3") or of a standard of vit. D_3(23). It is likely that this fraction, which was much smaller than the one containing vit. D_3, was made up of esters of the vitamin: it ran near the solvent front on thin-layer chromatography in a solvent system similar to that used in the present study (as in Fig. 1) and was diminished by saponification, a process which hydrolyses ester linkages.

Summary. Intestinal absorption and esterification of 4-C^{14} vitamin D_3 and 4-C^{14} cholesterol were studied in rats with lymph fistulas. After oral administration, extracts of lymph, fractionated by thin-layer chromatography, were found to contain the sterols, their esters and unidentified more polar compounds. In contrast to cholesterol, very little vit. D was esterified. The esters of both sterols, separated by thin-layer chromatography on the basis of the degree of unsaturation of the fatty acid moiety, were found to consist principally of saturated and monounsaturated fatty acids.

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Effects of the Counter Ion and pH on Intestinal Absorption of Calcium and Strontium.* (31535)

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The absorptive capacity of different regions of the small intestine for the alkaline earth metals has been shown to vary considerably (1-5). In the duodenum the absorption rate seems to be at a maximum, while the ileal rate is much lower. The total absorptive capacity of each region depends on the period over which the element is in contact with the mucosa; thus the more slowly absorbing ileal region absorbs more than the duodenum(2).

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The profound differences in rate of absorption of Ca^{45} and Sr^{89} suggest that some local factor is responsible for this variation. McHardy and Parsons(6) have shown that the rate of absorption of the phosphate ion, introduced as the sodium salt, increases with increasing pH from 4.4-7.9. The effect of small variations in H^+ ion concentration on calcium and strontium absorption has not been reported.

The pH of the secretions of different regions of the small intestine varies, and as H^+ concentration influences the solubility of calcium and strontium salts, variation in the rate of absorption of calcium and strontium in the different segments of the intestine would be expected.

In particular, the solubility of calcium and strontium salts formed with the physiologically important anions, phosphate and bicarbonate, changes rapidly as the pH increases within the normal range (6.0-7.3) found in the rats' intestine. Concentrations of the anions also vary in different parts of the small intestine, and from species to species; in man, the highest concentration of bicarbonate is 19 mEq per litre (middle ileum) whereas the corresponding figure for the dog is 91 mEq/litre(7). The ability of calcium to complex with other anions of biological importance such as citrate(8) alters markedly in this pH region. In the course of our studies on the inhibition of absorption of radioactive strontium(9), the nature of the counter ion and its solubility product with Sr or Ca was found to be of fundamental importance. It was thought therefore that a closer examination of the effects of pH and the role of the anion in Ca and Sr absorption should be carried out.

By an *in vivo* technic developed by Skoryna *et al*(10) in which the rats' small intestine was divided by ligation into 9 segments, the absorption of calcium and strontium may be studied in detail. In the experiments to be reported here, we have studied the effect of introducing excess of certain anions, which form soluble salts with Ca or Sr, on the absorptive capacity of the intestinal mucosa. Solutions, buffered sufficiently to maintain an almost constant value at the desired pH in the experimental period, were introduced into the lumen of each segment. After 30 minutes, absorption was measured by assessing residual radioactivity in the excised segment. The radioactivity levels in the blood and in the femur of each rat were also determined.

Methods. Rats of the R.V.H. strain of either sex, weighing 100-120 g, kept on a stock diet, were fasted for 24 hours before experiment. Ligated segments were prepared under pentobarbital anaesthesia without detaching the segment from the remainder of the intestine or disturbing the vascular or lymphatic supply. The duodenum, defined as segment II was ligated at the pylorus and at the ligament of Treitz. The jejunum and ileum were measured and divided into 8 segments of approximately 5 cm in length and numbered sequentially III-X. Only one segment was utilized from each animal.

To measure the pH of the intestinal lumen, 0.5 ml saline or buffer solutions were introduced into each ligated segment in separate groups of rats. After 30 minutes, the segment was excised and the contents of the lumen were collected. The pH was measured at 37° within 45 seconds, using a combination glass electrode with a Beckman pH meter. It was observed that the same pH was recorded irrespective of whether the sample was measured aerobically or under liquid paraffin.

