REFERENCES

- Agrell, I. (1953). *Acta physiol. scand.* **28**, 306.
- Bray, H. G. & White, K. (1957). *Kinetics and Thermodynamics in Biochemistry*, p. 222. London: J. and A. Churchill Ltd.
- Dawson, C. R. & Tarpley, W. B. (1951). In *The Enzymes, Chemistry and Mechanism of Action*, vol. 2, part 1, p. 454. Ed. by Sumner, J. B. & Myrback, K. New York: Academic Press Inc.
- Dennell, R. (1944). *Nature, Lond.*, **154**, 57.
- DuBois, K. P. & Erway, W. F. (1946). *J. biol. Chem.* **165**, 711.
- Heller, J. (1947). *Acta Biol. exp., Varsovie*, **14**, 229.
- Karlson, P. & Wecker, E. (1955). *Hoppe-Seyl. Z.* **300**, 42.
- Levenbook, L. (1950). *Biochem. J.* **47**, 336.
- Ludwig, D. & Barsa, M. C. (1956). *Ann. ent. Soc. Amer.* **49**, 103.
- Michaelis, L. & Menten, M. L. (1913). *Biochem. Z.* **49**, 333.
- Needham, D. M. (1929). *Biol. Rev.* **4**, 307.
- Patterson, D. S. P. (1956a). Ph.D. Thesis: University of London.
- Patterson, D. S. P. (1956b). *Arch. int. Physiol. Biochem.* **64**, 681.
- Patterson, D. S. P. (1957). *Biochem. J.* **65**, 729.
- Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
- Rees, K. R. (1954). *Biochem. J.* **58**, 196.
- Schwarzenbach, G., Gut, R. & Andregg, G. (1954). *Helv. chim. acta*, **37**, 937.
- Wigglesworth, V. B. (1949). *Annu. Rev. Biochem.* **18**, 585.
- Wojtczak, L. (1955). *Proc. 3rd Int. Congr. Biochem.* 12-31.
- Wojtczak, L. (1956). *Acta biochim. polon.* **3**, 197.

Studies on Human Synovial Membrane *in vitro*THE METABOLISM OF NORMAL AND RHEUMATOID SYNOVIA
AND THE EFFECT OF HYDROCORTISONE

By D. P. PAGE THOMAS AND J. T. DINGLE

Rheumatism Research Unit, Bath

(Received 23 May 1957)

Previous metabolic studies *in vitro* have shown that normal synovial membrane is a tissue with an extremely low oxidative metabolism, though its aerobic and anaerobic glycolysis are readily measurable. The presence of rheumatoid disease in the synovial membrane is accompanied by a marked increase in metabolism. The highest rate of carbohydrate metabolism is found in tissues where the villous proliferation which occurs in this disease is most intense. As the proliferative process wanes and the general reaction becomes more fibrotic during remission, the metabolic activity of the synovium diminishes (Dingle & Thomas, 1956). To investigate this increase in oxidation and glycolysis further, the metabolic effects of various intermediates of the glycolytic pathway and the citric acid cycle have been studied.

Articular biopsies of rheumatoid synovia have been performed before and during the administration of cortisone (Hench, Kendall, Slocumb & Polley, 1950). These authors reported that in each case synovial inflammation appeared to be reduced by this hormone, as indicated by a reduction in the cellular reaction, with a decreased number of lymphocytes and plasma cells, and a reduction in papillary villous tufting. Since the metabolic effects of cortisone and hydrocortisone in man are qualitatively similar (Laidlaw, Dingman, Arons,

Finkenstaedt & Thorn, 1955) and hydrocortisone is the more potent when administered locally (Hollander, 1951; Boland, 1955), the effect of the latter corticosteroid on synovial metabolism has been investigated. Preliminary observations have shown that hydrocortisone inhibits oxidation and glycolysis of rheumatoid synovial villi *in vitro* (Thomas & Dingle, 1955). This work has now been extended to include the effect of this hormone on synovial tissues of varying metabolic activity, and in the presence of various substrates.

METHODS

Synovial tissue. 'Normal' synovial tissue was obtained at the time of operation from patients undergoing meniscectomy. These cases had a traumatic aetiology and the effusion had subsided before operation. There was usually some residual subsynovial engorgement of the capillaries on histological examination.

Rheumatoid synovial tissue was similarly obtained from patients undergoing synovectomy or arthrodesis of the knee. All rheumatoid synovia used in this study were shown to have the histological changes characteristic of the disease.

Immediately after excision the synovial tissue was placed in ice-cold Krebs-Ringer-phosphate (Umbreit, Burris & Stauffer, 1951). The chilled tissue was dissected to remove the underlying fat and fibrous tissue from the membrane, and to separate the villi if present. Free villous processes of

0.25–0.5 mm. diam., or membrane strips 5 mm. $\times$ 2 mm. $\times$ 0.2 mm., after washing for 2–3 min. in ice-cold Krebs–Ringer-phosphate, were placed in Warburg flasks. This washing removed the majority of soluble nitrogenous material. Incubation for 1 hr. at 37° resulted in a very small increase of nitrogen in the suspending fluid, e.g. the average loss of nitrogen to the filtrate from samples containing approx. 3 mg. of nitrogen was less than 0.01 mg.

