The *in vitro* Synthesis of Glycogen in the Diaphragms of Normal and Alloxan-diabetic Rats

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Gemmill (1940, 1941) and Gemmill & Hamann (1941), confirmed by Hechter, Levine & Soskin (1941) and by Stadie (1944), showed that surviving rat diaphragm can synthesize glycogen from added glucose. The rate of this synthesis may be increased by addition of insulin *in vitro*. Following these findings the *in vitro* synthesis of glycogen from glucose as well as glycogenolysis by muscle were further investigated. It was proposed to examine (a) the influence of the composition of the medium, and especially the effect of potassium, on these reactions, (b) the influence of adrenaline, (c) the effect of insulin in normal animals, and (d) the synthesis of glycogen and glycogenolysis in diaphragms from alloxan-diabetic rats.

Cross & Holmes (1937) and Ostern, Herbert & Holmes (1939) found that liver slices can synthesize glycogen *in vitro* from added glucose. Hastings & Buchanan (1942) demonstrated that in rat-liver slices this synthesis takes place only in a potassium-rich, sodium-free medium, corresponding in ionic composition to intracellular fluid. In a medium resembling extracellular fluid no synthesis of glycogen takes place. Saxton & Miller (1944) showed that even under optimal conditions this synthesis of glycogen depends on the time interval which elapses between the death of the animal and the beginning of the experiment *in vitro*. On the basis of these experiments the synthesis of glycogen and glycogenolysis in rat-liver slices were studied *in vitro*, and were compared with the same processes in the diaphragm.

EXPERIMENTAL

Methods

Preparation of the diaphragm. Male albino rats of laboratory stock weighing 80–120 g. were used. The animals were anaesthetized with nembutal (pentobarbital) and bled. The diaphragm was quickly excised and two equal portions were weighed. In control experiments with both halves incubated under identical conditions, we observed the same deviations as were reported by Gemmill (1940). Dividing the diaphragm of older rats into three or more equal parts proved to be unreliable.

Media for incubation. (a) *Buffered salt solutions.* The diaphragm samples were incubated according to Gemmill (1940) at 38° in a medium described by Gey & Gey (1936)

and modified by Gemmill (1940). Its composition was: 0.7% NaCl, 0.037% KCl, 0.227% NaHCO₃, 0.017% CaCl₂, 0.015% Na₂HPO₄, and 0.003% KH₂PO₄ (all % w/v). This medium was similar to the solution described by Van Dyke & Hastings (1928), and resembled body fluid in its ionic composition. It is referred to here as 'extracellular solution'. In our experiments modifications of this solution by addition or removal of an ion were compensated for by corresponding changes in NaCl content. With a modification of this solution, containing only 0.74% NaCl, 0.015% Na₂HPO₄ and 0.003% NaH₂PO₄, we obtained optimal synthesis of glycogen by rat diaphragm *in vitro*. This solution is called 'phosphate buffered saline'. The glucose content of the media was usually 100 mg./100 ml., the concentration most favourable according to Hechter *et al.* (1941) for the demonstration of the insulin effect.

(b) *Serum.* For 'normal serum' pooled human serum was generally used, its glucose content being adjusted to 100 mg./100 ml. No differences were found with respect to synthesis of glycogen between sera of various animals, and fresh rabbit or rat sera were also used. When comparing synthesis in normal and diabetic serum, only freshly prepared rat serum was used, and the glucose content of the normal serum was adjusted to that of the diabetic serum. In another series of experiments the synthesis of glycogen in normal serum was investigated at a glucose concentration of 800 mg./100 ml., this being the mean serum glucose content in a series of alloxan-diabetic rats. The diaphragms of normal and alloxan-diabetic rats are termed 'normal diaphragm' and 'diabetic diaphragm', respectively, and the sera of these animals are similarly designated.

Bicarbonate-containing buffer media and sera were aerated with a gas mixture containing 95% O₂ and 5% CO₂, the bicarbonate-free phosphate-buffered saline with O₂. All solutions proved to be sufficiently buffered, and the experiments were carried out at a pH of approximately 7.4. No significant changes in pH were found after 3 hr. incubation with diaphragm even in unbuffered 0.9% NaCl. A concentrate of rat serum of approximately double strength was prepared according to Kanner (1946) by means of purified gelatin.

