concentration of chloride in the absorbed fluid is reduced under mersalyl, while the sodium concentration remains the same (Figure 1 and Table II).

A primary inhibition of the sodium pump would be the other possibility to be discussed. The following facts, however, do not speak in favour of this assumption. (1) In no object has an inhibition of the sodium pump by mersalyl yet been proved directly, i.e. by measuring the active transport of sodium. In contrast to this it could be shown in erythrocytes that mersalyl does not inactivate the sodium pump measured by the sodium efflux, whilst it diminishes the anion permeability as measured by ^{35}S-sulfate (FORTH, PFLEGER, and RUMMEL[29]). (2) The Cl/Na ratio remains unchanged when the absorption is reduced by typical inhibitors of the sodium pump. g-Strophanthin (2×10^{-3} *M*) diminishes the water absorption to 1.56 ml $\pm$ 0.58, $n = 11$, the Cl-concentration is 83.5 mEq/l $\pm$ 13.1, $n = 11$, the Na-concentration 126.1 mEq/l $\pm$ 11.6, $n = 11$, and the Cl/Na ratio 0.66 (RUMMEL and STUPP[30]). Digitonine (10^{-4} *M*), which blocks the sodium pump in erythrocytes like strophanthine (PFLEGER, RUMMEL, SEIFEN, and BALDAUF[31]), inhibits the water absorption to the same extent as mersalyl, but without diminishing the Cl/Na ratio (water absorption: 1.41 ml $\pm$ 0.34, $n = 8$; Cl-concentration: 82.4 mEq/l $\pm$ 14.0, $n = 8$; Na-concentration: 130 mEq/l $\pm$ 4, $n = 8$; Cl/Na ratio = 0.63; STUPP and RUMMEL[30]). (3) When the normal potassium concentration in the suspension fluid was reduced—a measure, which hampers the activity of the sodium pump—the water absorption is inhibited, while the Cl/Na ratio remains also unchanged (RUMMEL and STUPP[8]. (4) Dinitrophenol (5×10^{-5} *M*), finally, reduces the water absorption to 1.09 ml $\pm$ 0.21, $n = 10$. The Cl-concentration is 81 mEq/l $\pm$ 15.4, $n = 10$, and the Na-concentration 128 mEq/l $\pm$ 9.7, $n = 10$ (STUPP and RUMMEL[30]). In other words, the ratio remains 0.63.

Only copper (e.g. as *bis*-histidino-copper-II-dichloride 10^{-5} *M*) is known to diminish like mersalyl the Cl-concentration in the fluid absorbed by the intestine and therefore the Cl/Na ratio (water absorption 1.44 ml $\pm$ 0.3, $n = 14$; Cl-concentration 64.0 mEq/l $\pm$ 11, $n = 14$; Na-concentration 132 mEq/l $\pm$ 13, $n = 14$; Cl/Na = 0.48; BREYER[13]). In the frog skin, an inhibition of the Cl-permeability by $CuSO_4$ has already been described by USSING and ZEHRAHN[32].

The effect of mersalyl upon intestinal mucosal cells seems to be analogous to its action on the tubular epithelia of the kidney. In the proximal tubules, as in the intestinal epithelium, more sodium than chloride is absorbed (WALKER et al.[33]). Under the influence of mercurial diuretics, the Cl^- concentration in the extracellular fluid decreases and the HCO_3^- concentration increases (SCHWARTZ and WALLACE[34]). The diuretic activity, the inhibition of chloride absorption in the tubuli, is directly proportional to the concentration of chloride in the plasma, i.e. in the glomerular ultrafiltrate (AXELROD and PITTS[35]; RICE et al.[36]).

For explanation of the reported results a hypothesis may be offered on basis of the following facts: Normally, *in vivo* (PARSONS[37], CURRAN and SOLOMON[7]) as well as *in vitro* (WILSON[38], RUMMEL and STUPP[8]), more Na^+ than Cl^- ions are absorbed, and the quotient is about 0.6. The second anionic partner available for Na^+ pumped across the membrane is HCO_3^- (WILSON[38], PARSONS[37]). This ion can penetrate easily, partly as CO_2, and it is found accumulated on the serosal side (WILSON[38]). Consequently, the absorption of water is reduced to half in the absence of bicarbonate (SMITH and TAYLOR[16]). Therefore, both the availability of HCO_3^- and the membrane permeability for Cl^- seem to be limiting factors for the movement of sodium.

