STUDIES ON THE TRANSPLANTED HEART*

ITS METABOLISM AND HISTOLOGY

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PLATES 70 TO 72

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Homografts of the heart have been performed for over 50 years, since Carrel
performed the first of this procedure in 1907 (1).

In 1933 Mann and his coworkers (2) developed their technique for homotrans-
plantation of the intact mammalian heart. They anastomosed the carotid artery of
the recipient to the aorta of the graft and the pulmonary artery of the graft to the
external jugular vein of the recipient. Histologic studies of the graft revealed infiltr-
ation with lymphocytes, large mononuclears, and polymorphonuclear cells. In 1948,
Sinitsyn (3) maintained the coronary circulation of a homografted heart through the
action of its own left ventricle. In 1953, Luisada (4) maintained the circulation of the
homografted heart during the operative procedure by Anastomosing the left sub-
clavian artery of the homograft to the carotid artery of a donor animal. The technique
of Mann was later used by Downie (5) in 1953, Wesolowski (6) in 1953, Sayegh and
Creech (7) in 1957, and by Reemtsma and Creech (8) in 1960. Metabolic studies on
the transplanted heart were performed by Lee and Webb (9), and by Reemtsma,
Delgado, and Creech (8). Lee and Webb (9) found that in the normothermic homo-
grafit the coronary flow averaged 128 cc per 100 gm of left ventricle per minute, with
a range of from 80 to 140 cc. The myocardial oxygen consumption averaged 6.4 cc
per minute. Studies on the carbohydrate metabolism of these hearts showed myo-
cardial utilization of glucose, lactate, and pyruvate. An early negative balance of
pyruvate was a frequent occurrence. Reemtsma, Delgado, and Creech (8), using the
procedure of Mann, found marked variations in the oxygen consumption of the homo-
grasts. Myocardial lactate production rather than consumption was observed in the
majority of their experiments.

It is the purpose of this report to describe and define the metabolic changes
accompanying rejection of the homografted canine heart. In addition, observa-

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tions are reported describing the histologic and histochemical aspects of the tissue reactions observed in the homografted canine heart subjected to first and second rate rejection.

**Materials and Methods**

60 experiments were carried out on mongrel dogs anesthetized with pentobarbital (30 mg/kg weight). The method of Mann (2), together with the viviperfusion of Luisada (4), was followed. As a slight modification of Mann's procedure, the proximal end of the carotid artery of the recipient was anastomosed to the brachiocephalic artery of the graft. Adult dogs weighing from 15 to 30 kg served as blood donors and graft recipients; puppies weighing from 3 to 4 kg were used as graft donors. Blood clotting was prevented by the intravenous administration of from 20 to 60 mg of heparin sodium. Occasionally, grafts developed ventricular fibrillation immediately after transplantation, but electrical defibrillation was usually effective in re-establishing normal sinus rhythm. All animals received procaine penicillin (300,000 units b.i.d.) and 50 mg of depo-heparin sodium for a minimum of 3 days. Coronary arteriovenous differences were determined by direct sampling from the aortic arch and pulmonary artery of the graft. It was necessary to expose the graft to obtain the blood samples.

Blood samples were analyzed for oxygen, glucose, pyruvate, lactate, and for the activities of malic dehydrogenase and aldolase. Blood oxygen and carbon dioxide concentrations were determined with the manometric method of Van Slyke and Nell (10), and glucose by the method of Hugget and Nixon (11); pyruvate was also measured enzymatically (12). Lactate was determined by a method modified from Hohorst (13). Malic acid dehydrogenase activity in plasma was measured with the method of Siegel and Bing (14) and plasma aldolase activity was enzymatically determined (15). Hereafter, differences in concentration of substrates or the activity of enzymes between the arterial and coronary sinus blood will be referred to as myocardial balances. A positive myocardial balance indicates a lower value in the coronary sinus blood than in arterial blood; when the balance is negative, the value in the coronary sinus blood exceeds that in arterial blood.

