

the salt treatments of the first 2 days; all remained open longer than those of the controls.

Of the 22 experimental animals, only one failed to regenerate. None of the limbs of the 15 controls developed past the stage of a healed stump. The blastema on the limbs of the frogs whose arms were removed below the elbow began as a single mound of soft tissue. The single mound grew and differentiated into a wedge of tissue with the thin edge at the distal border. As growth proceeded a cleft converted the wedge into a double structure distally. Some of the regenerants developed further with new clefts appearing in each of the distal protuberances. By this time it was apparent that the resulting 4 protuberances were finger buds. Fig. 1 A shows such a regenerant. Alongside the regenerant is shown the corresponding limb of the other side. This animal was 5.2 cm long at the time of limb amputation in July and had metamorphosed the previous year or earlier. Fig. 1 B shows finger regeneration following amputation of two fingers from a first year frog. The regenerated first finger appears to be double and the second finger normal. Fig. 1 C shows the regenerated skeletal elements in a frog whose arm had been amputated through the radio-ulna. A movable wrist and imperfect hand regenerated. Skeletal elements along with other internal structures of the regenerants will be described elsewhere.

Adult frog limbs, therefore, regenerate when the wound is kept irritated with a strong salt solution.

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Nitrogen Content and Total Osmotic Activity of Gastric Juice.*

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In a previous communication¹ we reported the degree of hypo- or hypertonicity of gastric juice samples collected from chronic greater curvature (Heidenhain) and pyloric pouches in dogs. Histamine stimulated juices were 2-6 m.E./K hypertonic; pilocarpine stimulated juices were either iso- or hypotonic; and fasting juices were hyper-

* We are indebted to Mr. Charles Carr for carrying out many of the chemical determinations for this study.

¹ Lifson, Nathan, Varco, Richard L., and Visseher, M. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **47**, 422.

hypo-, or isotonic. Since pilocarpine results in more mucin production than does histamine, it seemed of interest to ascertain whether an inverse correlation existed between the nitrogen content and osmotic activity of these same secretion samples. The material studied in our previous work¹ was, therefore, used for the study reported here.

Methods. The osmotic activity data are the same reported in the previous paper, in which the experimental procedure, and analytical methods involved in the study of 31 samples from 7 dogs, have been described.¹ Free acid determinations were made directly upon collection; but the total nitrogen, chloride and total fixed base were measured on the same material after storage at freezing temperature for 60-100 days. Total nitrogen was estimated by the micro-Kjeldahl method without preliminary removal of constituents by centrifuging, filtering, or similar means. Free acidity was determined with dimethyl-amino-azo-benzene (Topfer's reagent) as the indicator. Total fixed base was measured on ashed samples by the electro dialysis method of Keys and Adair² as modified by Consalazio and Talbott.³ Chlorides were done by a micro-modification of the Volhard titration.

Results and Discussion. A. *Osmotic Activity and Nitrogen Content.* Tables I and II contain data for 30 samples, the following features of which will be noted:

(1) For greater curvature pouch samples, the histamine-stimulated juices had a total nitrogen content of 50 mg per 100 cc or less, whereas the pilocarpine-stimulated juices all had a total nitrogen content of 65 mg per 100 cc or more. Fasting samples possessed values in both ranges.

(2) Pyloric pouch juices had a total nitrogen content at least 100 mg per 100 cc higher than any of the greater curvature pouch juices.

(3) Samples with approximately the same total nitrogen content and collected under comparable circumstances may exhibit relatively large differences in osmotic activity. Compare, for example, Exp. VIIa with Exp. XVIa, or VIIb with XIIb. (Table I.)

(4) Samples with approximately the same osmotic activity and collected under comparable circumstances may exhibit relatively large differences in total nitrogen content. Compare, for example, Exp. IIIa with Exp. XIIa. (Table I.)

The anticipated inverse correlation between total nitrogen content

² Adair, G. S., and Keys, A. B., *J. Physiol.*, 1934, **81**, 162.

