Effects of Starvation on Plasma Pancreatic Glucagon in Normal Man

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SUMMARY

The role of pancreatic glucagon in starvation has been difficult to assess in humans because of the nonspecificity of antisera heretofore available for glucagon radioimmunoassay. The development of a relatively specific antiserum for pancreatic glucagon has now made possible valid measurements of pancreatic glucagon in human plasma and the effect of total starvation on plasma glucagon was, therefore, re-examined. Ten healthy male volunteers abstained from food for seventy-two hours or longer. During this period the mean level of glucose declined from 86 to 70 mg./100 ml., insulin declined from 10 to 3 μU./ml., while glucagon rose progressively from a mean prestarvation level of 126 μμg./ml. to 157, 189, and 178 μμg./ml. at the end of one, two, and three days' starvation, respectively. The responsiveness of the alpha cells to arginine stimulation was tested at the end of the starvation period by means of a forty-minute infusion of arginine at a rate of 11.5 mg./kg./min. Every one of the five subjects tested exhibited a prompt rise in glucagon secretion which reached a peak of 516 μμg./ml. in thirty minutes, significantly greater than the glucagon response of fed controls. Mean insulin concentration during the arginine infusion rose only 6.8 μU./ml. in contrast to a 29 μU./ml. rise in fed subjects. The biologic effect of the modest rise (about 50 per cent) in glucagon concentration would be exaggerated by the concomitant decline in insulin concentration. The magnitude and promptness of the glucagon response to arginine suggests that the pancreas contains abundant glucagon after three days of total starvation. DIABETES *18*:717-23, November, 1969.

The traditional view of glucagon as a hormone of "glucose need" is strongly supported by in vivo evidence that hypoglycemia, whether induced by insulin,[1-3] phloridzin,[1] or a sulfonylurea[3] stimulates its secretion. However, these forms of glucose need are highly unphysiologic experimental maneuvers, which have no counterpart in the healthy organism. Perhaps the only truly naturally occurring form of severe glucose need which can occur in a healthy individual is starvation, but efforts to demonstrate hyperglucagonemia during prolonged fasting have yielded conflicting results. Earlier work in this laboratory indicated that the glucagon level in healthy subjects rose threefold at the end of a three-day total fast,[4] and similar findings were later reported by Lawrence.[5] However, Samols et al.[6] were unable to demonstrate starvation hyperglucagonemia in man, and subsequent studies in our laboratory were also unsuccessful.[7]

In the foregoing studies all measurements of "glucagon" included glucagon-like immunoreactivity (GLI) of gastrointestinal origin, which may comprise close to 90 per cent of total plasma immunoreactivity,[8] as well as pancreatic glucagon, and their validity as a gauge of pancreatic glucagon secretion can be questioned. The accidental discovery of a glucagon antiserum of high sensitivity, which, unlike previously available antisera, cross-reacts only very weakly with gastrointestinal GLI, and gives zero readings in plasma from subtotally depancreatized dogs[9] made possible valid measurements of *pancreatic* glucagon in human plasma. The question of pancreatic glucagon secretion in starvation was, therefore, re-examined.

METHODS

Young, healthy, nonobese volunteers were recruited from among the house staff and medical student body for starvation experiments. They were observed for two days on their normal diet before starting three to four days of total fasting, save for water. Each patient was weighed daily and urine acetone was tested with Acetest tablets.

Each day of the experiment the subjects reported to the laboratory at either 8:00 a.m. or 9:00 a.m. and three blood specimens were collected at thirty-minute intervals. A 5 ml. blood sample was placed into

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chilled tubes containing EDTA and 5,000 U. of Trasylol in a volume of 0.5 ml. Blood specimens were centrifuged immediately at 4° C. and plasma was frozen and stored at —20° C. until the time of assay. Assays were performed within twenty days of the date of the experiments.

Glucagon was assayed by the previously reported technic[10] modified as follows: 20 μμg. glucagon-I-131 in 0.4 ml., 0.2 ml. of Trasylol (2,000 U.), and 0.2 ml. of either the glucagon standard or the unknown sample were incubated at 4° C. for four days with 0.4 ml. of the highly specific G-58 antiserum in a final dilution of 1:9,000. Separation of free and bound glucagon-I-131 was carried out by the charcoal method of Herbert[11] after it was found to give values identical to those obtained by chromatographic separation.