Measurement of glycogen synthesis. As measure of glycogen synthesis the difference in glycogen content between one test sample incubated for 6 min. and the other for 3 hr. is used. This value is termed 'glycogen synthesis value'. The influence of various factors, such as ionic composition and additions of drugs, was measured in most of our experiments by the difference in the glycogen content of two samples after 3 hr. incubation, one sample being incubated in the usual medium and the other in medium modified in the appropriate manner. This value is called 'glycogen

difference value'. The values are expressed in mg. increase/100 mg. diaphragm. The 'difference value' is also expressed as the percentage increase or decrease in glycogen.

The incubation of liver slices. Experiments on liver slices were carried out in the 'intracellular solution' described by Hastings & Buchanan (1942). The incubation time in these experiments was 4 hr., and glucose concentrations used were 0.25, 0.5 and 1%.

Experiments on glycogenolysis. These experiments were carried out in the extra- and intracellular solutions mentioned above without the addition of glucose. Serum was freed of sugar by incubation with yeast for 30 min. Glycogenolysis is expressed as the decrease in the glycogen content after 3 hr. incubation. Since the rate of glycogenolysis is dependent on the initial glycogen content, the values are given as the percentage decrease in glycogen as well as in mg./100 mg. tissue.

Respiration measurements. Determinations of oxygen uptake and respiratory quotient were carried out by Warburg's direct method (Dixon, 1943) in phosphate-Ringer. Measurements were made over periods varying from 2 to 3 hr. and the average value for 1 hr. was recorded.

Chemical procedures. Glycogen determinations in the diaphragm were carried out by a micromodification of Pflüger's method, employing liver slices according to Good, Kramer & Somogyi (1933). Glucose was estimated according to Shaffer, Hartmann & Somogyi (Somogyi, 1937).

Production of diabetes. Diabetes was induced by intravenous injection of 6-8 mg. of alloxan per 100 g. rat. Animals in various stages of diabetes were used, varying from those with severe coma to those with latent diabetes which exhibited glycosuria only on carbohydrate-rich food. Ordinary insulin (Squibb) was used, 2 units usually being added to 3 ml. of medium; this quantity corresponds approximately to the mean dose used by Gemmill. The ergotamine tartrate employed was Gynergen (Sandoz).

RESULTS

(1) Influence of the composition of the medium, and especially of the potassium content on glycogen synthesis and glycogenolysis

Removal of potassium from the 'extracellular solution' resulted in an increased synthesis of glycogen from glucose in rat diaphragm. Not only was the presence of calcium unnecessary for the synthesis of glycogen, but in a calcium-free medium the

synthesis was even slightly improved, the increase being $20\% \pm 8$ in 13 experiments. NH_4^+ had the same inhibiting effect as potassium. Optimal synthesis was observed in our experiments in phosphate-buffered saline. Two- or threefold increase in the potassium content of the 'extracellular solution' nearly always resulted in complete inhibition of glycogen synthesis (Table 1). In the 'intracellular' potassium-rich, sodium-free medium, described by Hastings & Buchanan (1942), intensive glycogenolysis occurred instead of synthesis, and was already manifest after 15 min. On the other hand, as demonstrated by these authors, glycogen synthesis in rat-liver slices takes place only in the intracellular medium. If in these experiments with liver slices part of the KCl is replaced by NaCl, glycogen synthesis is inhibited, but still demonstrable. With 'intracellular solution' glycogen can still be synthesized by liver *brei*, but only at the high glucose concentrations of 1.5-2%. In 13 experiments a mean increase of $0.63 \text{ mg.} \pm 0.19/\text{g. liver brei}$ was observed. Under no conditions could synthesis of glycogen be obtained in diaphragm *brei*. Contrary to our findings in the diaphragm, Ostern *et al.* (1939), working with liver slices, noted that the removal of calcium resulted in inhibition of glycogen synthesis.