³ Consalazio, W. V., and Talbott, J. H., *J. Biol. Chem.*, 1940, **132**, 753.

TABLE I.

Exp. No.	Condition of collection	Site of pouch	Osmotic activity of		OA (Osmotic activity of gastric juice minus plasma),	Total nitrogen of gastric juice, mg per 100 cc
			Plasma, m.E./K. H ₂ O	Gastric juice, m.E./K. H ₂ O	m.E./K. H ₂ O	
IIa	Fasting	Greater Curvature	157	155	— 2	50
IIIa	"	" "	158	156	— 2	8
Va	"	" "	155	151	— 4	63
Xa	"	" "	158	156	— 2	95
VIIa	"	" "	163	151	—12	67
VIIIa	"	" "	161	161	0	70
IXa	"	" "	161	167	+ 6	180
XIIa	"	" "	161	158	— 3	95
XIVa	"	" "	157	157	0	33
XVIa	"	" "	161	161	0	60
XIa	"	Pyloric	157	161	+ 4	340
XIIIa	"	" "	161	165	+ 4	500
XVa	"	" "	157	169	+12	350
XVIIa	"	" "	161	162	+ 1	280
Ib	Histamine	Greater Curvature	159	161	+ 2	28
IIIb	"	" "	161	167	+ 6	12
IVb	"	" "	156	162	+ 6	50
Vb	"	" "	152	155	+ 3	21
Xb	"	" "	160	163	+ 3	30
Vlb	Pilocarpine	" "	160	150	—10	90
VIIb	"	" "	162	154	— 8	160
VIIIb	"	" "	161	145	—16	90
IXb	"	" "	163	147	—16	160
XIIb	"	" "	162	162	0	100
XIVb	"	" "	163	162	— 1	65
XVIb	"	" "	165	165	0	130
XIb	Histamine	Pyloric	160	164	+ 4	500
XIIIb	Pilocarpine	" "	162	153	— 9	400
XVb	"	" "	163	161	— 2	400
XVIIb	"	" "	165	164	— 1	400

TABLE II.

Conditions of collection and type of pouch	No. of cases	OA		Total nitrogen of gastric juice	
		Mean m.E./K. H ₂ O	Range m.E./K. H ₂ O	Mean mg per 100 cc	Range mg per 100 cc
Fasting, Greater Curvature	10	—1.9	—12 to + 6	72	8-180
Histamine, " "	5	+4	+ 2 to + 6	28	12-50
Pilocarpine, " "	7	—7.3	—16 to 0	104	65-160
Fasting, Pyloric	4	+5.2	+ 1 to +12	368	280-500
Histamine, " "	1	+4		500	
Pilocarpine, " "	3	—4	— 1 to — 9	400	

and osmotic activity appears most clearly in the comparison between histamine- and pilocarpine-stimulated juices obtained from greater curvature pouches. The former juices all were higher in osmotic activity and lower in total nitrogen content than any of the latter. In this connection it may be mentioned that Gilman and Cowgill⁴ showed that in histamine-stimulated secretions from Pavlov pouches, hypotonicity up to 17 m.E./K. was associated with high mucus content. Our results indicate, however, that the relationship between the two variables measured is not a close one, especially if all the samples are considered as a single group without regard to conditions of collection or type of pouch. The nitrogenous constituents of gastric juice, by virtue of their buffer capacity, could participate in the production of hypotonicity in a manner previously described.^{5, 1} The absence of closer correlation between total nitrogen content and osmotic activity may be explained in part by (a) the fact that total nitrogen is probably not an accurate index of this nitrogenous buffer capacity,⁶ and in part by (b) the operation of quantitatively more important factors, in particular, buffering by non-nitrogenous constituents.

