built into the system. In addition, the electrophoresis process allows for the determination of the individual concentrations of all bases of the DNA molecule; data not available from the more sophisticated approaches. The limitation of *Tm* determinations to the range of 25–75 mol % G + C for which it has been established (3) is another advantage in favor of the electrophoretic method whose range is restricted only by the limits of determination of the individual bases by ultraviolet spectrophotometry.

A disadvantage of the described method is the requirement of a relatively large DNA sample in the analytical process. The mol % G + C values can be determined by thermal denaturation on 50 μg samples and by buoyant density on solutions containing 1–2 μg. The electrophoretic method requires the application of approximately 100 μg of hydrolyzed DNA in a volume of 25 μl. In these experiments, 1.0 mg of dry DNA was hydrolyzed and taken up in a total volume of 0.25 ml, thereby providing the means for replicate determinations. In the final analysis, the advantages of simplicity of instrumentation and technique must be weighed against the disadvantage of the increased DNA requirement.

Summary. The determination of DNA base pair ratios has been described using a rapid agar-gel electrophoresis method for the separation of DNA bases obtained from formic acid hydrolyzates. The actual mol % G + C values calculated from spectrophotometric data on the electrophoretically separated bases compared favorably with values obtained by thermal denaturation or buoyant density determinations on separate aliquots of the same DNA.

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Glucagon Clearance by the Isolated Perfused Rat Liver* (33081)

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The liver is often quoted as a major site for the degradation of glucagon (1,2). However, the evidence for this is only indirect, including destruction of glucagon-^{131}I by liver homogenates (3), loss of hyperglycemic properties of insulin after liver perfusion (4), and transhepatic gradients in glucagon levels in the intact animal (5). Since the introduction of precise and sensitive immunoassay methods for glucagon (6,7), the opportunity now exists to study the clearance of the hormone directly in a liver perfusion system. Immunoreactive glucagon (IRG) has been detected not only in the pancreas (PIRG) but also in the gut (GIRG) (8–11). It is not yet known if there are any similarities between PIRG and GIRG, but evidence is accumulating that they might not be identi-

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cal. There are immunological differences, although slight (8,9,11); they respond to different stimuli (9,11,12); they have dissimilar biological properties (12); and GIRG has a molecular weight twice that of PIRG based on Sephadex studies (12).

In view of the physiologic importance of these hormonal materials, the present experiments were designed to study the clearance in the liver perfusion of PIRG and GIRG. Because of the already well-established hepatic clearance of insulin (18), this hormone was also studied concomitantly to provide a comparison for the glucagon values obtained.

Methods. Intact livers weighing 14–15 gm from male Wistar rats (450 gm), fed *ad libitum,* were perfused cyclically according to the method of Miller *et al.* (13). Because hemolysis from red cells in the perfusion system was found to interfere with the immunoassay systems for insulin and glucagon, the medium used consisted of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 100 mg of glucose and 1 gm of albumin/100 ml. The total perfusate volume was 80 ml. Bood flow through the liver was maintained at 35–40 ml/min, and the perfusions were terminated after 1 hour. Sampling was done from the hepatic effluent and not more than 10 ml (12%) were removed during any single perfusion.

Acid–alcohol extracts of rat gut (jejunum and colon) and of rat pancreas were prepared according to the method of Kenny (14). The gut extracts contained 8 mμg of IRG equivalents and 20 μU of immunoreactive insulin (IRI)/mg of lyophilized weight; the pancreatic extracts had 20 mμg of IRG and 10,000 μU of IRI/mg.

After a 10-min control perfusion period, the material to be tested was added to the perfusate, and samples were taken over an hour period. PIRG was added in 2 forms: (i) crystalline beef–pork IRG (1.2 μg) (Eli Lilly), and (ii) rat PIRG (0.4 μg of IRG in 20 mg of extract). The GIRG was added as the rat gut extract (0.4 μg of IRG equivalents in 50 mg of extract). Measurements of IRG were made following the injection of rat pancreatic extract. Simultaneous with the rat GIRG clearances, pork insulin (Eli Lilly) (50,000 μunits added) clearances were measured in the same perfusions.

The IRI was assayed by the double antibody immunoassay technique of Morgan and Lazarow (15) and IRG by a similar technique (7) with Trasylol (Bayer Ltd.) added to the assay tubes to prevent proteolytic degradation of glucagon.

The results are plotted as percentage of maximal (initial) concentration determined 6 min after the insulin or glucagon had been added to the bath, when complete mixing had occurred (17,18). From semilogarithmic plots of this data, the half-lives ($t\frac{1}{2}$) of IRI and IRG in this system were estimated from the linear portion of the graph. No correction was made for the error introduced by the decreasing perfusate volume as this did not exceed 12%.

Results. The data for all hormone clearances are presented in Table I, and IRG clearances are also shown graphically in Fig. 1. The $t\frac{1}{2}$ values in Table I are expressed as a mean ± SD of the $t\frac{1}{2}$'s for each individual experiment performed, for that hormone. The $t\frac{1}{2}$ values in Fig. 1 are calculated from the single mean graph of all experiments for the hormone. The two methods give fairly similar results. The $t\frac{1}{2}$ values referred to below are those which appear in Table I. Clearance was mainly by first-order kinetics, but there was a tendency for a slower phase of clearance to appear later. For all clearances, the initial linear phase of the logarithmic plot was used to calculate the $t\frac{1}{2}$.

The $t\frac{1}{2}$ for crystalline beef–pork IRG was 19 ± 2.4 min (mean ± SD) (see Fig. 1). The clearances of rat PIRG and rat GIRG are shown in Fig. 1 and are similar, 23 ± 3.6 and 27 ± 9.3 min, respectively. Pork IRI had a $t\frac{1}{2}$ of 17 ± 4.2 min. There were no statistically significant differences between any of the clearances.