The standard deviation of the difference of each replicate determination from the mean of the replicates was ± 9.5 μμg./ml. From the standpoint of precision and minimal sensitivity it would appear that this assay can easily measure changes of 55 μμg./ml. and above with 95 per cent confidence.

The charcoal-dextran mixture is prepared by mixing equal volumes of 1 per cent charcoal and 0.5 per cent Dextran 80 solutions (both in 0.2M glycine at pH 8.8), and mixing gently for ten minutes at room temperature. The assay tubes are placed in a water bath of 4 to 6° C. To all tubes containing glucagon standard 200 μl. of a carrier protein mixture (normal sheep serum and Trasylol in a 9:1 ratio) chilled to 4 to 6° C. is added. No carrier protein is added to tubes containing samples of undiluted plasma. Using an Eppendorf automatic pipette, 0.5 ml. of the charcoal-dextran solution is added to each tube. These are then shaken several times and returned to the water bath. Forty-five minutes later the tubes are centrifuged at 4 to 6° C. for fifteen minutes at 2,000 rpm. After removal of the supernatant by suction, each tube is counted for radioactivity.

Insulin was assayed by the technic of Berson and Yalow[12] using the Herbert charcoal separation method.[11] Plasma glucose was measured on the Technicon Auto-Analyzer by the method of Hoffman.[13]

RESULTS

1. *"Within-hour" variability of glucagon levels*

The standard deviation of the difference of each of the trio of daily specimens from their mean was ± 10 μμg./ml., very close to the ± 9.5 standard deviation of the difference of replicates of a single blood specimen from their mean; this indicates that the variation as measured at three points within an hour is entirely attributable to the variability of the technic itself.

2. *Morning glucagon levels before and during starvation* (table 1)

A group of ten healthy male volunteers was assembled for starvation experiments. Twenty-four hours before and on the morning of the start of the fast plasma glucose averaged respectively, 89 (S.E.M. ± 1.4) and 86 mg./100 ml. (± 1.2), the glucagon averaged 126 (S.E.M. ± 10.4) and 126 μμg./ml. (± 12.3) and the insulin averaged 9 μU./ml. (S.E.M.. ± 1.5) and 10 μU./ml. (± 2.2). After twenty-four hours of starvation mean glucose fell to 78 mg./100 ml., glucagon rose to a mean of 157 μμg./ml. (± 18.6) while the mean insulin averaged 5 μU./ml. (± 1.0). After forty-eight hours of starvation, at which time the mean glucose level was 72 mg./100 ml., there were glucagon increments in six of the ten subjects; the mean glucagon rose to 189 μμg./ml., a statistically significant change ($p < 0.01$), and the insulin averaged 4 μU./ml. (± 0.6). After seventy-two hours the glucose level averaged 70 mg./100 ml. and all but two of the ten subjects had now exhibited a significant rise in glucagon on at least one of the days since the start of the fast. Glucagon averaged 178 μμg./ml. (± 18), a significant rise above day "0" ($p < 0.01$), and insulin averaged 3.0 μU./ml. (± 0.6). The mean insulin level after one day of starvation and on all subsequent days was significantly below the value on day "0" ($p < 0.001$).

The complete results of the study are recorded in table 1 and the mean values are shown in figure 1. Weight loss ranging 4 to 13 lbs. and ketonuria were observed in all subjects by the end of the fast.

3. *Arginine infusion test* (table 2)

Amino acid mixtures and arginine alone have been reported previously to stimulate the secretion of both insulin[14,15] and glucagon.[14,16,17] The glucagon response to the infusion of arginine monohydrochloride has re-

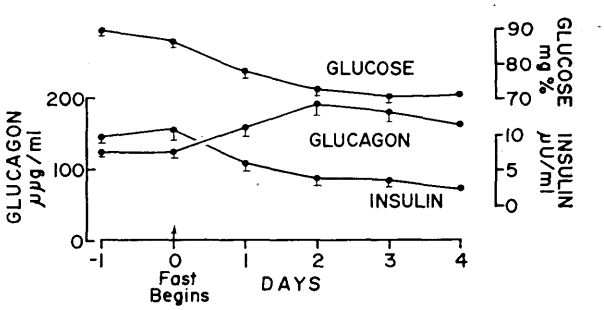

FIG. 1. Mean morning glucagon, insulin and glucose levels before and during three to four days of total starvation.