Accelerated (second set) rejection of the heart was produced in the following manner: The spleen of the donor animal was excised 5 to 7 days prior to the transplantation of the heart of this animal. The organ was cut into small pieces with a pair of scissors and pressed through a fine wire mesh. The tissue was suspended in approximately 10 cc of ice cold normal saline; the extract was then centrifuged in the cold at 2,000 rev/min. The supernatant was discarded and the sediment of spleen cells was resuspended in 10 cc of ice cold normal saline solution. The number of spleen cells per cubic millimeter were then counted in a white cell counting chamber. Usually, a suspension containing a billion spleen cells, suspended in 10 cc of normal saline, was injected intraperitoneally into the host 5 to 7 days prior to transplantation of the heart.

In experiments in which the effect of thiamine injection on the metabolism of the transplanted heart was studied, 200 mg of thiamine hydrochloride in distilled water was injected into the vessel leading to the graft. 30 minutes later, samples from the carotid artery and the pulmonary artery of the graft were collected and analyzed for oxygen, CO₂, glucose, pyruvate, and lactate.

The oxidation reduction potential (E₀, or redox potential) of the arterial and venous blood was calculated from the ratio of the molar concentration of lactate to that of pyruvate \( \frac{La}{Py} \). Klingenberg and Bücher (16) state that the oxidation reduction potentials of the lactate-pyruvate system in blood to that in liver bears a definite and direct relationship. It has also
been shown that the redox potential of the lactate-pyruvate system is different in coronary vein than in arterial blood. In normal dog hearts the redox potential of the lactate-pyruvate system is more positive in coronary vein than in arterial blood, and the redox potential of the heart muscle is close to that of the coronary vein blood. In an anoxic heart the redox potential of the coronary vein blood is more negative than that of the arterial blood (17).

The changes in the redox potentials across the heart (ΔEh) are defined by the equation:

$$\Delta E_h = E_{h \text{ vein}} - E_{h \text{ artery}}$$

In a normal heart ΔEh is positive, and in an anoxic heart ΔEh is negative.

The redox potential of the extramitochondrial DPN to DPNH system (Eo) has been estimated as $-240$ mv (16, 18). The redox potential is calculated from the formula:

$$E_o = E_o + \frac{RT}{nF} \ln \left(\frac{\text{oxidized substrate}}{\text{reduced substrate}}\right)$$

The per cent glucose-oxygen extraction ratio was determined from the myocardial extraction of glucose and oxygen:

$$\text{Glucose-oxygen extraction ratio (per cent)} = \frac{\text{O}_2 \text{ equivalent of extraction glucose}}{\text{Myocardial oxygen extraction}} \times 100.$$  

The oxygen equivalent of glucose is 0.75 mg per cent of glucose extraction. A glucose-oxygen extraction ratio of less than 100 per cent indicates that oxygen is available for the breakdown of substrates other than glucose. A ratio of more than 100 per cent suggests that a portion of glucose extracted by the heart is not metabolized through oxidative pathways (19).

Gross and histologic studies were performed on all donor and recipient hearts. In general, the majority of transplanted hearts was allowed to undergo spontaneous death before the preparation was taken for study. Immediately after death representative portions of the heart were fixed in 10 per cent formalin and Carnoy's solution. Stains employed were hematoxylin and eosin, methyl green-pyronine, and scarlet R. For the histochemical reactions the following enzymes were studied: DPNH diaphorase, lactic, malic, and succinic dehydrogenases. Small pieces of cardiac tissue 2 to 3 mm in diameter were frozen in a test tube and immersed in dry ice and acetone at $-65^\circ$C. They were transferred to a Linderström-Lang cryostat and cut at $-25^\circ$C until ready to be immersed into the substrate. The substrate for DPNH diaphorase consisted of: 5 mg DPNH in 12 ml phosphate buffer (0.2 M, pH 7.5), and 3 ml of nitro blue tetrazolium (2 mg/ml/H$_2$O). The substrate solution for lactic dehydrogenase consisted of: 5 ml of L- (+) lactate (ca. 0.2 M), and 5 mg DPN in 7 ml tris buffer (0.2 M, pH 7.4), and 3 ml nitro blue tetrazolium (2 mg/ml/H$_2$O). The substrate for malic dehydrogenase consisted of: L-malic acid (0.25 M), and 5 mg DPN in 7 ml tris buffer (0.2 M, pH 7.4). The substrate solution for succinic dehydrogenase consisted of: 5 ml disodium salt of succinic acid (0.1 M) in 7 ml phosphate buffer (0.2 M, pH 7.3), and 3 ml nitro blue tetrazolium (2 mg/ml/H$_2$O).