Glycogen synthesis in the diaphragm was optimal at pH 7.4, and decreased by $13\% \pm 7$ at pH 6.8. If serum was used as medium the synthesis of glycogen in the diaphragm was of the same order of magnitude as in phosphate-buffered saline (Table 1). Although the potassium content of serum is the same as that of the 'extracellular solution', no inhibitory effect could be observed with serum. Two- or threefold increase of the serum potassium, however, did result in a decreased synthesis of glycogen. Replacing one third to one fifth of the 'extracellular solution' by serum improved the synthesis of glycogen. No increase in synthesis could be induced by addition of serum to phosphate-buffered saline.

Glycogenolysis was of similar magnitude in phosphate-buffered saline and in serum. Much more intense glycogenolysis was observed with the 'extracellular solution' (Table 2).

Table 1. *Synthesis of glycogen in normal rat diaphragm in various media compared with synthesis in the 'extracellular solution'*

No. of exp.	'Extracellular solution', compared with	Glycogen difference	
		(mg./100 mg. tissue)	Increase or decrease (%)
9	'Extracellular', potassium-free	$+0.065 \pm 0.013$	$+48 \pm 13$
6	'Extracellular' + 0.01-0.015 M-KCl	-0.046 ± 0.020	-15 ± 6
10	Serum	$+0.057 \pm 0.018$	$+56 \pm 25$
6	'Extracellular' + 0.2-0.33 vol. of serum	$+0.089 \pm 0.018$	$+28 \pm 6$
12	Serum + 0.01-0.015 M-KCl	-0.046 ± 0.018	-13 ± 6
13	Phosphate saline	$+0.120 \pm 0.027$	$+87 \pm 25$

Table 2. *The influence of different media on glycogenolysis in rat diaphragm*

Medium	No. of exp.	Decrease in glycogen	
		(mg./100 mg. tissue)	(% decrease)
'Extracellular'	10	-0.126±0.028	-48±2
Phosphate saline	7	-0.049±0.017	-17±4.5
Serum	11	-0.068±0.024	-25±4

(2) *The influence of adrenaline*

The addition of adrenaline hydrochloride at a concentration of 0.33 µg./ml. regularly decreased glycogen synthesis. At concentrations of 0.033 µg./ml. the effect was irregular and at 0.0033 µg./ml. it disappeared. Inhibition could already be observed after 1 hr. incubation. Ephedrine at a concentration of 3.3 µg./ml. had the same effect as adrenaline, while tyramine at the same concentration did not change the rate of glycogen synthesis. The effect of adrenaline was not dependent on the presence of potassium. Ergotamine tartrate (8.3–10 µg./ml.) abolished the inhibitory effect of adrenaline on glycogen synthesis. Since ergotamine alone inhibits the synthesis of glycogen it must be assumed that its action under the conditions of this experiment is due to its antagonizing effect on adrenaline. Insulin weakened the effect of adrenaline in 8 out of 13 experiments (Table 3).

Table 3. *The influence of adrenaline and the antagonizing effect of ergotamine tartrate on synthesis of glycogen in diaphragm*

No. of exp.	Samples incubated	Glycogen difference	
		(mg./100 mg. tissue)	Increase or decrease (%)
8	With and without adrenaline	-0.085±0.027	-44±7
8	With adrenaline, with and without ergotamine	+0.060±0.012	+31±5.5
3	With and without ergotamine	-0.060±0.014	+16±4

The effect of adrenaline on glycogenolysis is of the same order of magnitude as its effect on glycogen synthesis. Adrenaline at a concentration of 0.33 µg./ml. increased glycogenolysis by 47%±7 (6 experi-

ments). Cross & Holmes (1937), working with liver slices, first demonstrated the inhibition *in vitro* of the synthesis of glycogen by adrenaline, albeit only at a concentration of 50 µg./ml. Bendall & Lehmann (1941) produced a twofold effect of adrenaline at a concentration of 0.45 µg./ml. on the synthesis of glycogen in liver slices: an initial reduction and a subsequent increase. No clear-cut effect of insulin on glycogenolysis produced by adrenaline could be observed.

