transamination mechanism. However, if a transamination reaction is taking place it is apparently concerned with some amino acid other than glutamic acid.

SUMMARY

1. Acetone-dried powders of a strain of *Nocardia globnerula* possess aspartic decarboxylase activity but are inactive towards all other amino acids tested.

2. The enzyme can be used for the quantitative estimation of L-aspartic acid in protein hydrolysates and is stable for long periods.

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Succinate as a Respiratory Inhibitor in *Tenebrio molitor* Pupal-Tissue Preparations

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When changes in the chemical composition of *Tenebrio* tissues during the pupal period were being observed (Patterson, 1956a) an effort was made to relate these findings to alterations in enzyme pattern which have been reported by various authors, e.g. Agrell (1953) for holometabolous insects during metamorphosis. Furthermore, as citrate was present in greatest concentration in *Tenebrio* pupal tissues at the stage of development corresponding to the lowest rate of oxygen consumption by the live pupa (Patterson, 1956b), any variations in the activities of the enzymes of the tricarboxylic acid cycle during metamorphosis were considered to be of interest.

The oxidation of succinate by *Tenebrio* tissues is the only step in the tricarboxylic acid cycle so far investigated. It has been found, surprisingly, that the addition of succinate to pupal-tissue homogenates does not uniformly stimulate respiration and, indeed, that with tissues obtained late in the pupal period a distinct inhibition is produced by adding succinate.

The investigation reported here shows that this diminished respiration was probably due to the

inhibition by the succinate of enzymes of the phenolase type, which become more active in pupal tissues towards ecdysis (Dennell, 1944; Karlson & Wecker, 1955; Wojtczak, 1956), and may make a quantitatively important contribution to the endogenous respiration by acting as a general end-system for oxidative processes (cf. Wojtczak, 1955). The oxidation of tyrosine by homogenates of *Tenebrio*-pupal tissues has been shown to be inhibited by succinate and, in the absence of phenolase activity (suppressed by the addition of ethylenediaminetetra-acetic acid), it was demonstrated that succinate was oxidized throughout metamorphosis although not at a uniform rate.

The inhibition of tyrosine oxidation by succinate was studied in acetone-dried-powder preparations, in which the endogenous respiration and succinoxidase activity were negligibly small but the tyrosine activity was high.

MATERIALS AND METHODS

Insects. Larval, pupal and adult tissues of *Tenebrio molitor* were used in these experiments. Fresh pupae were collected from a culture of larval insects (mealworms) and the metamorphosis was allowed to proceed in separate glass jars at 24° until the insects were required. The 'period

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of metamorphosis' as defined by Patterson (1957) lasted 10 days.

Homogenates. Insect tissues were minced with scissors and then homogenized with five times the weight of ice-cold water in an all-glass homogenizer (Potter & Elvehjem, 1936). Fragments of cuticle were carefully excluded by straining through fine gauze. An average 1:5 homogenate contained (in 1 ml.) 45 mg. of total solids and approx. 4.2 mg. of total nitrogen.

Acetone-dried-powder preparations of late-pupal tissue. *Tenebrio* pupae (89 g.; age not less than 5 days) were macerated with ice-cold water (270 ml.) in a previously cooled Waring Blendor. The resulting pulp (320 ml.) was strained through fine gauze and 10 vol. of ice-cold acetone were stirred in. The precipitate was filtered off at the pump after 30 min. The filter cake was thoroughly washed first with ice-cold acetone and then with cold ether. After air-drying the powder was finally desiccated *in vacuo* over liquid paraffin. An off-white powder (15.9 g.) was obtained. All operations were carried out in a room kept at 5°. For enzyme experiments 1 g. of acetone-dried powder was stirred with 10 ml. of water in an all-glass homogenizer.

Measurements of oxygen uptake. These were made in a Warburg microrespirometer. A 1:5 homogenate was diluted, 1 ml. to 3 ml., with sodium phosphate buffer (pH 7.4; final concn. 0.05M). Uptake of oxygen was measured over a period of not less than 30 min. with air as the gas phase in all experiments.