The slides with affixed tissue were incubated at $25^\circ$C for 15 minutes for DPNH diaphorase and 30 minutes for malic, lactic, and succinic dehydrogenase. In the presence of appropriate tissue enzymes and added coenzymes, the tetrazolium is reduced to form a lightly colored insoluble formazan which precipitates at sites of enzyme in tissue (20). The mean formazan intensities were visually recorded from one to four plus.

In five experiments a serial biopsy study of the homografted heart was performed hourly to determine the time of onset and progression of transplantation rejection. The biopsies were taken from the anterior wall of the left ventricle at different sites with a Vim Silverman needle.
RESULTS

The metabolic data are represented in Tables I and II. It may be seen that the average myocardial extractions of glucose by the homografted heart were positive in every instance. In contrast, myocardial pyruvate balances were negative in almost every case. The myocardial lactate extractions were usually negative for the period immediately following the transplantation. After that they became positive until the 2nd week following transplantation when lactate was again released by the heart. The activity of both malic acid dehydrogenase and aldolase was usually greater in the coronary vein than in arterial blood during the whole period of observation. Values for the respiratory quotients of the transplant ranged from 0.62 to 1.9. It is likely, as shown later, that the high respiratory quotients observed in the grafts were the result of conversion of carbohydrates to fats.

The metabolic data obtained on the transplanted hearts which were rejected at an accelerated rate (second set rejection) are illustrated in Table III. The severity of the metabolic derangement in these hearts is illustrated by the frequent finding of negative myocardial glucose extractions. Aldolase as well as malic acid dehydrogenase were released by the heart.