TABLE 1

Effect of starvation on the morning level* of glucagon and insulin

Subject	Measurement	Hours before —24	Start of fast 0	Hours after 24	48	72	96
	Glucose		80.0	68.0	72.6		
1118	Glucagon		196	287	260.0		
	Insulin						
	Glucose	89.0	91.3	98.0	88.3	74.6	74.3
1122	Glucagon	137	145	130.0	153	207	227
	Insulin	16.6	21.6	9.0	5.3	5.6	1.6
	Glucose	95.3	88.3	75.3	69.6	70.3	
1126	Glucagon	90	63	90	150	157	
	Insulin	16.3	13.0	8.0	3.0	2.6	
	Glucose	88.6	82.6	75.3	71.0	67.3	
1130	Glucagon	150	173	237	353	297	
	Insulin	9.3	15.6	4.0	2.0	1.6	
	Glucose	82.3	90.0	84.0	80.6	73.3	
1154	Glucagon	113	110	117	130	167	
	Insulin	11.0	17.6	10.6	7.3	7.0	
	Glucose	87.0	88.0	76.6	60.0	72.6	75.5
1157	Glucagon	90	93	113	127	103	105
	Insulin	7.3	6.6	2.3	2.3	3.3	4.0
	Glucose	89.3	82.0	67.6	62.6	59.0	63.5
1158	Glucagon	197	133	157	203	210	165
	Insulin	4.0	3.3	3.0	2.3	1.6	1.0
	Glucose	96.6	88.6	78.6	72.0	69.0	
1159	Glucagon	113	107	130	123	150	
	Insulin	5.3	6.3	3.3	5.3	4.0	
	Glucose	88.0	88.3	78.6	78.6	81.0	
1160	Glucagon	130	110	123	127	120	
	Insulin	8.0	6.0	2.6	4.3	3.0	
	Glucose	89.3	85.0	78.0	68.0	68.5	
1164	Glucagon	117	127	190	260	190	
	Insulin	6.6	4.3	3.0	2.6	1.5	
			Glucose				
	Mean	89.0	86.0	78.0	72.0	70.0	71.0
	±S.E.M.	1.4	1.2	2.6	2.6	1.9	
	p			<0.01	<0.001	<0.001	
			Glucagon				
	Mean	126	126	157	189	178	165
	±S.E.M.	10.4	12.3	18.6	23.5	18.0	
	p			n.s.	<0.01	<0.01	
			Insulin				
	Mean	9.0	10.0	5.0	4.0	3.0	2.0
	±S.E.M.	1.5	2.2	1.0	0.6	0.6	
	p			<0.01	<0.001	<0.001	

*Each individual hormone level is the mean of three levels obtained at 8:00, 8:30 and 9:00 a.m.

cently been studied in a similar group of healthy young volunteers.[18] To determine the effect of starvation on the glucagon response to arginine, five of the present group of volunteers were given a forty-minute infusion of arginine monohydrochloride* at a rate of 11.5 mg./kg./min. Within five minutes of the start of the infusion

*R-Gene, Cutter Laboratories.

TABLE 2

Insulin and glucagon response to arginine infusion in starvation

		Arginine, 700 mg./kg./hr. minutes											
Subject		—30	—20	—10	0	5	10	20	30	40	50	60	70
#1157	G	75	73	75	76	73	73	79	80	75	76	82	80
	Gl	100	110	100	110	350	520	620	550	480	460	320	230
	INS	5.0	4.0	3.0	3.0	14.0	4.0	4.0	3.0	3.0	3.0	2.0	2.0
#1158	G	64	62	74	63	64	60	72	74	80	76	78	79
	Gl	170	190	120	160	320	640	650	690	660	550	380	290
	INS	1.0	1.0	1.0	1.0	3.0	3.0	3.0	2.0	3.0	2.0	2.0	2.0
#1159	G	66	66	66	72	66	68	68	68	68	72	78	72
	Gl	150	140	130	150	260	400	360	460	560	280	150	140
	INS	1.0	1.0	2.0	2.0	7.0	4.0	3.0	3.0	3.0	2.0	3.0	2.0
#1160	G	77	86	85	85	85	86	87	83	82	83	70	80
	Gl	120	130	130	120	260	270	360	330	190	220	130	120
	INS	3.0	3.0	3.0	3.0	7.0	10.0	10.0	9.0	12.0	6.0	1.0	3.0
#1164	G	69	70	68	68	70	74	74	73	73	75	75	77
	Gl	190	180	180	190	420	440	550	550	580	560	400	280
	INS		2.0	2.0	1.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0
G Mean		70.2	71.4	73.6	72.8	71.6	72.2	76	75.6	75.6	76.4	76.6	77.6
±S.E.M.		2.5	4.0	3.0	3.7	3.7	4.2	3.3	2.6	2.5	1.8	1.9	1.5
p						n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Gl Mean		146.0	150.0	132.0	146.0	322.0	454.0	508.0	516.0	494.0	414.0	276.0	212.0
±S.E.M.		14.6	13.5	12.0	12.8	26.8	55.1	56.0	53.0	72.6	62.4	51.0	31.0
p						<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.05	n.s.
INS Mean		2.5	2.2	2.2	2.0	6.8	4.8	4.6	4.0	4.8	3.2	2.0	2.2
±S.E.M.		0.8	0.5	0.3	0.4	1.7	1.2	1.2	1.1	1.6	0.7	0.3	0.2
p						<0.01	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