The acetone-dried powder (0.5 ml. of a 10% suspension in water), diluted with phosphate buffer in the same way, showed only a small respiration (4–7 μ l. of O_2 /hr.). This could be neglected when the powder was being used as a source of phenolase.

All experiments described in this paper were carried out at 25° in phosphate buffer at pH 7.4.

RESULTS

During the course of some preliminary experiments, pupal-tissue homogenates were prepared in an isotonic sucrose (0.2M) homogenizing medium. For late-pupal tissue an average Q_{O_2} of about 2.67

was obtained for endogenous respiration at 25°. (This compares favourably with the values given in Table 1 for homogenates prepared with water.)

A simple separation of cell constituents into nuclei plus cellular debris, mitochondria and a supernatant fraction was carried out by the differential-centrifuging technique used by Rees (1954) for insect-muscle tissue. Of the separate tissue fractions prepared, only the final supernatant fraction exhibited a significant endogenous respiration. This amounted to at least one-half of the total respiration of the original homogenate.

The respiration of an homogenate of susceptible pupal tissue was depressed by added succinate and at the same time melanin-pigment formation was suppressed. Therefore it seemed likely that succinate, when it exerted an inhibitory effect, did so by acting upon tyrosinase or a similar enzyme. Being a water-soluble enzyme (Dawson & Tarpley, 1951) tyrosinase would be found in the supernatant fraction separated from pupal tissues.

Aqueous tissue extracts buffered at pH 7.4 were subsequently used in the present work in order to study the respiration of the water-soluble fractions and the inhibition of tyrosinase activity by succinate.

Respiration of pupal-tissue homogenates

When measured in 0.05M-phosphate buffer, pH 7.4, at 25°, the rate of endogenous oxygen uptake shown by the tissue homogenate was approximately constant for no longer than 30 min. and usually for only about 15 min. The substitution of a more complete medium such as that described by Rees (1954) in his metabolic studies of locust muscle did not prolong the period of linear oxygen uptake.

With the simple phosphate-buffer medium, the initial linear rate of oxygen utilization was dependent upon the age of the pupal tissue being used. This is shown in Table 1.

The increased respiration of pupal tissue at the beginning and at the end of metamorphosis is in accord with the familiar U-shaped curve of change in the rate of oxygen utilization by live metamorphosing holometabolous insects (Needham, 1929; Patterson, 1956b).

Effect of adding succinate to Tenebrio-tissue homogenates

As shown in Table 2, the addition of succinate to tissue homogenates gave rise to variable results, the magnitude and nature of the effect depending upon the stage of metamorphosis at which the tissue was used. The ratio of oxygen consumption in the presence of added succinate (measured in the first 30 min.) to that in the absence of added substrate was calculated from experimental data

Table 1. *Endogenous respiration of pupal-tissue homogenates at 25°*

Homogenate (1 ml.) in phosphate buffer at pH 7.4. Final concn. of phosphate, 0.05M. Final vol., 3 ml. Centre well of Warburg vessel contained 0.1 ml. of 20% KOH.

Age of pupal tissue (days)	Initial linear rate of O_2 uptake (μ l. of O_2 /hr.)	Initial Q_{O_2} (μ l. of O_2 /mg. dry wt./hr.)
0–1	118	2.6
	128	2.8
3–5	38	0.8
	50	1.1
	40	0.9
8–10	121	2.7
	158	3.5
	172	3.8
10	192	4.3
(during ecdysis)	252	5.6

obtained with homogenates of tissues of different stages of development and is referred to here as the 'stimulation coefficient'.

It can be seen that the actual depression of respiration by added succinate was apparently limited to homogenates of late-pupal tissues, and that homogenates of tissues obtained at other stages of development apparently oxidized succi-

nate normally. At all stages of development the respiration of tissue homogenates in the presence of succinate was characterized by a linear oxygen uptake lasting, in some cases, for 2 hr.