### Table I

*Myocardial Balances (Transplanted Heart), Metabolic Data*

<table>
<thead>
<tr>
<th>Time after grafting</th>
<th>Number of observations</th>
<th>Glucose A-V</th>
<th>Pyruvate A-V</th>
<th>Lactate A-V</th>
<th>Malic dehydrogenase</th>
<th>Aldolase A-V</th>
<th>Redox potential ΔEh</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 minutes</td>
<td>10</td>
<td>7.70</td>
<td>-0.171</td>
<td>-0.002</td>
<td>-8.7</td>
<td>0</td>
<td>+9.30</td>
</tr>
<tr>
<td>1 to 2 days</td>
<td>11</td>
<td>11.55</td>
<td>-0.125</td>
<td>0.271</td>
<td>-63.3</td>
<td>-0.8</td>
<td>+2.10</td>
</tr>
<tr>
<td>3 to 4 days</td>
<td>14</td>
<td>6.92</td>
<td>-0.032</td>
<td>0.222</td>
<td>-1.2</td>
<td>-0.6</td>
<td>+2.66</td>
</tr>
<tr>
<td>5 to 6 days</td>
<td>16</td>
<td>10.82</td>
<td>-0.109</td>
<td>0.035</td>
<td>9.2</td>
<td>0.2</td>
<td>+5.35</td>
</tr>
<tr>
<td>7 to 8 days</td>
<td>14</td>
<td>7.20</td>
<td>-0.313</td>
<td>0.071</td>
<td>-5.9</td>
<td>-1.4</td>
<td>+6.85</td>
</tr>
<tr>
<td>9 to 10 days</td>
<td>7</td>
<td>9.78</td>
<td>-0.128</td>
<td>0.175</td>
<td>-20.6</td>
<td>-2.7</td>
<td>+5.56</td>
</tr>
<tr>
<td>11 to 12 days</td>
<td>7</td>
<td>8.26</td>
<td>-0.147</td>
<td>0.150</td>
<td>-32.2</td>
<td>-5.3</td>
<td>+9.35</td>
</tr>
<tr>
<td>13 to 14 days</td>
<td>5</td>
<td>9.15</td>
<td>-0.361</td>
<td>-2.820</td>
<td>-415.5</td>
<td>-16.5</td>
<td>+8.60</td>
</tr>
<tr>
<td>15 to 17 days</td>
<td>4</td>
<td>7.55</td>
<td>-0.233</td>
<td>-0.070</td>
<td>8.1</td>
<td>-5.2</td>
<td>+10.51</td>
</tr>
<tr>
<td>19 to 20 days</td>
<td>2</td>
<td>12.20</td>
<td>-0.416</td>
<td>-4.360</td>
<td>-292.0</td>
<td>-1.7</td>
<td>+7.10</td>
</tr>
<tr>
<td>22 to 24 days</td>
<td>2</td>
<td>12.60</td>
<td>-0.135</td>
<td>-0.855</td>
<td>-54.5</td>
<td>-4.0</td>
<td>+8.00</td>
</tr>
<tr>
<td>27 to 30 days</td>
<td>2</td>
<td>5.50</td>
<td>-0.214</td>
<td>-0.650</td>
<td>-130.0</td>
<td>-1.0</td>
<td>+11.50</td>
</tr>
<tr>
<td>35 to 44 days</td>
<td>3</td>
<td>3.56</td>
<td>-0.075</td>
<td>-0.280</td>
<td>-0.7</td>
<td>2.0</td>
<td>+5.9</td>
</tr>
</tbody>
</table>

Ranges ............. 1 to 41.9  

* A-V, Arteriovenous difference.
The injection of thiamine resulted in a significant diminution in average release of pyruvate by the transplant (from $-0.210$ to $-0.124$, $p < 0.02$); the respiratory quotient of the homograft increased significantly (from 0.79 to 1.55, $p < 0.05$).

Two dissimilar pathologic processes occurred in the homografted canine hearts. The most prominent lesion consisted of a diffuse granulomatous pan-carditis which was strikingly similar to the proliferative stage of acute rheumatic fever (21). The second lesion was acute necrosis of the myocardium when the heart remained viable longer than 8 days. The host canine hearts displayed no pathologic changes. The granulomatous infiltrate was first seen at 3 hours in the subepicardial and subendocardial myocardium and consisted of lymphocytes perivascularly. At 5 hours, plasma cells, macrophages, and histiocytes appeared. By 19 hours, Anitschkow-like cells and Aschoff-like giant cells were present in the infiltrate. The large differentiated mesenchymal cells similar to Anitschkow-like cells had a distinctive nuclear chromatin pattern. The chromatin was aggregated in the center of the oval nucleus of the cell. The Aschoff-like giant cells generally had two nuclei or a folded multilobular nucleus. The entire granulomatous reaction at 19 hours was round or oval and surrounded the vessel. By 20 to 21 hours a lymphocytic and plasma cell infiltrate was present