To simplify the comparison of results, the concentration of inert $CaCl_2$ or $SrCl_2$ in the radioactive solutions was adjusted to the same value throughout the experiments. Radioactive solutions containing Ca^{45}, 5 μc, 0.55 μM $CaCl_2$, or Sr^{89}, 5 μc, 0.45 μM $SrCl_2$ in 0.5 ml saline or buffer were introduced into ligated segments. Groups of 12 rats were used to estimate absorption from each anatomical region. At the end of the experimental period the following samples were taken: 1) the excised segment (contents of the lumen were pooled with the intestinal wall); 2) one millilitre of blood; 3) one femur; these were removed from each animal and prepared for radioactive assay, using the method described previously(10). In one series with radioactive strontium (2 μc Sr^{89}, 0.45 μM per 0.5 ml), the loss of radioisotope from the different segments only was measured.

Materials. Sr^{89} and Ca^{45} in dilute hydrochloric acid were supplied by Atomic Energy

TABLE I. pH at 37° of Solutions Resting for 30 Min *in vivo* in Ligated Intestinal Segments.

Segment	Isotonic saline Init. pH 5.55 Mean pH	Tris, I = 0.1 Init. pH 7.95	Tris, I = 0.2 Init. pH 7.15	Phosphate, I = 0.2 Init. pH 5.85
II	6.48 (6.2 -6.6)	7.15 (6.85-7.4)	6.68 (6.55-6.75)	6.19 (5.9 -6.68)
III	5.96 (5.85-6.0)	7.03 (6.8 -7.25)	6.45 (6.3 -6.6)	5.95 (5.9 -6.1)
IV	6.06 (5.8 -6.1)	7.14 (6.95-7.35)	6.49 (6.35-6.65)	6.01 (5.9 -6.1)
V	6.03 (5.7 -6.5)	7.05 (6.8 -7.2)	6.98 (6.9 -7.1)	6.04 (5.9 -6.1)
VI	6.25 (6.1 -6.9)	7.2 (7.0 -7.5)	6.92 (6.8 -7.1)	5.95 (5.9 -6.01)
VII	6.1 (5.95-6.6)	7.2 (6.9 -7.5)	6.89 (6.9 -7.02)	5.95 (5.89-6.1)
VIII	6.56 (6.2 -7.15)	7.2 (7.2 -7.4)	6.99 (6.72-7.2)	6.1 (6.0 -6.35)
IX	7.01 (6.6 -7.5)	7.4 (7.2 -7.6)	7.32 (7.1 -7.5)	6.15 (6.05-6.35)
X	7.24 (7.05-7.4)	7.6 (7.5 -7.7)	7.12 (6.98-7.5)	6.14 (6.0 -6.5)

Means calculated on pH values obtained from groups consisting of 5 to 15 rats. Range of values given in parentheses.

of Canada Ltd. The pH of the buffers were chosen to lie at the extreme limits of the normal physiological range; a third lay at an intermediate value. The compositions of the buffer solutions were as follows: 1) sodium phosphate, ionic strength 0.2, pH 5.85 at 37°; 2) Tris (hydroxymethyl) aminomethane (HCl) Tris, ionic strength 0.1, pH 7.95 at 37°; 3) Tris (hydroxymethyl) aminomethane (HCl), ionic strength 0.2, pH 7.15 at 37°; 4) unbuffered saline (0.9%) pH 5.7 at 37°.

Results. The average pH of the saline washings from the ligated duodenal segments was 6.5, ranging between 6.2 and 6.6 (Table I). This final pH represents the contribution from the duodenal mucosa and glands and also bile and pancreatic secretions, as the common duct was not ligated. The proximal jejunal segment provided the lowest pH in the small bowel, followed by a very slight increase on proceeding towards the more distal segments. The stepwise progression of increasing pH continued throughout the ileal segments, reaching a maximum of 7.4 in the distal ileum.

On introducing the slightly alkaline or almost neutral Tris buffers (pH 7.95 and 7.15 respectively), acid production by the jejunal mucosa was sufficiently great to reduce the pH of the buffer solutions by approximately 0.9 unit. Acid production by the rats' jejunal mucosa was also observed by Wilson and Kazyak(11) on introducing bicarbonate-saline into intestinal loops.