In some experiments synovial tissues were depleted of endogenous substrates by pre-incubation in an oxygenated Krebs–Ringer-phosphate medium for 1 hr. at 37° after the method of Turner (1952). After the addition of the tissue and immediately before incubation, 0.5 ml. of the suspending fluid was removed for zero-time estimations on the various substrates, or metabolites, in each flask.

Splenic tissue. This was obtained from young male rats immediately after killing. It was kept ice-cold until use.

Tissue suspensions. Villous synovial tissue was cut into small fragments and homogenized in 0.145M-KCl and 0.024M-KHCO₃ with a motor-driven all-glass homogenizer. The suspension was filtered through glass wool to remove fibrous-tissue fragments. No intact cells were seen on microscopical examination. This preparation was diluted to give a final tissue concentration of about 10% (w/v). Splenic suspensions were similarly made and all preparations were used immediately.

Incubation media. In all aerobic and most anaerobic experiments on whole tissue, Krebs–Ringer-phosphate was used as the basal medium. In experiments where glycolysis was followed manometrically Krebs–Ringer-bicarbonate (Umbreit *et al.* 1951) was employed. Unless otherwise stated, substrates were used at a final concentration of 10 mm. Details of media used for tissue suspensions are given in the text.

Substrates. Commercial preparations of glucose, glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, fructose 1:6-diphosphate (Sigma Chemical Co., St Louis, Mo., U.S.A.), pyruvic acid, α -oxoglutaric acid, oxaloacetic acid, fumaric acid, succinic acid, malic acid, acetic acid and *cis*-aconitic acid (L. Light and Co.) were used as neutral solutions. Diphosphopyridine nucleotide and hexokinase were supplied by Sigma Chemical Co.

Incubation technique. For aerobic experiments oxygen was used as the gas phase, and for anaerobic experiments either nitrogen or nitrogen + carbon dioxide (95:5). The gas was introduced either by a rapid evacuation procedure, or by gassing the chilled flasks for 10 min. All flasks had a 10 min. equilibration period at 37° before manometric measurements were begun. The incubation period was 1 hr. unless otherwise specified. Conventional Warburg flasks were used, with a final fluid volume of 3 ml. For aerobic experiments 10% KOH was included in the central well. The agitation rate was 120 strokes/min. Approx. 30 mg. dry wt. of tissue, which corresponded to 3 mg. of tissue nitrogen, was used per flask.

Chemical analyses. After incubation the tissue was separated from the suspending fluid by filtration through glass wool. The tissue was washed and dissolved in 30% KOH. Samples were taken for estimation of total tissue nitrogen by the micro-Kjeldahl method (Hawk, Oser & Summerson, 1952) and glycogen by the method of Walaas & Walaas (1950). Little glycogen was leached into the suspending medium during incubation, e.g. approx. 0.2–0.4 μ g. of glucose equivalent/mg. of tissue nitrogen was

found as precipitable material in the filtrate after incubation. The filtrate volume was made up to 9 ml. and protein precipitated by the addition of 1.5 ml. of 5% (w/v) ZnSO₄·6H₂O and 1.5 ml. of approx. 0.3N-Ba(OH)₂. The latter was standardized so that 4.7–4.8 ml., when added to 5 ml. of the ZnSO₄ solution, was just pink to phenolphthalein. Glucose was estimated by the method of Nelson (1944), lactic acid by the method of Barker & Summerson (1941), glucose 1-phosphate and glucose 6-phosphate as reducing sugar by the method of Umbreit *et al.* (1951), fructose 6-phosphate and fructose 1:6-diphosphate by a modification of the method of Roe (1934). The results of all estimations were expressed on a tissue-nitrogen basis, the rate of oxygen uptake ($q_{O_2}^t$) being μ l. of O₂ uptake/hr./mg. of tissue nitrogen.

Hormone addition. Hydrocortisone (17-hydroxycorticosterone) was used. The preparation was a suspension in a saline medium (Merck). The suspending medium at the same dilution was present in the control flasks. Recent experience with various methods of rendering corticosteroids soluble (e.g. dissolving in ethanol, propane-1:2-diol or autoclaving or by using the hemisuccinate or dimethyl glutarate esters) has shown that such preparations may give rise to widely different results. In this work we have used one method only, by autoclaving at 20 lb./sq. in. for 10 min. (Miller, 1954).

RESULTS

These results were obtained from the synovia of ten normal subjects and 50 patients with rheumatoid arthritis.