(3) *The influence of insulin on glycogen synthesis and glycogenolysis*

The experiments of Gemmill (1940) on the influence of insulin on the synthesis of glycogen, in the solution used by him, were confirmed, and the same effect was also demonstrated in phosphate-buffered saline. In serum, however, no additional increase of synthesis could be induced by insulin (Table 5). No influence of insulin on glycogenolysis could be observed, either in the 'extracellular solution' or in phosphate-buffered saline.

(4) *Glycogen synthesis and glycogenolysis in the diaphragm of alloxan-diabetic rats: the influence of insulin*

In the 'extracellular solution' the synthesis of glycogen by diabetic diaphragm was certainly not less than that of normal diaphragm and may have been somewhat higher. In phosphate-buffered saline no difference was found between glycogen synthesis by normal and diabetic diaphragm. In serum the synthesis by the diabetic diaphragm was somewhat lower but not to a significant degree (Table 4). Glycogenolysis in the 'extracellular solution' was of the same order of magnitude in diabetic and normal diaphragms.

The addition of insulin to the 'extracellular solution' resulted in synthesis values of 0.130 mg./100 mg. diaphragm for the diabetic and 0.150 for the normal rat, synthesis values without insulin being 0.079 and 0.055, respectively. In phosphate-buffered saline the effect of insulin was markedly lower on the diabetic than on the normal diaphragm (Table 5).

Table 4. *Synthesis of glycogen in diaphragm of alloxan-diabetic and normal rats in different media*

Medium	Alloxan-diabetic rats		Normal rats	
	No. of exp.	Synthesis (mg./100 mg. tissue)	No. of exp.	Synthesis (mg./100 mg. tissue)
'Extracellular'	23	0.079±0.015	18	0.055±0.013
Phosphate saline	13	0.080±0.017	14	0.109±0.020
Pooled normal serum	31	0.057±0.012	29	0.084±0.012

Table 5. *The influence of insulin on synthesis of glycogen in diaphragm of alloxan-diabetic and normal rats*

Medium	Alloxan-diabetic rats		Normal rats	
	No. of exp.	Glycogen difference (mg./100 mg. tissue)	No. of exp.	Glycogen difference (mg./100 mg. tissue)
Phosphate saline	18	+0.044±0.037	20	+0.144±0.030
Normal pooled serum	22	+0.059±0.015	20	-0.032±0.022

In a further series of experiments the synthesis values of normal and of diabetic diaphragms, incubated in diabetic and in normal serum (glucose concentration 0.8%), were determined. The synthesis of glycogen in diabetic diaphragm was significantly diminished in diabetic serum, whereas in normal serum normal synthesis values were obtained. The synthesis of glycogen in normal diaphragm was markedly depressed in diabetic serum.

(5) *Respiration of normal and diabetic diaphragms**

In phosphate-Ringer solution, with and without glucose, the respiration of diabetic diaphragm was markedly higher than that of normal diaphragm. This result requires an explanation. Since glycogen synthesis differed in serum from that in phosphate-buffered saline, respiration experiments were carried out in normal and diabetic serum. In normal serum

Table 6. *Synthesis of glycogen in alloxan-diabetic and normal diaphragm incubated with alloxan-diabetic and normal serum and the effect of insulin*

Diaphragm	Serum	No. of exp.	Insulin	Synthesis (mg./100 mg. tissue)	No. of exp.	Difference (mg./100 mg. tissue)
Diabetic	Diabetic	18	-	0.122±0.022	7	+0.171±0.040
Normal	Normal	20	+	0.215±0.020		
Diabetic	Normal	12	-	0.204±0.036		
Normal	Diabetic	15	-	0.151±0.021†		
Diabetic	Diabetic	9	+	0.410±0.052		
Diabetic acidotic	Diabetic	6	+	0.135±0.027		
Diabetic	Diabetic, insulin-treated <i>in vivo</i>	7	-	0.290±0.050		
Diabetic	Diabetic (1/2) + normal serum concentrate (1/2)	7	-	0.201±0.017	10	+0.076±0.046
Normal	Normal	10	+	0.373±0.056		

† One experiment exhibited a distinctly higher synthesis value of 0.616 and is not included in this table.