At the end of some of these experiments, especially where late-pupal tissue had been used, the appearance of the contents of the two 'endogenous flasks' differed quite markedly from that of the flasks to which succinate had been added. Normally, freshly prepared homogenates of *Tenebrio* tissues were off-white to light brown in colour. After 30 or more minutes in a Warburg respirometer at 25°, there was usually considerable formation of a dark melanin-like pigment. Where differences were observed between 'endogenous' and 'succinate' flasks, the contents of the latter flasks were the less pigmented.

With graded amounts of added succinate, 1 ml. samples of 1:5 pupal-tissue homogenates showed maximum inhibition of respiration at a final concentration of about 0.1M-succinate, whereas at about the same concentration phenolase activity (indicated by melanin-like pigment formation) was minimal (see Table 3).

Effects of ethylenediaminetetra-acetic acid in the absence and in the presence of succinate

The addition of succinate apparently caused an inhibition of pupal-tissue phenolase activity, and it seemed likely that the inhibition of the endogenous respiration by succinate (which reaches a maximum of 88 %) might be due to its action on phenolase. Therefore, since phenolase is a copper-containing enzyme (Wigglesworth, 1949; Dawson & Tarpley, 1951) it was anticipated that ethylenediaminetetra-acetic acid (EDTA), which strongly binds copper (see Schwarzenbach, Gut & Andregg, 1954), would bring about a similar diminution of endogenous respiration by the elimination of phenolase activity.

This was found experimentally to be the case. An endogenous respiratory rate of 165 μ l. of O₂/hr. was reduced to 32 μ l./hr. by 10 mM-EDTA, and to 28 μ l./hr. by 30 and by 50 mM-EDTA, giving a maximum inhibition of 82 %. At the same time 'melanin' formation was abolished.

In view of the fact that succinate inhibited the endogenous respiration of pupal-tissue homogenates during the latter stages of metamorphosis, it was not possible to assess directly the succinoxidase activity of tissue samples. With maximum inhibition of the phenolases by EDTA, however, the respiration of *Tenebrio*-tissue homogenates was found to be stimulated by the addition of succinate at all stages of tissue development (see Table 4). The degree of stimulation was taken as an index of the succinoxidase activity of the tissue samples.

Table 2. *Effect of added succinate on the respiration of Tenebrio-tissue homogenates*

Succinate (100 μ moles) was added to the medium which contained 1 ml. of homogenate. Conditions were as described under Methods.

Stage of development of tissue	Stimulation coeff.*
Larva	1.86
	1.88
	2.41
Pupa 3-5 days	1.49
	1.26
	1.36
Pupa 8-10 days	0.66
	0.96
	0.80
Pupa (during ecdysis)	0.46
	0.50
Adult	1.44
	1.58

* Ratio of rate of O₂ uptake with succinate to rate without succinate.

Table 3. *Inhibition of respiration and of phenolase activity in 1 ml. of late-pupal-tissue homogenate by added succinate*

Final concn. of added succinate (M)	Initial rate of respiration (μ l. of O ₂ /hr.)	Melanin-like pigment formed
Nil	310	++++
0.033	130	+++
0.066	60	+
0.100	38	—

Table 4. *Effect of adding succinate on the respiration of homogenate in the presence of ethylenediaminetetra-acetic acid*

Succinate (100 μ moles) was added to medium containing 1 ml. of homogenate and 50 mM-EDTA. Conditions were as described under Methods.

Stage of development of tissue	Stimulation coeff.*
Larva	1.38
Pupa: 0-1 day	1.16
Pupa: 3-5 days	1.07
Pupa: (during ecdysis)	1.31
Adult	1.19

* See footnote to Table 2.