Glycogen. We have been unable to measure accurately the glycogen content of normal synovial tissue owing to its low concentration and the small amount of tissue available at one time. In rheumatoid villous synovia, however, the change in endogenous tissue glycogen during incubation depended upon its initial glycogen content. These results are given in Table 1. When the initial glycogen level was high, and in the absence of substrate, considerable glycogenolysis occurred. When the initial glycogen content was low, however, and in the presence of 10 mm-glucose, a certain amount of glycogen synthesis took place. With a high initial glycogen, under similar conditions, no change in glycogen content was found. One rheumatoid villous tissue, when incubated alone or in the presence of 10 mm-glucose, gave markedly dissimilar rates of glycogenolysis. The effect of hydrocortisone on the changes in tissue glycogen is also given in Table 1. The presence of the hormone resulted in a marked increase in glycogenolysis, or a diminution of glycogen synthesis where this occurred.

Glucose. Under aerobic conditions, hydrocortisone was found to inhibit markedly glucose utilization (Thomas & Dingle, 1955). This inhibition has also been found to occur under anaerobic conditions. For example, in a tissue whose aerobic glucose utilization was 280 μ g./mg. of tissue

nitrogen/hr., the addition of 250 $\mu\text{g.}$ of hydrocortisone/ml. resulted in a utilization of 227 $\mu\text{g.}$ /mg. of tissue nitrogen/hr. Under anaerobic conditions the same tissue utilized 530 $\mu\text{g.}$ /mg. of tissue nitrogen/hr., and in the presence of hormone this was lowered to 346 $\mu\text{g.}$ /mg. of tissue nitrogen/hr.

Hexose phosphates. Villous rheumatoid synovia readily metabolized glucose 1-phosphate, glucose 6-phosphate and fructose 1:6-diphosphate, under aerobic conditions. In a typical experiment 800 $\mu\text{g.}$ of glucose 1-phosphate, 300 $\mu\text{g.}$ of glucose 6-phosphate and 450 $\mu\text{g.}$ of fructose 1:6-diphosphate, were utilized/mg. of tissue nitrogen/hr. The endogenous $q_{O_2}^0$ did not appear to be affected by the addition of glucose 6-phosphate, fructose 6-phosphate or fructose 1:6-diphosphate, and the inhibition of $q_{O_2}^0$ by hydrocortisone in the presence of these substrates was of a similar degree to that found with glucose as substrate. In one experiment glucose 1-phosphate appeared to increase the rate of oxygen uptake, but the $q_{O_2}^0$ was again inhibited in the presence of hydrocortisone. The effect of hydrocortisone on the catabolism of the hexose phosphates, under our experimental conditions,

has so far yielded erratic results. It seemed possible that the action of hydrocortisone on the metabolism of endogenous substrates might mask the effect of the hormone on the substrates added experimentally. In an attempt to investigate this possibility further, we have examined the effect of hydrocortisone on a rheumatoid synovium, part of which was depleted of endogenous substrates by the method of Turner (1952). These results are given in Table 2. Depleted tissues utilized more glucose but produced less lactate than did non-depleted tissues. Hydrocortisone inhibited glucose utilization and lactate production in both the depleted and non-depleted tissue. With pyruvate as substrate, more lactate was produced by the non-depleted tissue, and under these conditions there was again inhibition by hydrocortisone. In the depleted tissue, however, hydrocortisone showed no inhibitory effect on lactate production. The $q_{O_2}^0$ in the presence of pyruvate was not appreciably affected by tissue depletion; with glucose as substrate, however, it was lowered. The inhibition of $q_{O_2}^0$ by hydrocortisone was little affected by these experimental conditions.

Table 1. *Glycogen changes of rheumatoid synovia during incubation and the effect of hydrocortisone*

Hydrocortisone was used at a final concentration of 250 $\mu\text{g.}$ /ml. +, Indicates synthesis of glycogen by the tissue; -, indicates glycogenolysis. The number of determinations made on each tissue is given in brackets. Gas phase, O_2 . Medium, Krebs-Ringer-phosphate. Temperature, 37°.

Initial tissue glycogen ($\mu\text{g.}$ of glucose equiv./mg. of tissue N)	Substrate (glucose) concn. (mM)	Change in tissue-glycogen content ($\mu\text{g.}$ /mg. of tissue N/hr.)	Change in tissue-glycogen content in the presence of hydrocortisone ($\mu\text{g.}$ /mg. of tissue N/hr.)
67	0	-27 (2)	-43 (2)
49	0	-22 (8)	-25 (4)
49	5	-11 (3)	-26 (4)
27	5	-13 (6)	—
67	10	-7 (2)	-29 (2)
60	10	0 (3)	—
23	10	0 (3)	—
16	10	+9 (3)	-6 (3)
3	10	+13 (3)	+6 (3)

Table 2. *Effect of depletion upon rheumatoid synovial metabolism*

Figures in brackets are the numbers of determinations. Gas phase, O_2 . Medium, Krebs-Ringer-phosphate. Temperature, 37°. Depleted tissue was incubated for 1 hr. at 37°; stored tissue was kept for 1 hr. at 0°.

