any specificity for B_{12} is masked. This conjecture might explain why differing preference patterns for B_{12} in the presence of certain B_{12} analogues were expressed by gastric juice and IFC (Tables IV and V). Discrepancies between B_{12} binding and intrinsic factor activity may be due to varying amounts of B_{12} binding substances other than intrinsic factor.

Summary and conclusions. 1) This article reports some observations on the general nature of vit B_{12} binding by gastric juice and certain other biologic substances. The majority of vit B_{12} molecules bound by gastric juice did not dissociate in the presence of excess vitamin molecules added subsequently. 2) Our data show competition offered by certain vit B_{12} analogues for the cyanocobalamin binding sites of gastric juice, intrinsic factor concentrate, saliva, colostrum, and serum. Of these substances, gastric juice exhibited the greatest preference for cyanocobalamin in the presence of excess analogue. The intrinsic factor concentrate tested was much less fastidious than gastric juice. Saliva, colostrum, and serum exhibited little selectivity in their B_{12} binding mechanisms. 3) Occurrence of a sulfate, nitrate, or chloride ion in lieu of the cyanide ion in the cobalamin did not diminish competition for cyanocobalamin binding sites of gastric juice.

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Effects of Sodium and Potassium upon Cardiac Glycogen Fractions of the Rat Heart.*† (23549)

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A medium low in sodium and potassium stimulates glycogenesis in the rat heart(1) and similarly, a high potassium concentration initiates glycogenolysis(2). It has also been reported that a solution low in potassium will

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TABLE I. Effects of Sodium Ion upon Fractional Glycogen Synthesis in Rat Heart. Glucose osmolarity in solution = .055 except where indicated.*

Series	No. of determinations	Na (osmols)	Total osmolarity of solution	Glycogen as glucose (μM/g) Acid-insoluble	Acid-soluble	Total	O_2 consumption (μM/g wet wt/hr)
Controls	100			6.11 ± .155†	3.09 ± .137†	9.50 ± .257†	
Exp. 1-14	16	.00	.300*	3.95 ± .296	16.29 ± 1.177	20.30 ± 1.228	44.5
	19	.01	" *	4.38 ± .453	10.06 ± .589	14.75 ± .907	46.5
	16	.00	"	3.33 ± .289	15.05 ± .848	18.32 ± .999	30.9
	26	.02	"	3.15 ± .179	4.94 ± .210	8.02 ± .358	33.2
	31	.05	"	3.58 ± .310	8.39 ± .406	11.97 ± .605	38.0
	21	.06	"	2.90 ± .176	5.86 ± .460	8.70 ± .611	33.6
	22	.10	"	3.27 ± .236	6.11 ± .833	9.32 ± 1.012	37.0
	17	.13	"	2.65 ± .216	1.97 ± .211	4.63 ± .289	27.3
	8	.20	"	2.65 ± .275	2.10 ± .205	4.81 ± .477	21.9
	15	.29	.345	2.16 ± .335	2.34 ± .360	4.63 ± .722	19.7
	15	2.00	2.055	2.65 ± .165	2.28 ± .146	4.81 ± .231	0.0
	9	.02	.345	1.73 ± .193	2.65 ± .370	4.38 ± .463	25.3
	5	"	.400	2.47 ± .548	3.09 ± .436	5.55 ± .950	26.9
	6	"	.450	2.34 ± .565	3.33 ± .532	5.24 ± .949	23.1

* Glucose osmolarity = .287. † ± S.E. of mean.

produce glycogenolysis(3,4). Poppen, Green, and Wrenn(5) point out that if the glycogen content of the heart is high the potassium ion concentration will likewise be high. Experiments of a similar nature have been performed with other tissues in an effort to explain total glycogen changes. The results reported are variable presumably because tissues have permeability differences and respond differently in the same ionic environment. Marsh and Miller(6) demonstrated glycogen synthesis with rat kidney slices in a potassium medium. This is in general agreement with the results obtained by Hastings(7), Buchanan, Hastings, and Nesbett(8) and Hastings, Teng, Nesbett, and Sinex(9) who found that a potassium medium was best for glycogen synthesis from glucose by rat liver slices. Sodium had an inhibitory effect upon glycogen synthesis or stimulated the glycogenolytic processes. Stadie and Zapp(10) observed glycogenesis with rat diaphragm tissue in a sodium medium.