Each of the buffer solutions had sufficient capacity to bring the pH of all segments to within a fairly narrow range; the standard deviation within each segment group was very small. Tris, introduced at pH 7.95, increased the pH of the contents of all segments to the region of pH 7.3 (6.8-7.7). The Tris solution at pH 7.15 increased the pH of the jejunal segments to the range 6.3-6.65 (almost the same as that normally occurring in the duodenum). The ileal segments maintained a pH of 6.8-7.5 with the majority of readings at pH between 6.9 and 7.1.

In order to choose a phosphate solution at a pH which would not precipitate the calcium or strontium in the experimental solutions, a preliminary *in vitro* test was made. Solutions of inert calcium and strontium chloride of concentrations identical with those used *in vivo* were mixed with phosphate solutions of the same molarity but adjusted to different pHs. The solubility of calcium and strontium phosphate is illustrated in Table II.

A phosphate buffer, pH 5.85 (37°) was chosen because this pH lies just within the physiological range of the small intestine (segment III-V), and because no Ca or Sr salt is precipitated. The phosphate buffer, when introduced into the intestinal lumen, lowered the pH of all segments to about 6.0 (range 5.9-6.4) and thus below the pH at which precipitation occurs.

Although the solutions were hypertonic, none caused distension of the segments, nor were any lesions detectable on histological

TABLE II.

	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8
Ca	—	+	+	+	+	+	+	+
Sr	—	—	—	—	+	+	+	+

Visible precipitate: +

TABLE III. Ca^{45} Absorption. Percentage absorbed from ligated segments after 30 min.

Segment	Normal saline *Unbuffered	Tris, pH 7.95 (37°) *Mean ± S.D.	p	Phosphate, pH 5.75 *Mean ± S.D.	p
II	68.4 ± 9.6	65.3 ± 12.8	>.05	71.9 ± 14.1	>.05
III	60.0 ± 4.3	48.7 ± 13.7	<.01	51.0 ± 9.2	<.01
IV	26.9 ± 6.5	40.5 ± 12.8	<.01	29.4 ± 8.7	>.05
V	20.8 ± 5.6	31.5 ± 8.7	<.01	28.4 ± 11.7	≦.05
VI	23.5 ± 6.2	43.3 ± 13.1	<.01	29.2 ± 5.3	<.025
VII	23.7 ± 6.0	33.7 ± 7.1	<.01	33.8 ± 6.2	<.01
VIII	20.0 ± 3.6	33.1 ± 9.1	<.01	37.4 ± 9.5	<.01
IX	17.9 ± 5.6	25.3 ± 6.9	<.01	16.0 ± 6.4	>.05
X	17.7 ± 4.4	21.5 ± 11.7	>.05	14.1 ± 6.1	>.05

* Groups of 12 animals.

TABLE IV. Sr^{89} Absorption. Percentage absorbed from ligated segments after 30 min.

Segment	Normal saline *Unbuffered	Tris, pH 7.95 (37°) *Mean ± S.D.	p	Phosphate, pH 5.75 *Mean ± S.D.	p
II	38.6 ± 8.6	39.8 ± 10.2	>.05	29.9 ±13.0	<.01
III	25.7 ± 9.5	27.3 ± 13.1	>.05	16.1 ± 6.0	"
IV	17.1 ± 4.0	21.0 ± 7.1	≧.05	11.3 ± 5.4	"
V	23.9 ± 8.3	25.1 ± 9.6	>.05	10.6 ± 4.2	"
VI	16.6 ± 8.3	20.8 ± 8.5	>.05	9.7 ± 3.3	"
VII	14.9 ± 5.5	24.1 ± 7.0	<.01	9.4 ± 3.3	"
VIII	15.4 ± 3.4	22.6 ± 9.4	≦.01	11.1 ± 3.1	"
IX	15.7 ± 3.8	20.7 ± 9.0	≦.05	9.4 ± 3.4	"
X	15.2 ± 4.1	14.5 ± 6.1	>.05	10.1 ± 3.4	"

* Groups of 12 animals.

examination. Solutions of lower ionic strength had insufficient capacity to buffer mucosal secretions.