Addition of insulin to diabetic serum produced a marked elevation of the synthesis value in diabetic diaphragm. Since the usual dose of 2 units of insulin was rather high, some experiments were carried out with one-tenth of this dose, which was found to be still effective. Furthermore, insulin led to an increase in synthesis by normal diaphragm in normal serum at a glucose concentration of 0.8%. The effect of insulin under these conditions was, however, irregular; only in 5 out of 10 experiments could an increase in synthesis be induced. Incubation in the serum of alloxan-diabetic insulin-treated rats, or in a medium containing equal volumes of diabetic and of normal serum concentrate, resulted in an increase in synthesis in the diabetic diaphragm. Addition of insulin in the usual or even higher dose had no effect on the synthesis in the diaphragm of alloxan-diabetic acidotic rats (Table 6).

the respiration of normal diaphragm was higher than that of diabetic diaphragm, whereas in diabetic serum no difference could be observed. In experiments with serum only the oxygen uptake was measured, since the direct Warburg method proved unsuitable for the estimation of the CO₂ output (Table 7).

(6) *Glycogen synthesis in liver slices of alloxan-diabetic rats*

In this experiment we were handicapped by the fact that the livers of diabetic animals after fasting still contained appreciable quantities of glycogen (Tuerkischer & Wertheimer, 1946), and a glycogen content of 0.2-0.3% is considered to be the upper

* The respiration experiments were carried out by Mrs I. Salomonowicz-Parnes.

Table 7. *Respiration of diaphragm of alloxan-diabetic and normal rats*

Medium	Alloxan-diabetic rats				Normal rats			
	No. of exp.	O ₂	CO ₂	R.Q.	No. of exp.	O ₂	CO ₂	R.Q.
		(μ l./mg. dry wt./hr.)	(μ l./mg. dry wt./hr.)			(μ l./mg. dry wt./hr.)	(μ l./mg. dry wt./hr.)	
Phosphate-Ringer	10	-5.98 ± 0.29	4.83 ± 0.27	0.81 ± 0.010	17	-3.68 ± 0.80	2.68 ± 0.61	0.73 ± 0.016
Phosphate-Ringer + 0.1% glucose	16	-6.71 ± 0.33	5.90 ± 0.25	0.88 ± 0.013	16	-4.47 ± 0.67	3.90 ± 0.68	0.87 ± 0.024
Normal serum, glucose average 0.12%	9	-5.41 ± 0.22	—	—	9	-6.85 ± 0.29	—	—
Diabetic serum, glucose average 0.5%	5	-5.42 ± 0.32	—	—	9	-5.40 ± 0.22	—	—

limit for the demonstration of synthesis. In acidotic alloxan-diabetic rats, where no glycogen is to be found in the liver, synthesis of glycogen from added glucose was markedly diminished. In these animals the liver is already severely damaged, as shown by the high fat content. Rats suffering from a mild diabetes with normal fasting blood sugar, on the other hand, did not differ from normal rats in regard to the synthesis of glycogen.

DISCUSSION

The effect of potassium on the synthesis of glycogen and glycogenolysis

Potassium in concentrations which are present in extracellular fluid inhibits the synthesis of glycogen from added glucose in rat diaphragm, and complete removal of potassium produces optimal conditions for synthesis. In the liver, on the other hand, synthesis of glycogen will take place only in a medium containing appreciable amounts of potassium (Hastings & Buchanan, 1942), and with this organ the 'extracellular' medium favours glycogenolysis. The same medium thus has an opposite effect on identical processes in liver and muscle. Since even physiological quantities of potassium affect synthesis and favour glycogenolysis in the diaphragm, one must assume that potassium is of physiological importance in the regulation of synthesis and breakdown of glycogen. It is known that part of the body potassium occurs in a free condition, and is able to move about in response to changes in membrane equilibrium. For instance a decrease in the potassium content of working muscle has been adequately demonstrated by Fenn (1940) and Hahn & Hevesy (1941). Fenn assumes that, on its way from muscle to liver, potassium migrates together with lactic acid, and on its way from liver to muscle with glucose. Boyer, Lardy & Phillips (1942, 1943) explain the effect of potassium as being due to an acceleration of the transfer of phosphate from 2-phosphopyruvate to the adenylic acid system. Substitution of potassium by the ammonium ion in