### TABLE II

**Myocardial Balances (Transplanted Heart), Physical Data**

<table>
<thead>
<tr>
<th>Time after grafting</th>
<th>Number of observations</th>
<th>Oxygen A-V</th>
<th>CO₂ A-V</th>
<th>R. Q.</th>
<th>Oxygen extraction ratio glucose</th>
<th>pH A-V</th>
<th>pCO₂ A-V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vol. per cent</td>
<td>vol. per cent</td>
<td>per cent</td>
<td></td>
<td>mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes</td>
<td>4</td>
<td>3.94</td>
<td>4.85</td>
<td>1.290</td>
<td>162.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 to 2 days</td>
<td>8</td>
<td>5.81</td>
<td>4.44</td>
<td>0.830</td>
<td>97.00</td>
<td>0.033</td>
<td>4.65</td>
</tr>
<tr>
<td>3 to 4 days</td>
<td>6</td>
<td>3.57</td>
<td>2.96</td>
<td>0.860</td>
<td>263.00</td>
<td>0.025</td>
<td>4.45</td>
</tr>
<tr>
<td>5 to 6 days</td>
<td>8</td>
<td>4.63</td>
<td>4.14</td>
<td>0.965</td>
<td>176.00</td>
<td>0.070</td>
<td>3.00</td>
</tr>
<tr>
<td>7 to 8 days</td>
<td>8</td>
<td>3.98</td>
<td>3.52</td>
<td>0.898</td>
<td>109.00</td>
<td>0.047</td>
<td>5.25</td>
</tr>
<tr>
<td>9 to 10 days</td>
<td>3</td>
<td>3.89</td>
<td>3.79</td>
<td>0.960</td>
<td>202.00</td>
<td>0.080</td>
<td>1.45</td>
</tr>
<tr>
<td>11 to 12 days</td>
<td>2</td>
<td>5.52</td>
<td>4.62</td>
<td>0.838</td>
<td>189.00</td>
<td>0.050</td>
<td>7.30</td>
</tr>
<tr>
<td>13 to 14 days</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15 to 17 days</td>
<td>4</td>
<td>4.67</td>
<td>4.85</td>
<td>0.925</td>
<td>109.00</td>
<td>0.032</td>
<td>5.17</td>
</tr>
<tr>
<td>19 to 20 days</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>22 to 24 days</td>
<td>1</td>
<td>4.83</td>
<td>4.79</td>
<td>0.990</td>
<td>154.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>27 to 30 days</td>
<td>2</td>
<td>5.35</td>
<td>5.31</td>
<td>1.040</td>
<td>71.50</td>
<td>0.025</td>
<td>6.75</td>
</tr>
<tr>
<td>35 to 44 days</td>
<td>1</td>
<td>5.71</td>
<td>3.43</td>
<td>0.600</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ranges</td>
<td>1.4 to 7.38</td>
<td>1.52 to 6.6</td>
<td>0.60 to 1.91</td>
<td>16.2 to 350</td>
<td>0.01 to 0.08</td>
<td>—1 to 7.1</td>
<td></td>
</tr>
</tbody>
</table>

The figures represent average observations.
between the myocardial fibers giving the appearance of interstitial myocarditis; this process increased with marked intensity from this period.

Hourly serial biopsy material demonstrated vividly the development of the pathologic changes occurring during the early period of homograft rejection. The earliest change occurred at 3 hours and consisted of a slight infiltration of lymphocytes which was adjacent to the arterioles (Fig. 1). There was a swelling of the endothelium of the small vessels at this time which remained fairly constant during the following 19 hours. By 5 hours a polar distribution of the cellular infiltrate was present adjacent to the arterioles. This lesion consisted of lymphocytes, plasma cells, macrophages, and histiocytes (Fig. 2). Biopsy specimens during the ensuing hours up to the 19th hour were essentially the same as seen in 5 hours, except that the infiltrate was more intense. A distinct change in the infiltrate occurred at 19 hours; at this time multinucleated cells resembling Aschoff giant cells and Anitschkow myocytes appeared in the granulomatous lesion (Figs. 3 to 5). This early change was consistent and no major pathologic changes were seen until the 8th day when there occurred an intensification of the granulomatous myocarditis.

