rest. The total period of repeated stimulation was 30 seconds and the flow per minute was calculated.

The flow of blood from an open free flowing cannula was caught and measured, both during a period of control when the limb was resting and flaccid, and during a period of stimulation as described above. Near the end of one of the procedures, the cannula became partially occluded by clotted blood, and the flow was reduced to a slow drip. The flow during both exercise and rest was also recorded under these circumstances. Several observations were recorded on each dog, and averages were computed as shown in the tables. Measurements during stimulation were alternated with measurements during rest in an effort to eliminate after-effects of stimulation on blood vessels.

Results. Measurements are recorded in the table. The venous return was increased from 80 to 100% by stimulation of the muscles when the cannula was flowing freely. When there was a partial obstruction, the increase was considerably greater, in one observation.

TABLE I.

No obstruction	At rest cc	With stimulation cc
Dog No. 1	45	90
	39	79
	33	74
Avg	39	81
Dog No. 2	30	53
	27	50
Avg	28.5	51.5

Conclusions. From these observations it is concluded that the venous flow of blood from a limb is increased by muscular contractions elicited by periodic electrical stimulations. The increase in flow was from 80 to 100%. Results suggest that increased blood flow is one of the reasons why similar methods, when applied to a large number of postoperative patients, have effected a marked reduction in the incidence of venous thrombosis.

1 Krogh, August, The Anatomy and Physiology of the Capillaries, New Haven, Yale University Press, 1927.

2 Bülbring, Edith, and Burn, J. H., *J. Physiol.*, 1939, **95**, 203.

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Protection of Gastric Mucosa of the Rat against Ulceration by Prefeeding with Protein Hydrolysates.

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The beneficial effects which Co Tui and his associates[1] reported to have resulted from the addition of large amounts of protein hydrolysates to the dietary of patients with gastric or duodenal ulcers have been amply corroborated by subsequent clinical experience. However, no adequate explanation of the action of such preparations has as yet been found. It has been thought that the buffering action[2] of the protein hydrolysates and/or the resultant improvement in the nitrogen balance[1,3,4] may be responsible for the beneficial effects observed. Opinions based on clinical studies have been expressed in favor of each of the above mechanisms, but the evidence presented cannot be regarded as conclusive. While the administration of protein hydroly-

1 Co Tui, Wright, A. M., Mulholland, J. H., Galvin, T., Barcham, I., and Gerst, G. R., *Gastroenterology*, 1945, **5**, 5.

2 Levy, J. S., and Siler, K. A., *Am. J. Digest. Dis.*, 1942, **9**, 354.

3 Hodges, H. H., *Gastroenterology*, 1947, **8**, 476.

4 Kenamore, B., Lonergan, W., and Shy, J. C., *Gastroenterology*, 1948, **10**, 177.

sate to ulcer patients has given encouraging clinical results, the effect of the hydrolysate on peptic ulcer and the mechanism of its action, as far as we know, have not been investigated experimentally.

We have described a method for the uniform production of ulceration in the rumen of the rat.[5] With this method ulceration develops as a result of the action of acid gastric juice accumulating in the empty stomach after pyloric ligation. In addition, a certain minimal concentration of pepsin and of acid is necessary for the development of ulceration, since neutralization of the acid or inactivation of the pepsin prevents ulceration.[5] We realize that rumen ulcers in the rat represent lesions of the wall of a cavity lined with squamous epithelium which in their pathogenesis may differ somewhat from ulcers of the glandular mucosa. However, as we[5] have pointed out previously, this method is valuable in studying the resistance of the gastric mucosa to acid-pepsin ulceration under various experimental conditions. This method has been employed by others[6-9] as well as by ourselves[10] for assaying antacid and anti-ulcer agents. By its means we have studied the effect of protein hydrolysates on the development of experimental ulcers in the rat. In this communication we report results which indicate that the fortifying of an otherwise adequate diet with protein hydrolysate affords definite protection to the gastric mucosa of the rat against the ulcerative action of acid gastric juice.