Experimental conditions				Increase in lactic acid ($\mu\text{g.}$ /mg. of tissue N/hr.)	Utilization of glucose ($\mu\text{g.}$ /mg. of tissue N/hr.)
Treatment	Substrate (10 mM)	Concn. of hydrocortisone ($\mu\text{g.}$ /ml.)	$q_{O_2}^0$		
Depleted	Sodium pyruvate	0	23 (4)	8.0 (2)	—
Depleted	Sodium pyruvate	250	14 (2)	9.0 (2)	—
Stored	Sodium pyruvate	0	24 (4)	25 (5)	—
Stored	Sodium pyruvate	250	15 (2)	18 (5)	—
Depleted	Glucose	0	15 (3)	135 (3)	560 (3)
Depleted	Glucose	250	12 (3)	100 (3)	310 (3)
Stored	Glucose	0	21 (3)	150 (3)	330 (3)
Stored	Glucose	250	16 (3)	100 (3)	240 (3)

It has been shown in Table 1 that the initial glycogen content of rheumatoid synovium varies widely. This would appear to limit the usefulness of the depletion technique, as pre-incubation for a standard period would not necessarily cause the final carbohydrate levels of different tissues to be similar.

Production of lactate

The aerobic production of lactate by rheumatoid synovia (chemical estimation), with glucose as substrate, has been shown previously to be inhibited by hydrocortisone. The aerobic lactate formed in the presence of the various hexose phosphates is apparently similarly inhibited by hydrocortisone. In view of evidence from the depletion experiments, with pyruvate as substrate, these findings are of dubious value, since it is not known how much lactate is formed from endogenous sources, the metabolism of which could show inhibition by hydrocortisone.

Under anaerobic conditions the increased lactic acid production of rheumatoid synovia is again inhibited by hydrocortisone; for example, the production of 112 $\mu\text{g.}$ of lactate/hr./mg. of tissue nitrogen in the control flasks and 95 $\mu\text{g.}$ of lactate/hr./mg. of tissue nitrogen in the flasks containing 250 $\mu\text{g.}$ of hydrocortisone.

These findings have been confirmed by measuring the rate of acid production in Krebs-Ringer-bicarbonate solution. The rates of anaerobic acid production and the effect of the addition of hydrocortisone are shown in Fig. 1. The rate of acid production by the control flasks was linear for at least 100 min. This finding was similar to that for the rate of oxygen uptake of the tissue, which was also linear for at least 2 hr., provided that glucose was present as a substrate.

In view of the hormonal inhibition of anaerobic lactate production by whole-tissue preparations it was felt that further information as to the nature

of this inhibition might best be obtained by the use of broken-cell preparations. Initial experiments were made with synovial-tissue suspensions in bicarbonate-saline. Under these conditions the rate of anaerobic acid production fell off very rapidly, and it was impossible to decide whether hydrocortisone exerted an inhibitory effect. In an endeavour to produce better experimental conditions the addition of various substrates and co-factors were made to the medium. It is evident from Table 3 that the presence of diphosphopyridine nucleotide (DPN) was essential for a rapid rate of glycolysis. Though nicotinamide had only a slight effect on glycolysis by itself, its presence

Fig. 1. Effect of hydrocortisone on the anaerobic acid production of synovial tissue. Hydrocortisone (250 $\mu\text{g./ml.}$) was added from the side arm after incubation for 45 min. Medium, Krebs-Ringer-bicarbonate; glucose (10 mM). Temperature, 37°. Gas phase, $\text{N}_2 + \text{CO}_2$ (95:5). ■, Control; ●, hydrocortisone added after 45 min.

Table 3. *Anaerobic glycolysis of rheumatoid synovial homogenates*

The basic medium contained KCl (0.154M), KHCO_3 (0.024M) and MgCl_2 (0.01M). Additions: glucose (0.01M), nicotinamide (0.05M unless otherwise specified), DPN ($2 \times 10^{-4}\text{M}$), pyruvate (0.01M), hexokinase (20 units). 1 ml. of 10% homogenate in bicarbonate-saline. Temperature, 37°. Gas phase, $\text{N}_2 + \text{CO}_2$ (95:5).