In transplants which survived longer than 8 days one of the striking changes was the progression of the intensity of the swelling of the vascular endothelium. This reaction progressed rapidly to the 14th day of viability of the homograft when marked endothelial hyperplasia of the arterioles appeared (Fig. 6). At 8 days necrosis of the myocardium also was present and coexisted with the granulomatous myocarditis (Fig. 7).

Sections of the homografted canine heart which sustained accelerated rejection showed widespread necrosis as early as 4 hours after grafting (Fig. 8). An intense neutrophilic polymorphonuclear reaction was present in the areas of necrosis. In addition marked coronary arteriolar intimal proliferation and perivascular granulomatous infiltrate occurred. The larger branches of the coronary artery showed marked intimal proliferation; granulomatous infiltrate and necrosis of the adjacent myocardium was prominent (Fig. 9). All these findings occurred during the accelerated rejection in a period from 6 to 10 hours.

Scarlet R stains of the heart demonstrated an increase of neutral fat in the myocardium. Methyl green-pyronine stains of the donor dog heart demonstrated pyronophilic material in the cytoplasm of the cells comprising the granulomatous infiltrate. The plasma cells showed the most pyronophilia suggesting production of RNA by these cells (22).

In the histochemical studies the visual intensity of the formazan, indicating enzyme activity, was most intense in the DPNH diaphorase, less in the succinic and malic, and least in the lactic dehydrogenase. There was a significant reduction of malic dehydrogenase in sections of the donor hearts. The degree of cellular infiltration did not seem to affect the decrease of malic dehydrogenase; succinic and malic dehydrogenase could not be demonstrated in the cellular
infiltrate. The early morphological changes in these four enzymes consisted of coarsening of the granules of formazan within the myofibrils. The accentuation of granular size in form of "clumps" could be seen particularly in succinic dehydrogenase. The striking finding was that in spite of such morphologic enzyme changes the activity as demonstrated by the amount of formazan granules was not significantly reduced except in malic dehydrogenase.

Text-Fig. 1. Illustrates the relationship between the respiratory quotient and the percentage oxygen extraction ratio of glucose. It may be seen that as the respiratory quotient increases, the percentage oxygen extraction ratio of glucose also rises, suggesting that the increase in respiratory quotient is due to the conversion of glucose to fat.

DISCUSSION

The metabolic results illustrate that the homografted canine heart usually releases pyruvate and lactate as well as malic dehydrogenase and aldolase (Table I). As rejection is accelerated, the release of these substrates is more pronounced, and even glucose appears in increased concentration in coronary vein blood (Table III). In many experiments, the respiratory quotient of the heart is elevated (Table II). Text-fig. 1 illustrates that together with the respiratory quotient, the percentage oxygen extraction ratio of glucose increases; in most experiments the myocardial oxygen extraction ratio of glucose exceeds 100 per cent (Table II). It is likely that this is the result of conversion of carbohydrates to fat, which may also be responsible for the increased respiratory quotient of the graft. This diversion toward the fatty acid cycle may result from
a metabolic block into or within the tricarboxylic acid cycle. Thiamine hydrochloride appears to correct a block located at the level of cocarboxylase, because injection of thiamine results in many instances in an elevation in the respiratory quotient and a diminution of pyruvate released by the transplanted heart. As illustrated in Text-fig. 2, the difference in oxidation reduction potential between arterial and coronary vein blood of the graft ($\Delta E_h$) is positive and the glucose-

![Text-Fig. 2. Illustrates the relationship between $\Delta E_h$ and the percentage oxygen extraction ratio of glucose in the transplanted and the anoxic heart. $\Delta E_h$ is positive in the transplanted heart, indicating the absence of anoxia. The combination of high percentage oxygen extraction ratios of glucose and positive $\Delta E_h$ suggests the presence of aerobic glycolysis, particularly since lactate is released by the heart.]

...oxygen extraction ratio exceeds 100 per cent. The transplanted heart therefore glycolyzes in the presence of oxygen.