Experimental method and results. In the present study 106 rats were used. All the animals were females of the Wistar strain, bred in our own colony. After weaning, they were maintained on the Rockland Diet.* We confined our investigation to one sex in these experiments on account of the difference in the weight curves of the two sexes, and particularly since Zucker and Zucker[11] have shown that the more rapidly growing male requires more protein in the diet than the female. At the beginning of the experiments the weights of the animals ranged from 100 to 120 g. The females in each litter were divided into 3 groups, one control and 2 experimental. All the animals were fed the Rockland Diet *ad libitum* and the amount of food consumed by each animal during the experimental period of 28 days was recorded.

Two commercial preparations of protein hydrolysate were used as dietary supplements, each in a separate experimental series. Thus, in addition to the colony diet, animals in one series received by stomach tube 2 cc of hydrolysate A† in the morning and also in the late afternoon 6 days a week for a period of 4 weeks (28 days). The animals in the second experimental series received similar 2 cc quantities of a suspension of hydrolysate B,‡ which has a nitrogen content equivalent to that of hydrolysate A. Since hydrolysate A contains 22% of sucrose, a similar solution of sucrose alone was administered as a supplement to the animals in the control series to substitute for the protein hydrolysate. The experiments were done on 10 animals at a time, namely,

[5] Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., and Siplet, H., *Gastroenterology*, 1945, **5**, 43.

[6] Wick, A. N., Irish, A. J., Pauls, F., and MacKay, E. M., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 40.

[7] Pauls, F., Wick, A. N., and MacKay, E. M., *Gastroenterology*, 1947, **8**, 774.

[8] Risley, E. A., Raymond, W. B., and Barnes, R. H., *Am. J. Physiol.*, 1947, **150**, 754.

[9] Morris, C. R., Grossman, M. I., and Ivy, A. C., *Am. J. Physiol.*, 1947, **148**, 382.

[10] Shay, H., Komarov, S. A., Siplet, H., and Gruenstein, M., *Am. J. Digest. Dis.*, 1947, **14**, 99.

* The "Rockland Rat Diet" contains 24.83% of protein, 4.67% of fat, and 49.32% of carbohydrate, according to the statement of the manufacturers (Arcady Farms Milling Company).

[11] Zucker, T. F., and Zucker, L., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 136.

† Hydrolysate A, supplied by Sharp and Dohme, Inc., contains an equivalent of 35% of protein, as calculated from the nitrogen content (N × 6.25), and in addition 22% of sucrose. Control sucrose solution contained added vitamins present in hydrolysate A.

‡ Hydrolysate B, supplied by Mead, Johnson and Co., contains an equivalent of 75% of protein, as calculated from the nitrogen content (N × 6.25). Caloric value: 1 g = 3.75 C.

on 5 which had received supplemental hydrolyzed protein and on 5 controls. All the animals treated similarly have been grouped together in one series and the different series will be referred to as hydrolysate A-treated, hydrolysate B-treated, and sucrose-treated controls respectively.

Some modifications of our previously described technic were adopted in this study. Following the suggestion of Pauls, Wick and Mackay,[7] we used brief ether anesthesia during the operative period, not supplementing it with urethane as we had done in our original studies.[5] This resulted in more rapid recovery of the animal, a much earlier restoration of active gastric secretion, and a more rapid production of ulcers (97% ulceration in 6 hours when 2 cc of saline were instilled into the stomach after pyloric ligation).

The animals in all 3 series underwent the same preliminary treatment, which was as follows. After 48 hours' starvation the animal was placed under light ether anesthesia and the pylorus was ligated. The stomach was lavaged with distilled water, warmed to 38°C, and 2 cc of saline were then introduced. Each animal was placed in a separate clean cage, and was sacrificed 6 hours later.