Flask no.	Additions	Time (min.)	qCO_2 ($\mu\text{l./mg.}$ of tissue N/hr.)			
			0-15	15-30	30-45	45-60
1	Glucose		0	0	0	0
2	Glucose, nicotinamide (0.01M)		0.9	0.9	0.9	0.9
3	Glucose, nicotinamide		4.1	2.0	1.6	1.2
4	Glucose, DPN, nicotinamide		20.5	15.6	5.9	2.9
5	Pyruvate		8.9	6.7	3.3	2.2
6	Pyruvate, nicotinamide		9.2	3.1	2.6	2.0
7	Pyruvate, DPN, nicotinamide		7.6	4.7	3.8	3.8
8	Pyruvate, glucose, DPN, nicotinamide		13.0	5.2	3.5	3.5
9	Glucose, hexokinase, nicotinamide		11.2	2.8	1.9	1.4
10	Glucose, pyruvate, hexokinase, DPN, nicotinamide		15.8	6.3	3.2	3.2
11	Glucose, DPN		17.9	5.8	2.1	0.5

with DPN tended to stabilize the rate of glycolysis. In the presence of glucose, neither pyruvate nor hexokinase appeared to be essential. The presence of DPN and nicotinamide had no influence on the rate of acid production with pyruvate as substrate. Further results are presented in Table 4. A bicarbonate-saline containing glucose and DPN was used as the basic medium for these experiments. No stabilization of glycolysis occurred when the concentration of nicotinamide was increased. Fructose 1:6-diphosphate alone appeared to inhibit the rate of glycolysis, but in the presence of phosphate and nicotinamide this compound gave a high rate of glycolysis with somewhat improved stabilization. Further experiments with this medium (Table 5, medium no. 4) have in several cases given linear rates up to 45 min. Although this medium has given the best results to date it probably does not represent the optimum conditions for the glycolysis of synovial homogenates. We have tested the effect of hydrocortisone on glycolysis in the presence of this improved medium.

The results are given in Table 5. Under these conditions no inhibitory action of hydrocortisone could be demonstrated. The action of hydrocortisone on other tissue homogenates is also given in Table 5. It is evident that the acid production by a rat-spleen suspension in bicarbonate-saline (medium no. 1) is markedly inhibited by hydrocortisone. In the presence of nicotinamide no significant depression of acid production occurred in the first 15 min. With a fortified medium (no. 3) the rate of acid production was inhibited to a lesser degree. Significantly different values were obtained only after incubation for 30 min. Suspensions of rat kidney in the same medium gave similar results.

Oxidative metabolism

The effect on the qO_2 of normal and rheumatoid synovia of malate, fumarate, succinate, acetate, pyruvate and glucose is given in Table 6. Although the addition of acetate, pyruvate or glucose had no effect on the qO_2 of normal membrane, the addition

Table 4. *Anaerobic glycolysis of rheumatoid synovial homogenates*

The basic medium contained KCl (0.154M), $KHCO_3$ (0.024M), $MgCl_2$ (0.01M), glucose (0.01M), DPN (2×10^{-4} M). Additions: fructose 1:6-diphosphate (0.03M), potassium phosphate, pH 7.4 (3 mM), nicotinamide. 1 ml. of 10% (w/v) homogenate in bicarbonate-saline. Temperature, 37°. Gas phase, $N_2 + CO_2$ (95:5).

Flask no.	Additions	Time (min.)	$q\dot{CO}_2$ (μ l./mg. of tissue N/hr.)			
			0-15	15-30	30-45	45-60
1	Nicotinamide (0.05M)		31.0	18.2	12.7	12.7
2	Nicotinamide (0.01M)		35.6	25.0	16.0	10.7
3	Fructose 1:6-diphosphate, nicotinamide (0.03M)		14.2	11.8	9.5	9.5
4	Potassium phosphate		48.0	13.9	4.0	4.0
5	Potassium phosphate, nicotinamide (0.01M)		21.7	15.5	10.9	9.3
6	Potassium phosphate, fructose 1:6-diphosphate, nicotinamide (0.03M)		31.2	26.8	19.3	16.4

Table 5. *Effect of hydrocortisone on anaerobic glycolysis of various tissue homogenates*

The basic medium contained KCl (0.154M), $KHCO_3$ (0.024M), $MgCl_2$ (0.1M), glucose (0.01M). Medium no. 1: basic medium. Medium no. 2, basic medium with nicotinamide (0.02M). Medium no. 3, basic medium with nicotinamide (0.02M), potassium phosphate (0.003M), hexokinase (20 units), adenosine triphosphate (0.001M), DPN (2×10^{-4} M), pyruvate (0.01M). Medium no. 4, basic medium with potassium phosphate, fructose 1:6-diphosphate (0.03M), nicotinamide (0.03M), DPN (2×10^{-4} M). 1 ml. of 10% (w/v) homogenate. Temperature, 37°. Gas phase $N_2 + CO_2$ (95:5). Figures in parentheses are standard error of mean. Values for % P are given where the probability of the difference between hormone and control results being due to chance is 5% or less.