G = Plasma glucose mg./100 ml.
Gl = Plasma glucagon μμg./ml.
INS = Insulin μU./ml.

all five subjects exhibited a prompt rise in glucagon concentration, which reached a peak of 516 μμg./ml. (± 53) at thirty minutes. Figure 2 indicates the mean glucagon level during arginine infusion, which at twenty and at thirty minutes is significantly greater than ($p < 0.005$) that of the group of fed normal volunteers reported elsewhere.[19] The mean insulin concentration rose only to 6.8 μU./ml. (± 1.7) in response to arginine infusion, in contrast to a 29.1 μU./ml. (± 2.5) rise observed in fed volunteers.[19] The hyperglycemic response, which averaged 16 mg./100 ml. in fed subjects, rose only 3.2 mg./100 ml. All individual results are summarized in table 2.

4. *Alpha amino nitrogen concentration and arginine tolerance during starvation*

Because hyperaminoacidemia is a stimulus of pancreatic glucagon secretion, it seemed important to exclude the possibilities that the hyperglucagonemia of starvation was a consequence of hyperaminoacidemia, and that the enhanced glucagon response to arginine infusion was a consequence of diminished tolerance to arginine. For these reasons alpha amino nitrogen levels were measured on the last day of the starvation just before the start of the arginine infusion and during the infusion, and the values were compared with those of a similar group of fed healthy males. The results, shown in table 3, reveal no difference in the concentration of alpha amino nitrogen either before or during the arginine infusion. It is, therefore, concluded that starvation-induced changes in glucagon concentration cannot be a consequence of differences in alpha amino nitrogen concentration.

DISCUSSION

The sensitive assay system employed in this study measures little or no circulating gastrointestinal glucagon-like immunoreactivity, which in earlier assay systems accounted for as much as 90 per cent or more of the measured immunoreactivity.[8] This assay system has given readings of zero on plasma obtained from depancreatized animals[9] and from a patient with advanced