Absorption of Ca^{45} and Sr^{89}. The maximum rate of absorption of Ca^{45} is from the duodenum where 66% of the dose was absorbed within 30 minutes of introduction of the radioisotope. The absorption rate from the proximal jejunum is only slightly less; thereafter there is a sharp drop in the remaining segments, where approximately 20% only is absorbed in 30 minutes. The pattern for the rate of absorption of Sr^{89} is very similar, but the percentage absorbed in each segment is always less than that of Ca (Table III, IV). The difference is most marked in the duodenal and proximal jejunal segments but considerably reduced in the lower portion of the small bowel.

By increasing the pH of the duodenal segments to 7.3 with Tris, no change occurs in the rate of absorption of either Ca^{++} or Sr^{++}. There is a significant drop in rate of absorption of calcium from the proximal jejunum, whereas strontium absorption is not affected. On the other hand, absorption of both ions from all the other segments increases appreciably with the exception of the terminal ileum. Uptake of Ca^{45} in the femur and blood levels of the isotope reflect the changes in absorption rate from the control values (Table V, VII).

At the pH obtained by injecting the phosphate solution used as described, both calcium and strontium formed soluble salts. In spite of this, the absorption rate of Sr^{89} was uniformly reduced in the presence of acidic phosphate, throughout the small intestine. Blood levels and femur uptake (Table VI, VIII) were correspondingly lower. The absorption rate of calcium, on the other hand, was not so consistently altered as that of strontium. There was an insignificant increase in absorption from the duodenum while a small reduction was observed from the proximal jejunum. In all remaining segments except the terminal ileum, a slight increase of absorption occurred, significantly so in segments VII and VIII ($p < 0.01$). However, blood levels and bone uptake were lower than the control values.

Absorption from the duodenum was reduced from 40% to 25% in the presence of Tris pH

TABLE V. Ca^{45} Absorption. Blood levels (CPM) of Ca^{45} at the end of 30 min.

Segment	Normal saline *Unbuffered	Tris, pH 7.95 (37°) *Mean ± S.D.	p	Phosphate, pH 5.75 *Mean ± S.D.	p
II	20392 ± 6585	13904 ± 2773	<.01	14816 ± 2582	<.01
III	10100 ± 2847	8596 ± 3606	>.05	8031 ± 1782	<.025
IV	6998 ± 2246	6700 ± 2382	>.05	3517 ± 1614	<.01
V	4474 ± 2093	6249 ± 1318	<.01	1679 ± 533	"
VI	4770 ± 1760	6757 ± 2003	<.01	1556 ± 415	"
VII	3677 ± 1561	5203 ± 1542	<.025	1777 ± 628	"
VIII	3678 ± 915	6047 ± 2249	<.01	1920 ± 535	"
IX	3047 ± 915	5207 ± 2415	<.01	1393 ± 622	"
X	3160 ± 1193	3172 ± 1346	>.05	1335 ± 316	"

* Groups of 12 animals.

TABLE VI. Sr^{89} Absorption. Blood levels (CPM) of Sr^{89} at the end of 30 min.

Segment	Normal saline *Unbuffered	Tris, pH 7.95 (37°) *Mean ± S.D.	p	Phosphate, pH 5.75 *Mean ± S.D.	p
II	16142 ± 3309	16711 ± 5087	>.05	11804 ±2052	<.01
III	8184 ± 2676	8332 ± 3312	>.05	3578 ± 968	"
IV	4500 ± 1158	8505 ± 3125	>.01	2093 ± 446	"
V	5090 ± 1275	9642 ± 3072	<.01	2014 ± 396	"
VI	4229 ± 1066	6385 ± 1376	<.01	2446 ± 469	"
VII	5053 ± 1554	7094 ± 1565	<.01	2512 ± 465	"
VIII	4256 ± 690	6353 ± 2038	<.01	2155 ± 442	"
IX	3715 ± 940	5830 ± 1174	<.01	2042 ± 490	"
X	2910 ± 590	4051 ± 1002	<.01	2277 ± 426	"

* Groups of 12 animals.

TABLE VII. Ca^{45} Absorption. Femur uptake (CPM) of Ca^{45} at the end of 30 min.