Tissue	Medium no.	Hormone addition (μ g.)	$q\dot{CO}_2$ (μ l./mg. of tissue N/hr.)							
			0-15 min.	% P	15-30 min.	% P	30-45 min.	% P	45-60 min.	% P
Rat spleen	1	0	24.0 (± 2.8)	5.0	18.8 (± 2.7)	5.0	15.7 (± 1.3)	1.0	13.6 (± 1.6)	1.0
		500	14.9 (± 1.3)		10.6 (± 0.9)		9.1 (± 0.7)		7.2 (± 0.5)	
Rat spleen	2	0	12.2 (± 1.2)	1.0	7.8 (± 0.8)	—	—	—	—	—
		500	9.6 (± 0.7)		4.0 (± 0.4)		—		—	
Rat spleen	3	0	14.7 (± 0.7)	0.1	12.0 (± 0.6)	—	11.0 (± 0.4)	0.1	10.5 (± 0.2)	0.1
		500	14.2 (± 0.6)		9.2 (± 0.8)		8.5 (± 0.2)		8.2 (± 0.2)	
Rat kidney	3	0	17.2 (± 0.4)	5.0	8.6 (± 0.4)	—	4.9 (± 0.4)	5.0	4.0 (± 0.2)	1.0
		500	16.1 (± 0.8)		5.8 (± 0.7)		2.9 (± 0.3)		1.3 (± 0.2)	
Rheumatoid synovium	4	0	44.6 (± 1.4)	—	39.6 (± 3.3)	—	35.4 (± 5.0)	—	—	—
		500	45.0 (± 2.6)		43.5 (± 2.3)		42.0 (± 2.9)		—	

Table 6. Influence of substrate upon the $q_{O_2}^0$ of normal and rheumatoid synovial tissues

Figures in parentheses are the number of determinations. Medium, Krebs-Ringer-phosphate. Temperature, 37°. Gas phase, O_2 .

Substrate	Substrate concn. (mM)	$q_{O_2}^0$		
		'Normal' synovial tissue	Rheumatoid synovial membrane	Rheumatoid synovial villi
Malate	0	<1.0 (2)	2.1 (2)	—
	10	7.5 (2)	3.7 (2)	—
Fumarate	0	<1.0 (1)	2.0 (2)	22.0 (3)
	10	5.0 (1)	2.6 (2)	28.0 (3)
Succinate	0	<1.0 (4)	2.0 (2)	25.0 (5)
	10	6.0 (4)	4.3 (2)	40.0 (5)
Acetate	0	3.0 (1)	—	21.0 (2)
	10	3.0 (1)	—	21.0 (2)
Pyruvate	0	<1.0 (1)	2.0 (2)	24.5 (4)
	10	<1.0 (1)	3.7 (2)	23.0 (4)
Glucose	0	<1.0 (6)	1.7 (3)	30.0 (5)
	10	<1.0 (6)	1.2 (3)	31.9 (5)

Table 7. Influence of hydrocortisone on the substrate metabolism of a rheumatoid synovium

Substrates were used at a concentration of 10 mM. Medium, Krebs-Ringer-phosphate. Gas phase, O_2 . Temperature, 37°.

Substrate	$q_{O_2}^0$		Inhibition (%)
	Control	250 μ g. of hydrocortisone/ml.	
None	16.9	9.2	46
Acetate	16.4	7.6	54
Oxalacetate	28.8	12.7	55
cis-Aconitate	18.7	10.8	42
Citrate	18.2	10.2	44
α -Oxoglutarate	20.0	11.4	43
Succinate	18.8	11.5	39
Fumarate	25.1	12.0	52
Malate	18.0	10.3	43

of malate, fumarate and succinate markedly stimulated respiration. The rate of oxygen uptake by both rheumatoid synovial membrane and villi showed a similar stimulation by malate, fumarate and succinate, but not by acetate, pyruvate or glucose. As different tissues were used with each substrate to obtain the values given in Table 6, it was not possible to compare the relative oxidative stimulation by each substrate. We therefore tested the effect of various members of the tricarboxylic acid cycle on a single rheumatoid tissue. These results are given in Table 7. Acetate again had no stimulatory effect on $q_{O_2}^0$, though all the other substrates stimulated oxygen uptake; the most active in this instance were oxalacetate and fumarate. During this experiment hydrocortisone was introduced from the side arm after 46 min. and the rate of oxygen uptake measured for a further 60 min. The percentage inhibition of oxygen

Fig. 2. Influence of hydrocortisone on the oxygen uptake of various synovial tissues of rheumatoid origin. Hydrocortisone, 250 μ g./ml. Medium, Krebs-Ringer-phosphate; glucose (10 mM). Gas phase, O_2 . Temperature, 37°. Number of experiments, 57. Regression equation, $y = 30.3x - 3.31$.

uptake, in the presence of hydrocortisone, was of roughly the same order for all flasks. There was no fall in respiration in the absence of hydrocortisone.

We have previously shown that the $q_{O_2}^0$ of rheumatoid synovia varied greatly, and that the oxidative metabolism might be correlated with the proliferative state of the tissue.