The hepatic clearances (k) of the hormones were also calculated from the formula (1) of Mortimore and colleagues (16) and of Burgi *et al.* (17)

$$k = 2.3 \times V\ \Delta t \qquad (1)$$

where $k \times$ hepatic clearance or reaction velocity constant; $V \times$ volume of perfusion

TABLE I. Hormone Clearances.[a]

Hormone	No. of expts.	Hormone conc/ml at 6 min (mean ± SEM)	Minutes after hormone injection									$t_{1/2}$ (min)
			6	9	12	15	20	25	30	40	50	60	
Beef-Pork IRG	3	7760 ±1350 μg	100	87 ± 3	81 ± 4	65 ± 2	56 ± 5	—	36 ± 9	28 ± 4	22 ± 5	15 ± 4	19 ± 2.4
Rat PIRG	6	1795 ± 102 μg	100	93 ± 2	73 ± 4	78 ± 3	68 ± 4	56 ± 7	47 ± 6	39 ± 5	28 ± 6	26 ± 5	23 ± 3.6
Rat GIRG	6	1240 ± 93 μg	100	94 ± 4	87 ± 5	84 ± 6	70 ± 7	60 ± 7	58 ± 7	45 ± 7	39 ± 8	32 ± 5	27 ± 9.3
Pork IRI	6	303 ± 57 μunits	100	93 ± 4	76 ± 2	66 ± 3	59 ± 6	46 ± 6	41 ± 5	33 ± 6	30 ± 6	26 ± 6	17 ± 4.2

[a] Results are expressed as the percentage (mean ± SD) of hormone concentration measured 6 min after injection.

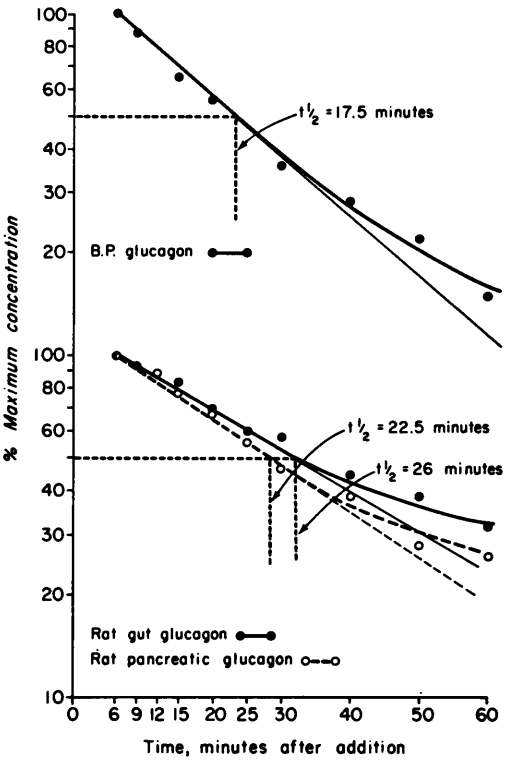

FIG. 1. Hepatic clearances of crystalline beef–pork glucagon (upper); and rat gut glucagon and rat pancreatic glucagon (lower) are plotted as the percentage of the T_6 maximum (initial) concentration of immunoreactive glucagon.

fluid during sampling time Δt; $Ca \times$ hormone concentration at beginning of time interval Δt; and Cf = hormone concentration at end of time interval Δt. The k values for pork IRI beef-pork IRG, rat PIRG, and rat GIRG are shown in Table II.

Discussion. The $t_{1/2}$ for pork IRI of 17 min agrees well with the value of 14 min obtained by Solomon *et al.* (18) in this laboratory, using the same perfusion technique but a red cell containing media as the perfusate. This would appear to indicate that our substitution of albumin buffer in the system still results in a liver perfusion system adequate for hormone clearance studies.

Our data for glucagon clearance confirm previous reports (3,4,19) suggesting that the liver is a major source of glucagon degradation. Several workers have, however, suggested that glucagon may be cleared more

TABLE II. Hepatic Clearances of Hormones.[a]

Hormone:	Beef–Pork IRG	Rat PIRG	Rat GIRG	Pork IRI
k (hepatic clearance) (ml/min)	2.91	2.40	2.04	3.24

[a] For definition of k see text.

rapidly by the liver than insulin (3,20), but there was no evidence from the results in the present report that this was the case.

Based on the assumption that hepatic inactivation is primarily responsible for clearance of these hormones in the intact rat, a theoretical $t\frac{1}{2}$ for glucagon can be calculated using the approximation of Farris and Griffith (21) and formula B of Burgi *et al.* (17). By calculation, this value is 5.1 min for beef-pork IRG, 6.2 min for rat PIRG, and 7.3 min for rat GIRG. These approximatic values are a little lower than the estimated $t\frac{1}{2}$ of glucagon in man of 10 min (22).

Unger *et al.* (12) postulated that IRG extractable from the gut differs from pancreatic IRG. The similar clearances of the two materials which we obtained in these studies, at least suggest identical handling by the liver. The rapid clearance by the liver is in keeping with the quick rise and fall in concentration noted in GIRG after oral glucose (23).

Summary. In the isolated perfused rat liver the biological half-life for pork immunoassayable insulin was determined to be 17 min. The corresponding figures for beef–pork, rat pancreatic, and rat gut, immunoassayable glucagon were 19, 23, and 27 min, respectively. The data are in keeping with the liver, which is a major site for degradation of insulin and glucagon.

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