equimolar concentration had the same effect. In the experiments of Boyer *et al.* calcium counteracted the effect of potassium. In our experiments, on the other hand, no antagonism between potassium and calcium was found. This might be because the above authors used minced or homogenized muscle, while in our experiments tissue was used which had suffered minimal damage and calcium might be unable to enter the intact cell. However, the role of potassium in the phosphorylating process of the adenylic acid system does not provide an explanation of the opposite effect of this ion on identical processes in liver and muscle tissue. Serum, on the other hand, contains a factor which counteracts the effect of potassium. Owing to the presence of this factor, serum is an optimal medium for glycogen synthesis in the diaphragm. The cause of this effect is still to be explained.

The effect of adrenaline on the synthesis and breakdown of glycogen

The complete inhibition of synthesis and the increase of glycogenolysis in rat diaphragm on addition of small doses of adrenaline is in accord with the findings of Hegnauer & Cori (1934), Cori & Cori (1936) with muscle and Nachmansohn (1937) with muscle *brei*. These authors found that adrenaline increased the formation of hexosemonophosphate and of lactic acid in frog muscle under aerobic as well as under anaerobic conditions. However, whereas in their experiments these metabolic changes were not inhibited by ergotamine, in our experiments the inhibition of the synthesis of glycogen by adrenaline was completely abolished by ergotamine; this may be considered as evidence that in this case adrenaline acts by way of the sympathetic nervous supply still present in the preparation.

Synthesis of glycogen in alloxan-diabetic rats

The *in vitro* synthesis of glycogen in diaphragm from alloxan-diabetic rats showed the following characteristics: the difference between normal and

diabetic diaphragm became apparent only when both serum and diaphragm from alloxan-diabetic rats were used. Under such conditions there was a marked decrease in synthesis with diabetic animals, whilst addition of insulin to diabetic serum greatly increased the synthesis. A similar increase in synthesis could be produced by incubation with serum of insulin-treated alloxan-diabetic rats, with serum of normal rats, or by addition of a normal serum concentrate to the diabetic serum. Incubation with diabetic serum, on the other hand, diminished synthesis of glycogen in the normal diaphragm.

This effect of insulin on diabetic diaphragm incubated with diabetic serum failed to appear when the diaphragms of severely diabetic rats with acidosis were used. In these experiments the known resistance of comatose alloxan-diabetic rats to insulin (Kaplan, Franks & Friedgood, 1945) was corroborated *in vitro*. In buffered salt solutions, however, no significant difference existed between the synthesis of glycogen in normal and diabetic diaphragms. The effect of insulin on the synthesis in diabetic diaphragm with these media was even smaller than that with normal diaphragm. The insulin effect, always present in buffered salt solution, could not be produced in the normal diaphragm incubated with normal serum. In the diabetic diaphragm this effect was definitely present.

An explanation of most of these findings can be given as follows: in normal serum a factor favouring synthesis of muscle glycogen, possibly insulin, is present, and synthesis cannot be improved by addition of insulin. At high glucose concentrations the amounts of this factor present may not be sufficient, and under these conditions addition of insulin may produce an increase in the synthesis of glycogen. In buffered salt media, therefore, an insulin effect could always be produced. In diabetic serum the concentration of this factor is either diminished, or the factor is absent and synthesis of glycogen is therefore decreased. However, this synthesis can be increased by addition of insulin, or by incubation with serum of insulin-treated alloxan-diabetic rats, or by addition of a normal serum concentrate. We can offer no explanation for the diminished effect of insulin in the diabetic diaphragm in buffered salt media.