Segment	Normal saline *Unbuffered	Tris, pH 7.95 (37°) *Mean ± S.D.	p	Phosphate, pH 5.75 *Mean ± S.D.	p
II	38110 ±13249	25201 ± 9354	<.01	38625 ± 6934	>.05
III	17722 ± 4951	12038 ± 5943	<.025	13250 ± 5585	<.025
IV	8350 ± 5238	8062 ± 2999	<.025	8062 ± 2999	>.05
V	8452 ± 2795	16204 ± 3373	<.01	2837 ± 744	<.01
VI	8232 ± 2033	10895 ± 4246	<.05	2828 ± 902	"
VII	7442 ± 2399	8826 ± 3251	>.05	3303 ± 863	"
VIII	6479 ± 2616	7163 ± 1859	>.05	3072 ± 820	"
IX	5625 ± 2054	5945 ± 2533	>.05	1921 ± 692	"
X	4982 ± 1848	3781 ± 1622	=.05	1803 ± 655	"

* Groups of 12 animals.

TABLE VIII. Sr^{89} Absorption. Femur uptake (CPM) of Sr^{89} at the end of 30 min.

Segment	Normal saline *Unbuffered	Tris, pH 7.95 (37°) *Mean ± S.D.	p	Phosphate, pH 5.75 *Mean ± S.D.	p
II	31051 ±14099	31822 ±10903	>.05	24372 ± 6878	>.05
III	9658 ± 2451	14249 ± 4540	<.01	6690 ± 1948	<.01
IV	6488 ± 2305	9594 ± 3411	<.01	4527 ± 1252	<.01
V	7251 ± 1834	18140 ± 4481	<.01	3535 ± 670	<.01
VI	7909 ± 3325	8295 ± 1171	>.05	2428 ± 547	<.01
VII	9669 ± 3941	8792 ± 2543	>.05	2036 ± 493	<.01
VIII	7125 ± 2480	9053 ± 1460	<.025	2117 ± 485	<.01
IX	9967 ± 2925	8255 ± 1923	>.05	2155 ± 567	<.01
X	8248 ± 1832	8653 ± 1811	>.05	8653 ± 1811	<.01

* Groups of 12 animals.

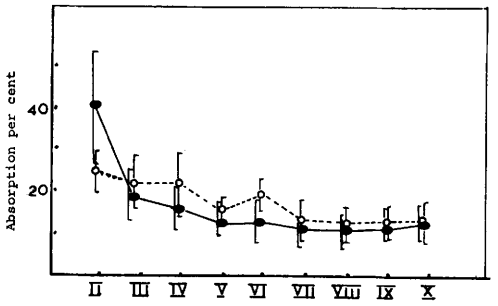

FIG. 1. Absorption of 2 μc Sr^{89} in the presence of Tris (pH 7.15 at 37°) from ligated intestinal segments. Percentage of injected dose absorbed from intestinal segment. ●—●, control; ○---○, Tris. Each point represents mean and SD on values from 12 rats.

7.15. In all other segments there is a small increase in absorption ranging from 0.25% of that of the normal saline (Fig. 1). These changes from the normal absorption rate are less than with the more alkaline Tris, and suggest that pH as well as the presence of the Tris anion influences the rate of absorption.

Total absorption of Ca^{45} and Sr^{89}. The enhancing effect of Tris and the depressant effect of acid phosphate on the amount of Sr^{89} and Ca^{45} absorbed from various segments of the lower small intestine reflects their action on the rates of absorption. These experiments suggested that Tris and acid phosphate might also influence total absorption from the entire small intestine, under physiological conditions.

Solutions (1 ml) of Ca^{45} and Sr^{89}, of the same composition as used in the ligated segment studies, were introduced through an orogastric tube to lightly anaesthesized rats. At the end of 2 hours, the animals were sacrificed. The results confirmed the previous findings (Table IX). By introducing Sr^{89} in the presence of Tris buffer, blood levels and bone uptake were increased by about 250% of the saline control value; on the other hand, blood and bone counts were depressed by about 50% by the acid phosphate solution. Blood levels of Ca^{45} were enhanced but bone uptake was increased very little by the alkaline reagents. Acid phosphate also reduced Ca^{45} levels both in blood and in bone.