The relationship between metabolic disturbance as expressed by negative myocardial pyruvate balances and increased permeability illustrated by the release of enzymes is illustrated in Text-fig. 3. Negative balances of pyruvate are associated with increased release of malic acid dehydrogenase or aldolase. This finding suggests a causal relationship between enzyme loss and metabolic function.

The metabolic changes in the transplanted heart in which homograft rejection has been accelerated are more severe. Negative glucose, pyruvate and lactate balances and a considerable release of enzymes by the heart muscle are ob-
TEXT-Fig. 3. The relationship between myocardial balance of pyruvate and malic acid dehydrogenase. Negative pyruvate balances are accompanied by the release of the enzyme.

served (Table III). In two experiments, $AE_b$ is negative (Experiments 178 and 193 Table III), indicating the presence of myocardial anoxia. The oxidation reduction potential of tissue obtained from hearts in which rejection had been accelerated shows the same trend (oxidation reduction potential, $-285$); in ad-
dition, values for ATP and ADP in the myocardium are low in these specimens (Table IV). Glycolysis is present in several transplants (Experiments 178 and 193) as illustrated by the negative $\Delta E_h$ (Table IV) and release of lactate and pyruvate.

The histopathology of the homografted heart indicates that cardiac tissue is extremely antigenic and induces an immune response with marked rapidity. Evidence of tissue hypersensitivity is present within 3 hours after grafting; within 5 hours there is evidence of an immune response in the presence of perivascular island cells which have been described by Gell (23) and Waksman (24).

Waksman has recently compared the various types of delayed tissue hypersensitivity reactions. These are the tuberculin reaction, delayed allergic reactions in other bacterial, other than tuberculosis, mycotic and viral infections, homograft rejection, experimental auto-allergies, contact allergy, and delayed reactions to purified proteins. Gell identified three different pathogenic components of hypersensitivity lesions, which were designated as perivascular island reaction, vasculo-necrotic reaction, and plasma cell transformation. Waksman has added a fourth component, the specific histiocytic invasive destructive reaction. The perivascular island reaction and the specific histiocytic invasive destruction reaction were prominent features of each type of delayed tissue hypersensitivity reactions. These two types of reactions also are the prominent lesions in the homografted heart.

Therefore, the basic reaction which occurs in the homografted heart is the infiltration of lymphocytes, plasma cells, and histiocytes around vessels with an increase in the number of these cells by further accumulation or proliferation.

### TABLE III

**Myocardial Balances (Accelerated Reaction), Metabolic Data**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Time after grafting</th>
<th>Glucose A-V (mg per cent)</th>
<th>Pyruvate A-V (mg per cent)</th>
<th>Lactate A-V (mg per cent)</th>
<th>Malic dehydrogenase A-V (units/min)</th>
<th>Aldolase A-V (units/min)</th>
<th>Redox potential $Ae \Delta E_h$ (units/rain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>174</td>
<td>12</td>
<td>14.5</td>
<td>-0.737</td>
<td>-5.8</td>
<td>12</td>
<td>-17.5</td>
<td>+10.6</td>
</tr>
<tr>
<td>175</td>
<td>12</td>
<td>-6.6</td>
<td>-0.545</td>
<td>-3.99</td>
<td>-44</td>
<td>-14</td>
<td>+7.8</td>
</tr>
<tr>
<td>176</td>
<td>15</td>
<td>4.5</td>
<td>-0.185</td>
<td>-0.50</td>
<td>-50</td>
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<td>-30.7</td>
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The cells then invade the interstitial portions of the heart. A number of cells in the heart strongly resemble cells which compose the Aschoff nodule in acute proliferative rheumatic pancarditis. These cells are the Anitschkow-like cells and Aschoff type giant cells. The presence of these cells in the homografted heart is additional evidence of a hypersensitivity tissue immune response.