The preceding experiments involve total glycogen only and indicate it is influenced considerably by changes in the electrolytic pattern. It has been established that glycogen exists in more than one form and in this paper the effects of variable sodium and potassium concentrations upon these glycogen fractions of the rat heart were investigated.

Materials and methods. Male and female adult white rats, unfasted, were decapitated and the heart quickly excised. The great vessels and auricular tissue were discarded. The ventricular tissue was placed in a Stadie-Riggs tissue slicer and slices approximately 0.5 mm in thickness and weighing between 45 and 65 mg were prepared. Each slice was placed in a Warburg flask containing the appropriately gassed solution. Two or more slices were weighed quickly and then frozen between blocks of dry ice for later analysis of reference control levels of the myocardial glycogen fractions. Standard Warburg procedures were employed and the tissue in the flasks was shaken for 2 hours. Oxygen utilization was recorded every one-half hour. When the flasks were removed, tissue from 2 flasks was combined in order to insure adequate mass for glycogen analysis. The tissue was homogenized immediately in cold 10% trichloracetic acid and the acid-soluble glycogen determined by a procedure described by Bloom, Lewis, Schumpert, and Shen(11). The acid-insoluble glycogen fraction was determined by digesting the residue, obtained by centrifugation of the soluble moiety, in hot 30% KOH. Both glycogen fractions subsequently were hydrolyzed in 95% sulfuric acid and quantitatively determined by the anthrone method as reported by Seifter, Dayton, Novic, and Muntwyler(12). The *media* used may be noted in Tables I and II. In most experimental situations the osmolarity of the solution was maintained at 0.300. With the

TABLE II. Effects of Potassium Ion upon Fractional Glycogen Synthesis in Rat Heart. Glucose osmolarity in solution = .055 except where indicated.* Sodium osmolarity in solution = .020 except where indicated.†‡

Series	No. of determinations	K (osmols)	Total osmolarity of solution	Glycogen as glucose (μM/g) — Acid-insoluble	Acid-soluble	Total	O_2 consumption (μM/g wet wt/hr)
Controls	100			6.11 ± .155§	3.09 ± .137§	9.50 ± .257§	
Exp. 1-10	16	.000†	.300*	3.95 ± .296	16.29 ±1.177	20.30 ±1.228	44.5
	16	.000†	"	3.33 ± .289	15.05 ± .848	18.32 ± .999	30.9
	19	.000‡	" *	4.38 ± .453	10.06 ± .589	14.75 ± .907	46.5
	12	.012	"	3.15 ± .243	6.60 ± .352	9.69 ± .356	37.2
	7	.025†	"	2.41 ± .179	5.55 ± .286	7.96 ± .398	32.7
	12	.025	"	2.96 ± .215	4.81 ± .495	7.77 ± .666	39.2
	11	.050	"	2.22 ± .111	3.52 ± .178	5.61 ± .195	38.2
	8	.220	"	1.48 ± .182	2.10 ± .158	3.58 ± .306	25.5
	4	.400	.475	1.11 ± .154	1.85 ± .229	2.96 ± .358	.0
	2	2.460	2.535	1.05 ± .215	1.67 ± .062	2.71 ± .151	.0

* Glucose osmolarity = .287. † Sodium osmolarity = .000. ‡ Sodium osmolarity = .010.
§ ± S.E. of mean.

appropriate electrolyte and glucose osmolarities, the total osmolarity desired was obtained by the addition of sorbitol, a non-ionic, metabolically inert compound(1).