Discussion. The results obtained by utilizing *in vivo* techniques confirm the previous findings in our laboratories by Dukay(4) and Paul(5) that the maximum rate of absorption of both Ca^{45} and Sr^{89} with carrier salt in physiological saline occurs in the duodenal segment, with a sharp drop in the jejunal and subsequent segments. The maximal rate of absorption from the duodenum could not be increased by altering the pH, nor by the presence of the solubilizing anions. A similar conclusion was suggested by Schacter and Rosen(12) who found that transfer of Ca^{45} by everted duodenal sacs is limited by a maximal rate.

The introduction of phosphate which buffered the duodenum at pH 6.0 or of Tris (buffered at pH 6.5-7.05), decreased the rate of Sr^{89} absorption considerably. On the other hand, the rate of absorption from the other segments can be both decreased or increased. Both Ca^{45} and Sr^{89} absorption rates from segments V to IX were increased in the presence of Tris which buffered the intestinal lumen to a relatively stable value at pH 7.3.

TABLE IX. Absorptive Capacity of the Small Intestine. Influence of acid phosphate (pH 5.85) and Tris (hydroxymethyl) aminomethane (Tris) (pH 7.95) on absorption of Ca^{45} and Sr^{89} introduced by orogastric intubation.

	Strontium^{89}	Exp / Control	Calcium	Exp / Control
	CPM	%	CPM	%
		Blood		
Control	6,178 ± 1,871		7,008 ± 1,130	
Tris	14,663 ± 6,202	237	12,696 ± 1,724	185
Phosphate	3,209 ± 1,378	52	5,242 ± 1,379	75
		Bone		
Control	55,145 ± 18,727		121,091 ± 29,653	
Tris	142,609 ± 23,730	258	130,050 ± 18,098	107
Phosphate	32,412 ± 10,973	59	98,175 ± 31,503	81

Each figure represents mean counts per min and standard deviation in groups of 6 animals.

The Ca^{45} absorption rate from two of these segments was also significantly increased in the presence of the phosphate buffer, whereas Sr^{89} absorption was consistently reduced.

Blood levels and bone uptake of Sr^{89} and Ca^{45} reflected, in general, variations in the loss of radioisotope from the intestinal segments. However with phosphate, Ca^{45} levels were decreased both in the bone and in the blood, whereas absorption from segments IV-VIII was increased, significantly so in segment VII and VIII ($p < 0.01$). It is possible that high phosphate derived from the acidic gut contents caused the formation of colloidal calcium phosphate in the hepatic veins, subsequently removed by the liver. This would account for the decreased blood and bone levels of Ca^{45} against the increase observed in the absorption rate. Further studies on the distribution of Ca^{45} in the soft tissues are necessary to confirm this hypothesis.

The intubation studies have shown that total absorption as well as rate of absorption of Ca or Sr is affected by the presence of Tris or phosphate buffer; apparently absorption under physiological conditions depends strongly on the anionic character of the metal salt as well as on the pH. As it is impossible to alter one variable at a time in experiments of this kind, it is difficult to determine whether alteration of pH or the presence of the solubilizing anion is directly responsible for the observed change from the normal absorption pattern. A third factor is introduced by the possible side effects of the buffer ions: Tris is a primary aliphatic amine of considerable reactivity.

Kshirsagar *et al*(13) have recently reported an overall discrimination in favour of calcium over strontium in the transfer from blood to bone. It is interesting to note that the Tris buffer used in our experiments reversed blood levels and bone uptake in favour of strontium (Table III). These alterations in absorption mechanisms may be of importance in the induction of malignant bone tumours by radioactive strontium(14).

Summary. Ligated intestinal segments in rats were prepared without disturbing the vascular or lymphatic supply. The pH of the contents of the lumen, measured immediately after excision of the segment, ranged from 5.96 in the proximal jejunum to 7.24 in the terminal ileum. Introduction of an acid phosphate buffer reduced the pH of all segments to the range 5.95-6.19. Tris (hydroxymethyl) aminomethane increased the pH to the range 7.03-7.6. Acid phosphate decreased the absorption rate of Sr whereas the alkaline Tris reagent increased absorption of both Sr and Ca from all segments except from the duodenum, which appears to have a maximum limiting rate. Intubation experiments confirmed the results obtained with ligated segments: the total absorptive capacity of the small intestine for these ions is decreased by acid phosphate and increased by the alkaline Tris reagent.

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