In no case was the stimulation of respiration as great as that obtained in the absence of EDTA at stages of development where stimulation would occur (see Table 2). Clearly, at this concentration EDTA was partly, at least, inhibiting enzyme systems other than the phenolases, and it would have been better to have used a more specific inhibitor of tyrosinase such as α -naphthylthiourea (DuBois & Erway, 1946).

Inhibition of pupal-tissue tyrosinase by succinate

Tyrosine, when added to *Tenebrio* pupal-tissue homogenates, was readily oxidized, but when succinate was also present the tyrosine was oxidized at a reduced rate (Table 5). The minimum

Table 5. *Inhibition by succinate of tyrosinase activity in 1 ml. of late-pupal-tissue homogenate*

Tyrosine (8 μ moles) was added. Conditions were as described under Methods.

Concn. of succinate (M)	Initial rate of oxidation (μ l. of O_2 /hr.)
Nil	221
0.08	117
0.12	86
0.15	82

Table 6. *Inhibition by succinate of tyrosinase activity in pupal-tissue acetone-dried powder*

Tyrosine (10 μ moles) was added. Conditions were as described under Methods.

Concn. of succinate (mm)	Initial rate of oxidation (μ l. of O_2 /hr.)
Nil	490
0.33	493
5.0	444
9.9	416
17.0	367
33.0	300
61.0	241
133.0	197

Table 7. *Inhibition of tyrosinase activity in pupal-tissue acetone-dried powder by some di- and tri-carboxylic acids*

Tyrosine (10 μ moles) was added. Conditions were as described under Methods.

Inhibitor	Concn. (M)	Initial rate of oxidation (μ l. of O_2 /hr.)
Nil	—	525
Succinate	0.033	433
Malonate	0.033	348
Citrate	0.033	266
α -Oxoglutarate	0.007	69

rate of oxidation was obtained with a concentration of 0.12–0.15 M-succinate.

With aqueous suspensions of acetone-dried powders of pupal tissues, the initial rate of tyrosine oxidation was found to decrease logarithmically with concentrations of up to about 0.13 M-succinate when maximum inhibition was obtained (Table 6). This is in close agreement with the result for the inhibition of tyrosinase in homogenates and with the earlier finding that maximum inhibition of the endogenous respiration of late pupal-tissue homogenates was obtained with about 0.1 M-succinate.

The inhibition of tyrosinase by succinate is not peculiar to this one acid. Inhibition of the oxidation of tyrosine by pupal-tissue acetone-dried powder is caused also by malonate, citrate and α -oxoglutarate (Table 7).

The succinate inhibition was selected for somewhat closer study. With a constant level of 100 μ moles of succinate/flask, the initial rate of tyrosine oxidation catalysed by 50 mg. of pupal-tissue acetone-dried powder was obtained graphically from manometric data for substrate concentrations of 0.66–3.96 mm. For comparison, similar data were obtained for uninhibited tyrosine oxidation. Two experiments were carried out with different suspensions prepared from the same stock of acetone-dried powder.

Following the theory of enzyme action of Michaelis & Menten (1913), the relationship between v , initial reaction velocity, S , substrate concentration, and the constants V , the maximum initial-reaction velocity at infinite substrate concentration, and K_m , the Michaelis constant, may be expressed in the form:

$$S/v = S/V + K_m/V.$$

Accordingly, the values of the ratio of substrate concentration to the corresponding initial velocity of the tyrosinase reaction (S/v) were plotted against the substrate concentration (S) for the two sets of conditions and, where applicable, average values of duplicate determinations were used. Two straight lines were calculated as regression lines of S/v upon S (Fig. 1). The slopes for the uninhibited and succinate-inhibited oxidation of tyrosine are $+1.19 \pm 0.18$ and $+1.81 \pm 0.16$ (95 % limits) respectively, and are numerically equal to 10^3 times the reciprocals of the respective maximum initial velocities $1/V$ and $1/V'$. The ratios of the Michaelis constants to the maximum initial velocities are given by the intercepts on the ordinate. Thus $K_m/V = 1.80$ and $K'_m/V' = 2.27$, whence the values of K_m and K'_m are 1.59×10^{-3} and 1.25×10^{-3} respectively. The calculated maximum initial velocity was reduced from 839 μ l. of O_2 /hr. (V) to 553 μ l. of O_2 /hr. (V') in the presence of 33 mm-succinate, the difference being significant.