Results. Sodium. The information presented in Table I indicates absolute values and allows one to compare raw data. Statistical analysis of the data shows there is a decrease in the acid-insoluble glycogen fraction in all experimental conditions when compared to the control animals. The P value in all cases was $<.001$. The effects observed with the acid-soluble fraction are more diversified. There is a significant increase ($P = <.001$) in this glycogen fraction when the experimental medium contained no sodium or sodium in the amounts of 0.010 to 0.100 osmol (Table I, Exp. 1 through 7). A solution with a sodium concentration of 0.130 osmol or more (Exp. 8 through 11) or a condition in which the osmolarity of the solution is increased (Exp. 12 through 14) does not significantly alter the level of the acid-soluble fraction. Total glycogen, obtained by the algebraic sum of the 2 fractions, is significantly different ($P = <.001$) from the control levels in all experiments except 6 and 7.

In these particular experiments oxygen utilization was higher in solutions containing 0.287 osmol of glucose or containing 0.100 osmol or less of sodium than in a more concentrated sodium medium.

Potassium. When the acid-insoluble component of the experimental series was compared statistically to the reference control values there was a significant decrease in this glycogen fraction ($P = <.001$) in all cases (Table II). The changes observed in the acid-soluble fraction followed a rather precise pattern. Solutions containing from zero to 0.025 osmol of potassium (Exp. 1 through 6) produced a significant increase in this glycogen fraction when compared to the control value ($P = <.001$). There was no significant difference when the solution contained 0.050 osmol or more of potassium (Exp. 7 through 10). Total glycogen remained unchanged only when a solution contained 0.012 or 0.025 osmol of potassium (Exp. 4 through 6). In all other experiments it was significantly higher or lower than the reference control level.

Addition of phosphate (as sodium phosphate) in the amount of 0.020 osmol to a medium containing 0.055 osmol glucose and 0.025 osmol potassium had no significant effect upon either glycogen fraction (Table II, Exp. 5 and 6). In essence this corroborates results reported on diaphragm and liver(8,10, 13) to the effect that glycogen synthesis in a constant electrolytic environment is not influenced by the addition of phosphate. Consequently, a small amount of phosphate may serve as a buffer in the maintenance of pH of the medium without influencing the experiment.

Oxygen utilization by rat cardiac tissue slices in the presence of potassium is not in-

fluenced particularly until such time as the electrolyte concentration is greatly increased. Respiration does not occur at high osmolar concentrations.

Discussion. The acid-insoluble cardiac glycogen fraction, which is the more stable component, is readily lowered in these *in vitro* experiments regardless of the electrolytic concentration or the osmolarity of the medium. This effect was observed in all experimental series when the comparison was made to the reference control values. It is not improbable that enzymatic degradation may account for at least a portion of this decrease. The glycogenolytic effect becomes more pronounced as the concentration of sodium or potassium is increased or as the osmolarity of the solution is raised. The acid-soluble form of glycogen in rat cardiac tissue is the fraction principally affected. Glucose promotes synthesis of this component(14) which accounts for the increase in total glycogen. These findings were confirmed in this investigation. The optimal medium for synthesis of this glycogen fraction by rat heart tissue slices is one that has an osmolarity of 0.300 and contains either 0.287 or 0.055 osmol of glucose and no electrolytes. A statistical analysis revealed there was no significant difference between solutions containing 0.287 and 0.055 osmol of glucose (Table II, Exp. 1 and 2) and synthesis proceeded equally well in both. In a 0.300 osmolar solution containing 0.010 to 0.100 osmol of sodium and approximately 1% glucose, synthesis of the labile glycogen form also occurs (Table I, Exp. 2 and 4 through 7). However, as sodium concentration is increased to a level of 0.130 osmol or more, or osmolarity of the solution is elevated above that of the normal body fluid, glycogenolysis takes place. It has been shown(15) that this glycogenolysis, observed with tissue slices incubated in a relatively high sodium medium, is due to an increased phosphorylase activity.