In this study we routinely inspected the full length of the esophagus in each animal. We believe this to be essential, for in some cases perforating ulcers of the esophagus develop rather early. This causes a large part of the gastric juice to be lost from the stomach, and as a result the residual volume of gastric juice within the stomach becomes so small that it is insufficient to bathe the rumen and cause ulceration. A few cases of perforating esophageal ulcers occurred in our experiments and these were discarded.

Table I shows the number of animals with rumen ulceration and the mean values for the composition of the gastric contents of the animals in each series. It is clear that the fortifying of the already adequate diet with protein hydrolysate produced a rather striking increase in the resistance of the rumen mucosa to the destructive action of the very potent gastric juice. In a considerable pro-

TABLE I.

Incidence of Rumen Ulcers and Composition of the Gastric Contents in Rats Pretreated with Protein Hydrolysates and in Sucrose-Treated Controls.

Series	Ulcer incidence		Animals used†	Composition of gastric contents (mean values)					
	No. of animals*	%		Volume (ml/hr per 100 g body wt)	pH	Acidity Total	Acidity Free (m.eq/l)	Cl (m.eq/l)	Pepsin Conc. Act. (Mett units)
Sucrose control									
Total	35		32	.801	1.19	80	67	157	81 10.7
With ulcers	34	97	31	.803	1.19	80	67	157	80 10.8
Without ulcers	1	3	1	.741	1.05	94	82	166	108 9.6
Amisate treated									
Total	46		44	.840	1.15	86	71	158	77 10.4
With ulcers	28	59	26	.854	1.15	85	71	157	80 10.3
Without ulcers	18	41	18	.783	1.14	87	71	158	73 10.5
Protolysate treated									
Total	30		30	.868	1.14	88	75	156	61 7.1
With ulcers	22	73	22	.898	1.14	86	73	156	65 7.3
Without ulcers	8	27	8	.793	1.11	94	82	156	49 6.5

* % of animals with ulcers in each series was calculated from all animals used.

† For analysis of gastric contents several animals were not available due to accidents or heavy contamination of the gastric contents with blood.

TABLE II.
Severity of Ulceration in Protein Hydrolysate-Treated* Rats and in Sucrose-Treated Controls.

Series	No. of animals	Rumen lesions per animal: Single mean	Coalescent mean	Mean	Total S.D.†	P‡
Sucrose-treated controls	34	6.2	8.5	14.7	11.60	0.003
Protein hydrolysate-treated*	50	4.2	4.6	8.8	8.30	

* Amisate and protolysate treated series combined.
† Standard deviation.
‡ Probability of both groups belonging to same population.

TABLE III.
Average Daily Food Consumption and Caloric Intake and Average Weight Gain per Animal During Experimental Period (28 Days).

Series	No. of animals		Food intake (g): Protein	Carbohydrate	Fat	Caloric intake	Wt (g): Initial	Gain
Sucrose-treated (control)	32	*a*	3.27	6.51	0.61	47	110	38.8
		b	—	0.68	—			
		c	100%	100%	100%			
Amisate-treated	44	*a*	2.90	5.79	0.55	47	111	43.7
		b	1.17	0.68	—			
		c	125%	89.9%	90.1%			
Protolysate-treated	30	*a*	2.89	5.72	0.54	44	111	38.9
		b	1.16	—	—			
		c	124%	79.8%	88.4%			

a: Intake with Rockland diet.
b: Intake with hydrolysate (values for protein equivalent calculated from nitrogen content (N × 6.25)).
c: Total intake expressed as percentage of intake of sucrose-treated control series.

portion of the protein hydrolysate-treated animals, namely, 41% in the hydrolysate A-treated series and 27% in the hydrolysate B-treated series, no ulceration developed, while of the control animals only 3% had no ulcers. This difference was found to be highly significant statistically. Furthermore, those animals in the hydrolysate-treated series that did develop ulcers showed significantly fewer lesions per animal than the animals in the sucrose-treated series (Table II).