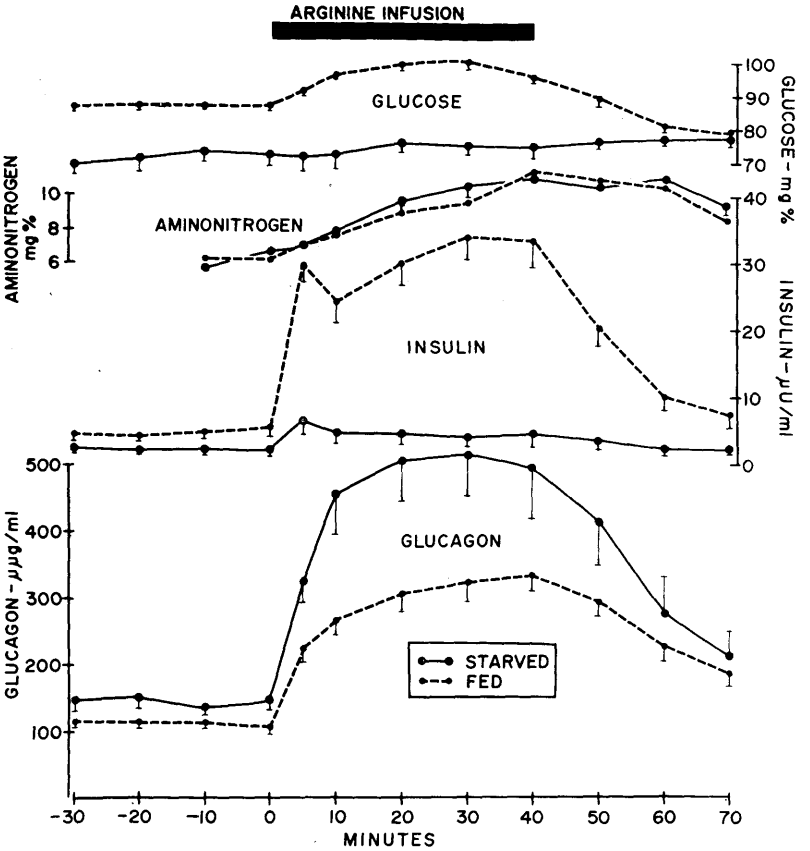

FIG. 2. The mean response of glucagon, insulin, glucose, and alpha amino nitrogen to arginine infusion after three or four days' total starvation compared with that of normal subjects.

pancreatic acinar and insular insufficiency secondary to severe calcific pancreatitis.[19] Consequently, it is believed that the plasma levels measured with the present assay system reflect largely, if not exclusively, the true level of circulating pancreatic glucagon, and that the contribution of extrapancreatic immunoreactivity is, at most, small.

During starvation the mean of the measurements of plasma glucagon made on the three blood specimens drawn during a one-hour period each morning rose by

TABLE 3

Comparison of plasma and aminonitrogen levels and arginine tolerance in starved and fed healthy males

		Arginine infusion 700 mg./kg./hr. minutes									
Subject		—10	0	5	10	20	30	40	50	60	70
1157	Fed	5.4	5.4	—	8.0	9.4	9.6	11.4	12.6	—	9.0
	Starved	5.8	6.8	7.0	8.0	10.5	9.5	9.0	11.0	8.5	8.0
1158	Fed	6.6	6.4	8.2	7.8	9.6	—	12.0	12.2	11.6	—
	Starved	5.4	6.3	7.0	8.5	11.0	12.0	12.5	12.0	11.5	11.0
1159	Fed	8.0	7.6	7.6	8.6	11.0	—	12.6	15.0	12.0	14.6
	Starved	—	6.4	6.3	7.3	8.0	9.0	12.0	9.0	9.5	9.0
1160	Fed	6.2	7.4	7.2	8.2	8.4	9.4	12.0	8.0	8.6	6.4
	Starved	6.0	6.6	8.0	8.3	10.5	11.5	14.3	10.3	9.5	9.3
1164	Fed	4.8	4.2	5.6	6.4	6.4	9.8	9.6	8.0	—	5.0
	Starved	6.0	6.2	—	—	9.0	11.0	—	—	16.0	10.3
Fed mean		6.4	6.2	7.1	7.8	9.0	9.6	11.5	11.2	10.7	8.7
±S.E.M.		0.5	0.6	0.5	0.4	0.7	0.1	0.5	1.3	0.9	1.8
Starved mean		5.8	6.5	7.1	8.0	9.8	10.6	11.9	10.6	11.0	9.5
±S.E.M.		0.1	0.1	0.3	0.3	0.5	0.6	0.9	0.6	1.2	0.5

a readily detectable amount in eight of the ten subjects on at least one of the three to four days of starvation. The mean glucagon level of the group was significantly ($p < 0.01$) above the prestarvation level on the second and third days of starvation. As previously reported,[4] insulin declined significantly during the fast.

The findings suggest that in healthy, young males a modest degree of hyperglucagonemia may be detected between the hours of 8:00 a.m. and 9:00 a.m. during the first four days of total starvation.* While such a change in concentration does not furnish absolute proof of an increase in the twenty-four-hour secretion of glucagon, a determination which is not, as yet, feasible, the frequency of hyperglucagonemia and its often progressively increasing pattern would tend to favor such a conclusion.

Even though in absolute terms the starvation-induced 50 per cent rise in glucagon is quantitatively unimpressive, the likelihood that pancreatic glucagon plays an important role in starvation is difficult to dispute on this ground. The opposing actions of glucagon and insulin on hepatic glucose production, emphasized by recent experimental data in humans[19] and in the perfused rat liver,[20-22] suggest that the level of glucagon's activity upon the liver will be a function of the glucagon and insulin concentrations, rather than of the glucagon concentration alone. If this is true, the decline in insulin concentration, which characterizes starvation, would create a relative hyperglucagonemia in terms of glucagon's biological activity on the liver, even if absolute hyperglucagonemia had been absent.