Glycogenesis is observed, under the conditions of the potassium experiments, if the electrolyte in question is maintained at a concentration of 0.025 osmol or less. This synthesis is confined to the acid-soluble glycogen fraction. An increase above this level depresses glycogen synthesis or promotes glycogenolysis. Wajzer(16) inferred this when he stated that excess potassium accelerates the decomposition of the lyoglycogen (acid-soluble) in liver or muscle pulp and will involve the desmoglycogen (acid-insoluble) at the same time or shortly thereafter. Potassium also induces greater synthesis of the acid-soluble constituent than does sodium. This was confirmed by a comparison of experiments 4 in each table in which the P value was $<.001$. Cahill and co-workers(15) believe the low level of phosphorylase activity is a factor in the ability of tissue to synthesize glycogen in a potassium medium.

Oxygen consumption in a solution containing glucose and sorbitol is higher than when an electrolyte is added. The general trend of oxygen utilization is toward depression as the concentration of sodium or potassium is increased.

Summary. Rat cardiac tissue slices incubated in a 0.300 osmolar solution containing up to 0.100 osmol sodium or up to 0.025 osmol potassium and 1% glucose, synthesized acid-soluble glycogen. Greater synthesis of this glycogen fraction occurred in a glucose medium if the electrolytes were not present. A further increase of either ion induced glycogenolysis or depressed synthesis of this fraction. The acid-insoluble glycogen fraction was not especially sensitive to the electrolyte content of a solution but glycogenolysis was observed under all experimental conditions and increased as the electrolyte was increased. Oxygen consumption was highest in a 0.300 osmolar solution that contained approximately 1% glucose and 0.010 osmol of sodium and no potassium. As concentration of the electrolytes was increased oxygen utilization decreased. To extract the most information from glycogen synthesis studies the acid-soluble and acid-insoluble glycogen fractions should be investigated.

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Serum Properdin Levels and Cancer Cell Homografts in Man.* (23550)

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Homotransplantation studies with cultivated human cancer cells revealed differences in the ability of healthy persons and patients with advanced cancer to reject the implanted cells(1). The present paper reports parallel differences in serum properdin(2) levels in these same persons. The implantation of cancer cells into normal volunteers elicited a marked local inflammatory reaction and rapid rejection of the implant. In patients with advanced cancer the initial inflammatory reaction was minimal and growth of the implanted cells occurred at almost all sites. Spontaneous regression occurred in some cancer patients but in others growth of the implanted cells was progressive. Attempts have been made to determine the cause of the difference of response of healthy persons and cancer patients to cancer cell implants. None of these studies, except the present investigation, has thus far revealed any gross alterations in cellular or humoral factors that might explain the impairment of homotransplant rejection in the cancer patients (see Discussion). It has been reported elsewhere that treatment of mice and other experimental animals and man with large doses of zymosan or certain polysaccharides derived from bacteria or mammalian tissue cells caused temporary depression of properdin level, while small doses caused transient increases(6-10). It has also been observed that alterations in properdin levels and resistance to infections(11,12), to roentgen irradiation(13), and to hemorrhagic shock(14,15) can be induced by these high molecular weight polysaccharides. Studies in experimental animals also suggest a relationship between serum properdin levels and growth of transplantable tumors. Palm(16) found that rats treated intensively with zymosan, an agent known to influence properdin levels *in vivo*, became temporarily receptive to heterotransplants of the human epidermoid cancer cell line HEp #3. Herbut and Kraemer(17) reported similar results using their human colon adenocarcinoma HR 132 in rats, and postulated that the properdin system might be one of the natural defense factors against neoplasms. Bradner and co-workers (18) found that the transplantable mouse sarcoma S 180 grew faster in mice treated with large doses of zymosan which depressed the properdin levels of these mice, but less well in mice treated with a small dose of zymosan which elevated their properdin levels. Neither these animal studies nor the present clinical

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