The assumption that mersalyl inhibits the penetration of chloride through the membrane by restriction of the permeability would thus explain a secondary decrease in the absorption of sodium. It is possible that the mersalyl molecules attach themselves with their mercury atoms to sulfur-bearing groups of the negatively charged pores, through which Cl^- ions have to pass. According to CURRAN and SOLOMON[7], the negative charge barrier in the membrane rises by such a

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process. Due to the increased charge density on the pore wall, the diffusion of Cl^- is more restricted.

The increased transfer of calcium, seen under the same conditions as the decreased transfer of chloride, may have the same cause: the increase of negative charges in the pores caused by an increase in fixed anions in the pores. If the movement of calcium through membranes does not occur mainly by diffusion but involves intermediate reactions with fixed anionic groups in the walls of the pores (DUMONT et al.[39]), an increase in these fixed anionic groups by mersalyl could result in an increased transfer of calcium. Hydrochlorothiazide cannot act by the same mechanism, because the calcium absorption is inhibited, not activated. Apart from its interpretation regarding mode of action, the difference between mersalyl and hydrochlorothiazide may be helpful for further investigations.

Zusammenfassung. (1) Unter dem Einfluss von Mersalyl (5×10^{-4} *m*) und Hydrochlorothiazid (2×10^{-3} *m*) nimmt die Cl-Konzentration in der resorbierten Flüssigkeit ab, während die Na-Konzentration gleich bleibt und die Glucose-Konzentration ansteigt. Gleichzeitig kommt es zu einer osmotisch äquivalenten Einschränkung der Wasserresorption.

(2) Die prozentuale Hemmung der Wasserresorption durch Mersalyl nimmt zu bei höherer Cl^--Konzentration in der angebotenen Flüssigkeit und hängt zwischen pH 6,5 und 7,4 nicht von der H^+-Konzentration ab.

(3) Die Wirkung von Mersalyl lässt sich mit Cystein aufheben und ist auch *in vivo* an der abgebundenen Darmschlinge nachweisbar.

(4) Der Glucosetransport und die Calciumresorption werden durch Mersalylkonzentrationen, die die Wasser- und Natriumresorption um 30% hemmen, nicht beeinträchtigt. Der Durchtritt von Calcium durch die Darmwand ist auf das 1,5fache erhöht, es häuft sich dabei in der resorbierten Flüssigkeit an.

(5) *p*-Chloromercuribenzoat hemmt in Konzentrationen, die die Natrium- und Wasserresorption um 40% vermindern, den Glucosetransport ebenfalls um 40%. Im Unterschied zu Mersalyl setzt es die Cl^--Konzentration der resorbierten Flüssigkeit nicht herab.

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Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

Steroidal Cyclic Ketals[1]: The Preparation of Steroidal Δ^4-3-Ethyleneketals

Ketalization of steroidal Δ^4-3-ketones by the Salmi method[2] gives Δ^5-3-ethyleneketals[3–5]. We now describe a modification of this procedure which gives Δ^4-3-ethyleneketals.

When oxalic acid was substituted for *p*-toluenesulfonic acid as the catalyst in a series of ketalization reactions of Δ^4-3-ketones (see Table), the product was found to be either the Δ^4-3-ethyleneketal or a mixture of the Δ^4- and Δ^5-3-ethyleneketals. When the weaker adipic acid was used as the catalyst only the Δ^4-3-ethyleneketal was obtained. The presence of the Δ^4-3-ethyleneketal grouping was established when oxidation of 3,20-bis-ethylenedioxypregn-4-ene with osmium tetroxide in pyridine[6] gave 3,20-bis-ethylenedioxypregnane-4ξ,5ξ-diol from which the known[7] 4-hydroxyprogesterone was obtained by treatment with formic acid.

These Δ^4-3-ethyleneketals exhibit a sharp, weak band in the infrared at about 1668 cm^{-1} which is not shown by Δ^5-3-ethyleneketals. The Δ^4-3-ethyleneketals are stable to base but they are extremely sensitive to acid. In contrast to Δ^5-3-ethyleneketals, they are hydrolyzed almost quantitatively in benzene by magnesium sulfate, and, by comparison of the ultraviolet absorption before and after such treatment of a sample, this was found to be an excellent method of determining the proportions of Δ^4-3-ethyleneketal and Δ^5-3-ethyleneketal in a crude reaction mixture.

DJERASSI and GORMAN[8] proposed a mechanism for the formation of Δ^5-3-ethyleneketals in which an intermediate 3,5-dienol ether I is formed which ring-closes by 1,2-addition to the 3,4-double bond to give the Δ^5-3-ethyleneketal II. Similarly, the preparation of Δ^4-3-ethyleneketals IV probably involves the formation of an intermediate

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