Fig. 2 shows a scatter diagram of the rates of oxygen uptake from a variety of rheumatoid tissues, plotted against the log of the depression of $q_{O_2}^0$

produced in that tissue by 250 μg . of hydrocortisone/ml. The values given in the diagram have accumulated over a period of 3 years, and in consequence the experimental technique with regard to the addition of hydrocortisone was not uniform. In one-third of the cases hydrocortisone was tipped from the side arm during incubation and the rate measured. In the remaining two-thirds hydrocortisone was present in the flasks during the whole experimental period, and an equal number of control flasks were put up. The pairs of observations in this latter group were randomly selected from all the observations on each tissue. The 'true-pair' regression line for q_0^0 on log depression q_0^0 was $y = 29.65x - 3.89$ for 21 pairs. The regression line for the 'random-pair' experiments was $y = 29.23x - 3.29$ for 36 pairs. There is not a statistically significant difference between these slopes and we have therefore treated all observations as if the experimental method was uniform. The resulting regression equation is $y = 30.3x - 3.31$. The correlation coefficient is 0.538 ($p < 0.1\%$).

The same level of hormone gave increased inhibition with increasing synovial oxidative metabolism. The level of hydrocortisone used in these experiments (250 μg /ml.) is well within the range expected in the synovial fluid after intra-articular injection.

DISCUSSION

Intact synovial tissue was able to utilize a number of glycolytic intermediates *in vitro*. It was also capable of glycogen synthesis or breakdown. Glycogenolysis was increased in the presence of hydrocortisone, while aerobic and anaerobic glucose utilization and lactate production were markedly inhibited by the hormone. These inhibitory effects of this corticosteroid have been demonstrated on a number of other tissues [Bacila & Barron (1954), Miller (1954) and Martin, Chaudhuri, Green & McKinney (1954)].

After homogenization of synovial tissue it was necessary to make a number of additions to the medium to achieve even a moderate rate of glycolysis. The most important of these additions appeared to be DPN. The increased stabilization of the glycolytic rate in the presence of DPN and nicotinamide was presumably due to inhibition of breakdown of DPN. Under these conditions no inhibition by hydrocortisone of acid production could be observed. Splenic homogenates, however, showed rapid rates of glycolysis without the addition of cofactors and were inhibited by hydrocortisone. When the splenic homogenates were fortified, the degree of inhibition was often reduced, and was apparent only at a later period of incubation. Thus it would appear that the inhibitory action of hydrocortisone may be modified by the

presence of various glycolytic intermediates and cofactors. The marked stimulatory effect of DPN upon the glycolysis of synovial homogenates suggests that the synovial content of this coenzyme may be low. Branster & Morton (1956) have suggested that a decreased rate of synthesis of DPN may be one aspect of rapid cell proliferation. Jedeikin & Weinhouse (1955) have reported that the total DPN contents of a variety of transplanted solid and ascites tumours were considerably lower than those of normal tissues. As the rheumatoid synovia used in our experiments were in an active state of proliferation it would be of interest to know the level of the tissue DPN. Our findings with suspension of rat spleen appear to indicate that the inhibitory action of the hormone can be demonstrated in broken-cell preparations. These results are at variance with the views expressed by Thorn *et al.* (1953), who stated that no effect of cortisone on enzyme systems had been clearly demonstrated in the absence of the intact cell, and with the findings of Martin *et al.* (1954), who showed that the inhibitory effect of cortisone and hydrocortisone on aerobic lactate formation was seen only in intact human leucocytes, which had been handled with care to prevent injury.

Our results indicate that various intermediates of the tricarboxylic acid cycle can be readily oxidized, and that their respective enzyme systems can be presumed to be present. The question of the complete operation of the citric acid cycle in normal and rheumatoid synovia is of considerable importance, as it has been shown that the presence of rheumatoid disease in the synovial membrane is accompanied by a very considerable increase in oxidative metabolism, e.g. < 1.0 up to q_0^0 37.0 (Dingle & Thomas, 1956). This minimal oxidative rate found in normal synovia might be due to a very low level of oxidative enzymes, or an incomplete operation of the tricarboxylic acid cycle, or to the effect of some rate-controlling factor. In the presence of rheumatoid disease can be presumed to alter any of these conditions, the oxidative level of the tissue might be raised. As the addition of malate, fumarate, and succinate raised the q_0^0 of some normal tissue to a value approaching that of some rheumatoid tissues, it is unlikely that the minimal synovial respiration normally present is due to a low level of oxidative enzymes. Whether or not the Krebs's cycle is fully operative in both normal and rheumatoid synovia, however, must await further investigation. The inhibition of oxygen uptake by hydrocortisone appeared relatively constant regardless of the added substrates, which minimizes the possibility of inhibition of a specific enzyme system within the cycle.

In view of its inhibitory action on both glycolysis and oxidation, it is possible that the action of

hydrocortisone is upon some form of metabolic pacemaker.