It is clear that this is not a case of competitive inhibition because, where competition between substrate and inhibitor for the same enzyme site exists, the theoretical maximum reaction velocity at infinite substrate concentration will be unaltered by the presence of an inhibitor. Here, the inhibitor is not a structural analogue of the substrate and competition for the same enzyme sites would not be expected.

From theoretical considerations of the general case of enzyme inhibition by an inhibitor which may combine with both the free enzyme and the enzyme-substrate complex, the following rate equation may be deduced (cf. Bray & White, 1957):

$$V/v' = K_m/S(1 + I/K_I) + (1 + I/K_{SI}),$$

where V is the calculated maximum initial velocity of the reaction in the absence of the inhibitor, v' is the initial inhibited-reaction velocity and S and I are the substrate and inhibitor concentrations. K_m is the Michaelis constant for the enzyme-substrate complex and K_I and K_{SI} are the dissociation constants of the enzyme-inhibitor complex and the enzyme-substrate-inhibitor complex respectively.

Fig. 1. Inhibition of the tyrosinase activity of 50 mg. of pupal-tissue acetone-dried powder, in 0.05M-phosphate at pH 7.4 and 25°, caused by adding 100 μ moles of succinate. Total vol., 3 ml. O, Average experimental points obtained during the uninhibited oxidation of tyrosine (curve A); ●, average experimental points for the inhibited oxidation (curve B).

Values for V/v' were plotted against the corresponding values for K_m/S , the figures for V and K_m , calculated from curve A, Fig. 1, being used. A straight line was drawn through the experimental points, which had a slope of 1.24 ± 0.12 . The intercept at the ordinate had a value of 1.50 ± 0.12 (95% limits). The 95% confidence limits for the numerical values obtained for the slope and the intercept did not overlap and were therefore significantly different. This excluded non-competitive inhibition where $K_I = K_{SI}$. Both values were greater than unity and consequently I/K_I and I/K_{SI} were greater than zero. Competitive and uncompetitive types of inhibition were thus ruled out, since in these cases the values of K_I and K_{SI} respectively are very large and the expressions $(1 + I/K_I)$ and $(1 + I/K_{SI})$ are approximately equal to unity (Bray & White, 1957). The inhibition of pupal tyrosinase by succinate was therefore considered to be in all probability one of the general cases of enzyme inhibition.

The dissociation constant of the enzyme-inhibitor complex (K_I), calculated from the observed slope of the graph in Fig. 2, is approx. 139×10^{-3} , and K_{SI} , the dissociation constant of the enzyme-substrate-inhibitor complex, was calculated from the value of the intercept to be approx. 66.6×10^{-3} . Thus succinate when acting as an inhibitor has a greater affinity (about twice) for the tyrosinase-substrate complex than for the free tyrosinase.

Values for V and K_m obtained from experimental observation of uninhibited tyrosine oxidation

Fig. 2. Plot of V/v' against K_m/S for the oxidation of 0.66–3.96 mM-tyrosine by pupal-tissue acetone-dried powder in the presence of 33.3 mM-succinate.

Table 8. *Initial rates of tyrosine oxidation by pupal-tissue acetone-dried powder inhibited by succinate*

Succinate (100 μ moles) was added. Conditions were as described under Methods.