The exaggerated glucagon response to arginine after three days of fasting is perhaps the most dramatic finding of this study. Its promptness suggests that at the end of three days of fasting the alpha cells contain abundant quantities of glucagon available for rapid release. This could be a consequence either of diminished glucagon release during the fasting period, of an increase in glucagon biosynthesis during starvation, or of a combination of both. Since hyperglucagonemia was observed in most of these subjects during the starvation period, a reduction in glucagon release seems unlikely. Rather, it would seem more probable that in starvation glucagon biosynthesis increases but its release is carefully titrated in relation to need to avoid unnecessary gluconeogenesis and protein wastage, leaving an abundance of intracellular hormone.

The lack of any insulin rise in response to the arginine infusion is in sharp contrast to the delayed but exaggerated and prolonged insulin response to oral glucose loading reported previously in similar subjects after three days of starvation.[4] From the teleologic viewpoint it is highly appropriate that the beta cell, although able to release insulin in response to glucose, fails to respond during glucose need to nonglucose stimuli such as hyperaminoacidemia and hyperglucagonemia. Whatever the mechanism by which the beta cell becomes unresponsive to betacytropins other than glucose during acute cataglycemia[15] and chronic glucose need,[23] it is clear that it very effectively protects the organism from inappropriate insulin secretion during such circumstances.

*There is reason to suspect that subject #1160 did not observe a total fast. He lost only five pounds and had only moderate ketonuria at the end of the fast.

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Association of Coca Leaf Chewing with Malnutrition

It has been estimated that up to 45 per cent of the Quechua Indian population of Peru and, to a lesser extent, the mixed Indian-Spanish Mestizos, are chewers of coca leaf. The active ingredient of the leaf is cocaine, and this centuries-old habit fulfills various psychological and social functions. To what extent the deleterious effects on health of this pernicious habit counterbalance its social and emotional gratifications has been a matter of some speculation. It has even been suggested that the habit may increase work performance by reducing the sensations of fatigue.

A. A. Buck *et al.* (*Am. J. Epidemiol.* 88:159, 1968) have reported on a health survey of two carefully matched groups of coca chewers and nonchewers. Some comparisons were made of the health status of these two groups: fifty-one age, sex, and ethnically matched pairs of subjects drawn from the 492 residents of the community of Cachicoto, Peru. For most of the health parameters assessed, only the mean differences between the pairs were reported and these differences were the basis for the statistical testing. On the other hand, frequency distribution graphs of several of the characteristics are shown for each of the two groups of subjects, and these allow for some qualitative comparisons.

The simple ratio of weight to height pointed to a slight but not significant degree of relative thinness in the chewing group, a difference supported by a smaller triceps skinfold thickness (1.17 mm.) among chewers.

The mean level of serum total protein was 0.53 gm. per 100 ml. less in chewers and the serum albumin was 0.36 gm. per 100 ml. less; the latter difference was significant at the 5 per cent level of confidence. Serum total cholesterol was 20.3 mg. per 100 ml. less in the chewers ($p < 0.01$).

Hemoglobin and hematocrit values were low even among the nonchewers, but were still more depressed among users of coca leaf. Mean levels of hemoglobin were 10.4 and 12.2 gm. per 100 ml. in chewers and controls respectively ($p < 0.01$). For hematocrit, the difference between the two groups was 5.5 per cent packed cells ($p < 0.01$). On the basis of stool egg counts, estimates were made of hookworm burden. The two-way comparisons of egg counts and hemoglobin levels were especially interesting. Although coca chewers had slightly heavier infections than controls, at each level of hookworm load the mean hemoglobin was less in chewers, and this gap became progressively greater as the infection level increased.

For example, with stools negative for hookworm eggs, the hemoglobin levels were 10.6 in chewers and 12.4 in controls; in the groups with moderately infested stools, levels were 9.6 and 11.2 respectively; with heavy infestations, 7.7 and 10.8. The high prevalence of hookworm and other intestinal parasites doubtlessly contributed to the over-all iron deficiency in these subjects.

A morbidity survey was carried out and showed that the rate of illness was almost twice as great among chewers.

The studies support the three hypotheses tested, namely that (a) coca chewing is associated with an inferior nutritional state, (b) that chewers have a higher prevalence of conditions resulting from poor personal hygiene, and (c) that work performance of chewers is inferior. Coca chewing may blunt the sensations of hunger and cause more malnutrition. However, the authors note that alcoholism was more common among the chewers. The association seems clear in this group; whether there is a causal relationship remains to be proven.

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