It has previously been shown that the $q\dot{O}_2$ of rheumatoid synovia is increased in proliferating tissues. Fig. 2 indicates that the inhibitory effect of the hormone is greatest in the most metabolically active synovia. It is likely therefore that the inhibition of oxidation and glycolysis must result in a marked deprivation of energy to the most actively proliferating tissues. This is substantiated somewhat by clinical experience of intra-articular injections of hydrocortisone, which appear to be most effective when the proliferative phase is most marked. It is of interest in this connexion that Roberts & Szego (1953) found that the initiation of true growth in the rat uterus, occurring 20 hr. after administration of oestradiol, was associated with a substantial increase in respiration as well as in glucose utilization, and that hydrocortisone was effective in inhibiting these metabolic changes.

SUMMARY

1. The effect of various substrates on the metabolism of normal and rheumatoid synovial tissues *in vitro* has been investigated in the presence and absence of hydrocortisone.

2. Rheumatoid synovial membrane has been shown to be capable of glycogen synthesis or breakdown. The presence of hydrocortisone resulted in an increase in glycogenolysis or a diminution of glycogen synthesis where this occurred.

3. Glucose utilization of synovial tissue is inhibited by hydrocortisone under both aerobic and anaerobic conditions.

4. Rheumatoid synovial tissue utilized glucose 1-phosphate, glucose 6-phosphate and fructose 1:6-diphosphate under aerobic conditions. The utilization of these substrates gave variable results in the presence of hydrocortisone, though the inhibitory action on $q\dot{O}_2$ was unaffected.

5. Aerobic and anaerobic lactic acid formation was inhibited in the presence of hydrocortisone.

6. The effect on the $q\dot{O}_2$ of normal and rheumatoid synovia by malate, fumarate, succinate, acetate, pyruvate and glucose has been investigated. Malate, fumarate and succinate stimulated oxygen uptake. None of these substrates affected the respiratory inhibition produced by hydrocortisone.

7. The inhibitory effect of a standard hydrocortisone concentration, on $q\dot{O}_2$ of rheumatoid

synovial tissues of varying degrees of metabolic activity, was greatest in those tissues which had the highest rates of oxygen uptake.

8. Synovial homogenates showed only slight anaerobic acid production unless diphosphopyridine nucleotide was added. With optimum conditions for glycolysis, hydrocortisone showed no inhibitory action.

9. Splenic homogenates showed significant inhibition of anaerobic acid production by hydrocortisone.

We should like to thank Dr G. D. Kersley for his interest and encouragement; the orthopaedic surgeons of the Bath clinical area for the tissue specimens and Dr G. Herdan for his advice on statistical analysis. The hydrocortisone suspension was generously supplied by the Medical Research Council. Thanks are also due to Miss A. Langley for technical assistance.

REFERENCES

- Bacila, N. & Barron, E. S. G. (1954). *Endocrinology*, **54**, 591.
 Barker, J. B. & Summerson, N. H. (1941). *J. biol. Chem.* **138**, 535.
 Boland, E. W. (1955). *Ann. N.Y. Acad. Sci.* **61**, 349.
 Branster, M. V. & Morton, R. K. (1956). *Biochem. J.* **63**, 640.
 Dingle, J. T. & Thomas, D. P. Page (1956). *Brit. J. exp. Path.* **37**, 318.
 Hawk, P. B., Oser, B. L. & Summerson, W. H. (1952). In *Practical Physiological Chemistry*, p. 820. London: J. and A. Churchill Ltd.
 Hench, P. S., Kendall, E. C., Slocumb, C. H. & Polley, H. F. (1950). *Arch. intern. Med.* **85**, 545.
 Hollander, J. L. (1951). *Rheum. Dis.* **2**, 3.
 Jedeikin, L. & Weinhouse, S. (1955). *Abstr. Proc. Amer. Ass. Cancer Res.* **2**, 26.
 Laidlaw, J. C., Dingman, J. F., Arons, W. L., Finkenshaedt, J. T. & Thorn, G. W. (1955). *Ann. N.Y. Acad. Sci.* **61**, 315.
 Martin, S. P., Chaudhuri, S. N., Green, R. & McKinney, G. R. (1954). *J. clin. Invest.* **33**, 358.
 Miller, Z. (1954). *J. biol. Chem.* **208**, 327.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Roberts, S. & Szego, C. M. (1953). *J. biol. Chem.* **201**, 21.
 Roe, J. H. (1934). *J. biol. Chem.* **107**, 15.
 Terner, C. (1952). *Biochem. J.* **50**, 145.
 Thomas, D. P. Page & Dingle, J. T. (1955). *Brit. J. exp. Path.* **36**, 195.
 Thorn, G. W., Jenkins, W., Laidlaw, J. C., Goetz, F. C., Dingman, J. F., Arons, W. L., Streeten, D. H. P. & McCracken, B. H. (1953). *New Engl. J. Med.* **248**, 232.
 Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1951). *Manometric Techniques and Tissue Metabolism*, p. 119. Minneapolis: Burgess Publishing Co.
 Walaas, O. & Walaas, E. (1950). *J. biol. Chem.* **187**, 769.