The adopted classification of the rumen lesions was, from the very nature of the ulcerative process, somewhat arbitrary. However, under magnification of 10.5 X, the differentiation between "single" and "coalescent" multiple lesions is not difficult. The former are characterized by even, circular margins; the latter by scalloping or irregularity in outline and, as a rule, by greater dimensions. The relatively greater reduction in the "coalescent" ulcers in comparison with single lesions in the hydrolysate treated animals increases the significance of the results.

The most striking feature of these experiments is the absence of any significant differences between the hydrolysate A- or the hydrolysate B-treated series and the sucrose-treated controls in respect of the volume, acidity, and peptic power of the gastric contents. The difference in the incidence of ulceration manifestly cannot be attributed to concurrently lower digestive power of the gastric juice in the hydrolysate-treated animals. We believe that our results indicate this effect of protein hydrolysate to represent a true increase in the mucosal resistance and that it is to be distinguished from the protective effect obtained with other agents, *e.g.*, antacids, which act by depressing the digestive power of the gastric juice. The increased resistance of the mucosa in the hy-

drolysate-treated animals was not due to a greater caloric intake, since the average amounts of food consumed daily by the animals in all series were isocaloric, although the diets differed in composition. This is illustrated by the data presented in Table III, which also shows the proportions of protein, carbohydrate, and fat in the different diets, so that the food intake of the hydrolysate A- and the hydrolysate B-treated series may be compared with that of the sucrose-treated control series. The rate of growth was adequate in all groups and no statistically significant .difference was found between the weight gain of the sucrose-treated controls and that of the protein hydrolysate-treated animals.§ This indicates that the protein in the Rockland Diet was adequate for the animals at the weight levels at which the experiments were started, and that the addition of protein hydrolysate did not result in any significant increase in the nutritional value of this diet.

Nevertheless, these experiments show that the prefeeding of approximately 25% more hydrolyzed protein to the experimental animals than was fed to the controls for the relatively short period of 28 days resulted in a very marked increase in the resistance of the rumen mucosa to peptic ulceration. This may be regarded as a true prophylactic action. The only other preparation that has been reported to be effective in the prevention of peptic ulceration is enterogastrone, as demonstrated in experimental studies on dogs.[12]

It is noteworthy that this prophylactic effect of protein hydrolysate was exerted against the ulcerating action of acid and pepsin, the agents which were shown to be responsible for the type of experimental ulcer produced by our method in the rat, and which also are generally accepted as factors of primary importance in the development of peptic ulceration in man. All concepts regarding the etiology of gastric and duodenal ulcers in man recognize that factors other than acid and pepsin are also involved. Among these are included differences in the resistance of tissues to the destructive action of gastric juice. If the prophylactic action of protein hydrolysate, which is demonstrated in this study for the the rat, should be found to apply also to man, the first step in the solution of the all-important problem of ulcer prevention will have been accomplished by the simple expedient of fortifying the diet. Experiments are now being carried out to determine whether the addition of native proteins to the diet will produce the same effect as hydrolysates.

Summary. The supplementing of an already adequate diet with protein hydrolysates in amounts equivalent to 25% of additional protein produced in rats a marked increase in the resistance of the gastric rumen to peptic ulceration without concurrent changes in the volume, the acidity, or the peptic power of the gastric contents. This protective effect was not accompanied by any significant changes in the rate of growth of these animals as compared with animals on a control diet supplemented with sucrose alone.

§ Hydrolysate A-treated *versus* Hydrolysate B-treated: $t = 1.85$, d. f. $= 75$, $P = 0.06$. Hydrolysate A-treated *versus* sucrose-treated control: $t = 1.64$, d. f. $= 81$, $P = 0.10$. Hydrolysate B-treated *versus* sucrose-treated control: $t = 0.02$, d. f. $= 64$, $P = >0.55$.

12 Hands, A. P., Greengard, H., Preston, F. W., Fauley, G. B., and Ivy, A. C., *Endocrinology*, 1942, **30**, 905.