Concn. of tyrosine (mm)	Mean initial rate of oxidation (observed) (μ l. of O ₂ /hr.)	Mean initial rate of oxidation (calculated) (μ l. of O ₂ /hr.)
0.66	187	187
1.32	274	291
1.98	356	338
2.64	370	418
3.30	400	400
3.96	422	420
Mean difference		= 7.5
S.D. of mean difference		= 22.7

(curve A, Fig. 1) and the values for K_i and K_{si} obtained graphically from Fig. 2 were finally substituted in the general equation for enzyme inhibition. The initial velocities of inhibited tyrosine oxidation were calculated for tyrosine concentrations of from 0.66 to 3.96 mm in the presence of 33.3 mm-succinate. They were in good agreement (see Table 8) with the mean experimental values obtained with acetone-dried powders of pupal tissue (i.e. 102.2 % of the observed figures; mean difference 7.5, s.d. 22.7). In confirmation of the results obtained by the graphical methods, the experimental data for the inhibition of pupal tyrosinase by succinate appear to conform to the general equation for enzyme inhibition.

DISCUSSION

Succinate acts as a respiratory inhibitor for late-pupal tissue (i.e. pupal tissue of age more than 5 days at 24°). It is apparently at this stage that the titre of phenolase is highest if this insect resembles some other holometabolous insects cited in the literature (Dennell, 1944; Karlson & Wecker, 1955; Wojtczak, 1956).

If, as has been suggested by Heller (1947) and by Wojtczak (1955), the phenolases behave as general end-systems for oxidations in insects and these phenolase-coupled oxidations account for a large proportion of the endogenous respiration, then the demonstrable antagonism between succinate and tyrosine will account for the large diminution (about 88 %) in the endogenous respiration when succinate is added to late-pupal-tissue homogenates. The addition of EDTA to homogenates brings about a similar degree (82 %) of maximum inhibition. Although the mechanism of action of EDTA is almost certainly different, it is the phenolase-coupled respiration which is largely eliminated, as shown by the inhibition of melanin-pigment formation. In late-pupal tissue of *Tenebrio* therefore it is estimated that respiration

coupled to tyrosinase or other phenolases or to them both amounts to about 85 % of the total.

This is not to say that succinate is not utilized at all. In fact, all *T. molitor* tissues were found to catalyse the oxidation of succinate in the presence of sufficient EDTA to abolish phenolase activity.

At other stages of development it is probable that the titre of the phenolases is lower than in the later stages of pupal life. Thus although some of the added succinate would inhibit tyrosinase activity, a larger proportion would also be vigorously oxidized by succinoxidase present (Ludwig & Barsa, 1956), which would account for the net increase in oxygen uptakes observed.

The fact that a number of organic acids besides succinic act as inhibitors of the enzyme tyrosinase is of interest because the extracellular fluids of insects contain, by mammalian standards, a high concentration of organic acids [e.g. in the blood of *Gastrophilus intestinalis* larva there are 58 m-equiv. of organic acids/l. (Levenbook, 1950)]. At such concentrations *Tenebrio*-pupal tyrosinase inhibition was found to be almost maximum. It will therefore be interesting to determine whether the content of organic acids in insect extracellular fluid is decreased when tissue-phenolase activity increases. In this way it will be possible to decide whether the inhibitory action of organic acids such as succinate on tyrosinase is likely to be of significance *in vivo*.

SUMMARY

1. Succinate was oxidized by *Tenebrio molitor* tissue preparations at most stages of development, but late in the pupal period the endogenous respiration of tissue homogenates was greatly reduced by added succinate.

2. The inhibition was probably due to the abolition of phenolase activity, which is at a high titre at the later stages of pupal life.

3. With suspensions of acetone-dried powder as a source of pupal enzyme, the inhibition of tyrosinase by succinate was readily demonstrated. The inhibition followed a general rate equation for enzyme inhibition.

4. Malonate, citrate and α -oxoglutarate also inhibit pupal tyrosinase.

5. Since about 85 % of the endogenous respiration of late-pupal tissue may be abolished either by succinate or ethylenediaminetetra-acetic acid, both of which inhibit tyrosinase activity, it is suggested that this proportion of the endogenous respiration is due to oxidations coupled to a phenolase end-system.

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

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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