So far mention has been made only of a factor present in normal and absent from diabetic serum which favours the synthesis of glycogen. But there still exists the possibility that in normal serum two substances may be present, one favouring and the other inhibiting synthesis. Such an assumption would explain the fact that syntheses by the normal diaphragm in the optimal buffered saline and in serum are of the same order of magnitude.

Further experiments are required to investigate the validity of this assumption. On the other hand, it is probable that alloxan diabetes may also produce changes in muscle, even if such changes are not apparent in buffered salt media. That a disturbance must exist in the diabetic diaphragm itself is proved by the absence of the insulin effect in the diaphragm of alloxan-diabetic comatose rats incubated with serum from alloxan-diabetic non-comatose rats, and by the effectiveness of insulin on the diabetic diaphragm incubated with normal pooled serum.

In any case these results support the view that important factors influencing the normal carbohydrate metabolism of tissue slices are present in serum. Experiments on synthesis of glycogen as well as on respiration demonstrate that the use of serum in place of buffered salt media may lead to entirely different results (Shipley, 1944). Disturbances in metabolism may become detectable *in vitro* only when the experiments are carried out in the abnormal pathological environment and not in indifferent salt solutions.

SUMMARY

1. Glycogen synthesis and glycogenolysis by rat diaphragm and liver have been studied *in vitro*.

2. Potassium, even in physiological concentrations, inhibited synthesis of glycogen from added glucose by rat diaphragm. At higher concentrations of potassium glycogenolysis took place instead of synthesis. For glycogen synthesis in rat-liver slices, however, it is essential to have potassium present in the medium.

3. Adrenaline completely inhibited synthesis of glycogen in diaphragm and favoured glycogenolysis, whilst ergotamine abolished the effect of adrenaline.

4. Insulin favoured synthesis of glycogen from added glucose in all buffered salt media. With serum, however, no additional synthesis of glycogen could be induced by addition of insulin.

5. Incubation with 'diabetic' serum decreased the synthesis of glycogen in the 'diabetic' as well as in the 'normal' diaphragm. The synthesis in the 'diabetic' diaphragm could be increased by addition of insulin or of a normal serum concentrate to 'diabetic' serum, or by incubation with serum of insulin-treated alloxan-diabetic rats. In normal pooled serum a distinct increase in synthesis was obtained in 'diabetic' but not in 'normal' diaphragm by addition of insulin.

6. No differences, however, between the synthesis of glycogen in diaphragms of alloxan-diabetic and normal rats were observed in the different salt media. The effect of insulin in the 'diabetic' diaphragm in buffered salt media was even smaller than that observed in 'normal' diaphragm.

7. Glycogenolysis in diaphragm was favoured by potassium but unaffected by insulin. The order of magnitude of glycogenolysis was the same in 'normal' and 'diabetic' diaphragm.

8. Metabolic changes of tissues *in vitro* may become manifest only in a correspondingly changed

medium and may be undetectable in an indifferent salt medium.

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Hydrochloric Acid Production by Isolated Gastric Mucosa*

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The experiments described in this paper were undertaken to throw light on the mechanism of hydrochloric acid production in the stomach. Isolated mucosa was used because it is easier to control the experimental conditions in excised tissue than in the intact organism.

The oxyntic cells of rats secrete continuously (Friedman, 1943), hence the starting materials for the production of HCl must come eventually from the blood and must be neutral. Several mechanisms which produce hydrogen ions have been envisaged and can be divided roughly into two types. (1) Those

* A part of the present work was communicated to the Biochemical Society (Davies, 1946).

producing the acid together with approximately neutral compounds. (2) Those producing the acid together with basic compounds (Fig. 1). Many manometric experiments have been carried out in Warburg cups with sheets of sheep, toad and frog gastric mucosa to test the possibility that mechanisms of type 1 are involved. In no case was evidence obtained that HCl was being formed, and liberating CO₂ from the HCO₃⁻ of the medium, although the rate of respiration of the tissues was normal and was increased by histamine. Since several glands can function *in vitro* (Delrue, 1930; Deutsch & Raper, 1936) it seemed unlikely that the failure to detect acid was due to the inability of the