B. *Comparison of Calculated and Observed Osmotic Activities.* Table III contains the results of determinations of free acidity, total fixed base, and chlorides, all expressed in m.E. per liter of gastric juice, for nine of the samples, arranged in the order of increasing total nitrogen content. From these additional data, the total osmotic activity of the samples in question has been calculated on the assumptions that (1) free acidity is equivalent to hydrogen ion concentration, (2) all the fixed base is univalent, (3) the difference in units employed is negligible, *i. e.*, non-solvent volume has been ignored, and (4) no other osmotically active constituents are present. It will be noted in Table III that for the six samples in which free acid occurred (all from greater curvature pouches) the agreement between calculated and observed osmotic activity values is reasonably close, *i. e.*, within 6 m.E./K. However, for the 3 samples with no free acid (all from pyloric pouches) the calculated values are 12, 24, and 24 m.E./K., respectively, less than the observed values. Such discrepancies as the last two suggest that one or more of the assumptions involved in the calculation may be erroneous. But the first of these assumptions appears to be exonerated by the absence of free

⁴ Gilman, Alfred, and Cowgill, George R., *Am. J. Physiol.*, 1933, **103**, 143.

⁵ Hollander, Franklin, *Am. J. Dig. Dis. and Nut.*, 1936, **3**, 651.

⁶ Bonis, A., *Z. f. Klin. Med.*, 1930, **113**, 611; Mitchell, T. Carlyle, *J. Physiol.*, 1931, **73**, 427.

TABLE III.
Comparison of Calculated and Observed Osmotic Activities of Gastric Pouch Juices.

Exp. No.	Condition of collection	Site of pouch	Free acidity, fixed base, Chlorides, m.E./L.	Total m.E./L.	Calculated osmotic activity, m.E./L.	Observed osmotic activity, m.E./L.	Calculated nitrogen minus observed	Total nitrogen content, mg/100 cc
Xb	Histamine	Greater Curvature	141	15	170	163	0	30
XIVa	Fasting	"	141	25	156	161	+ 4	33
XVIa	"	"	144	24	165	166	+ 5	60
VIIb	Pilocarpine	"	122	21	159	151	+ 1	90
XVIIb	"	"	152	19	167	169	+ 4	130
IXa	Fasting	"	2	160	168	165	- 2	180
XVa	"	Pyloric	0	160	154	157	-12	350
XVb	"	"	0	130	144	137	-24	400
XVIIb	"	"	0	124	153	138	-24	400

acid, and the second by the fact that any polyvalent base would yield fictitiously high rather than fictitiously low calculated osmotic activities. Correction for non-solvent volume would not account for more than 6-7 m.E./K. However, it may be that the fourth assumption does not hold for anacid juices, particularly of pyloric origin. The high total nitrogen content of such secretion samples suggests that nitrogenous constituents of relatively low molecular weight may account for an appreciable part of the osmotic deficit.

Summary. 1. In general, pilocarpine-stimulated greater curvature gastric pouch juice has a lower osmotic activity, and high total nitrogen content than does histamine-stimulated juice. 2. Fasting juices show highly variable relations between total nitrogen content and osmotic activity. 3. Pyloric pouch fasting juice has in our experience very much higher total nitrogen content than does greater curvature pouch juice. The osmotic activity of the former is nevertheless hypertonic with respect to blood. 4. There appears to be a partial correlation between total nitrogen content and osmotic activity of gastric juice. 5. Calculated and observed osmotic activities are in reasonable agreement for six greater curvature pouch samples in which free acid was present. 6. Calculated values were 12 to 24 m.E./K. lower than the observed osmotic activity values for three pyloric pouch juices without free acid. It is suggested that part of the deficit results from neglecting in the calculation the contribution of the nitrogenous constituents.

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An Analysis of Tobacco Mosaic Virus for Biotin, Riboflavin and Pantothenic Acid.

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Investigation into the structure of filterable viruses has revealed the presence of some amino acids,^{1, 2} certain enzymes such as catalase

¹ Ross, A. F., *J. Biol. Chem.*, 1941, **138**, 741.

² Knight, C. A., and Stanley, W. H., *J. Biol. Chem.*, 1941, **141**, 39.