Tissue necrosis with associated polymorphonuclear neutrophilic infiltration may result from the histiocytic infiltration of the myocardium, as Waksman has suggested (24). Damage to the arterioles of the homografted heart may play an equal role. In primary rejection endothelial hyperplasia of the arterioles is seen approximately 14 days after grafting and this reaction is intensified as the graft remains viable. In the 47 day preparation arteriolar occlusion is one of the more prominent findings. This occurs secondary to extreme initial hyperplasia and hypertrophy, and necrosis of the myocardium results which coexists with the granulomatous myocarditis.

The experiments in which accelerated rejection was produced lend additional support to the concept that transplantation rejection is partially on a vascular occlusion basis. Prominent arteriolar endothelial proliferation with occlusion occurs with extreme rapidity in these hearts contributing to necrosis of the myocardium.

Reports from other laboratories have demonstrated that anoxia was probably present during transplantation, as necrosis appeared earlier than would have been expected (5, 7). Wesolowski and Fennessey (6) found that homografted canine hearts exhibited an initial "take" period of about 6 days. Evidence of necrosis appeared on the 3rd day. They ascribed this to both a time lag of establishing coronary circulation and obliteration of coronary circulation of the graft by an immune intolerance. The results presented in the preceding report indicate that anoxia does not exist in the homografted heart which undergoes primary rejection; the histological and histochemical studies support these metabolic data. Necrosis of the myocardium is present only after 8 days, and at this time occurs probably on the basis of transplantation immunity.

### TABLE IV

<table>
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<tr>
<th>Experiment No.</th>
<th>Lactate</th>
<th>Pyruvate</th>
<th>Glycogen*</th>
<th>Pi</th>
<th>ATP</th>
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<td>1060</td>
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* Glycogen determined as glucose.
The methyl green-pyronine stain indicates that the plasma cells in the infiltrate are actively producing or storing RNA which may represent antibody protein synthesized in the graft.

Histochemical reactions indicate a reduction of malic dehydrogenase in the myofibrils. This finding correlates with the results presented in the preceding paper which described malic dehydrogenase release by the heart. In addition, fat stains of the myocardium of the homografted heart demonstrated an increased amount of fat. The possibility of conversion of carbohydrates to fat has been discussed in the preceding report. It is possible that the finding of increase of fat in the heart muscle is the result of this process.

**SUMMARY**

The metabolic changes in the homografted canine heart were studied in order to define the biochemical alterations accompanying homograft rejection. In several experiments, homograft rejection was accelerated by prior sensitization of the host animal.

The homografted heart released pyruvate and lactate as well as malic dehydrogenase and aldolase. Extraction of glucose by the graft usually remained positive. During the accelerated rejection, the release of pyruvate and lactate was more pronounced, and even glucose appeared in increased concentrations in coronary vein blood. In many experiments the respiratory quotient of the transplanted heart as well as its glucose-oxygen extraction ratio were elevated.

It seemed likely that the elevated respiratory quotients were the result of conversion of carbohydrates to fat, since the injection of thiamine hydrochloride resulted in further elevation of the respiratory quotient and in an increased myocardial pyruvate extraction. Apparently, thiamine corrected a metabolic block at the level of the cocarboxylase.

The metabolic block or blocks present in the transplanted heart are likely to be the result of diminution in intracellular enzymes and coenzymes resulting from increased cellular permeability. The redox potential across the transplanted heart was positive, indicating the absence of anoxia. The results illustrate that glycolysis proceeds in the transplanted heart in the presence of oxygen.

Histopathologic and histochemical studies show the earliest lesion to be an accumulation of lymphocytes around vessels at 3 hours. Swelling of vascular endothelium occurs. By 5 hours a polar perivascular cellular infiltrate of lymphocytes, plasma cells, macrophages, and histiocytes exists. Changes following at 19 hours show the appearance of Aschoff- and Anitschkow-like cells. Granulomatous myocarditis which was first perivascular became interstitial with lymphocytic and histiocytic invasion of the myocardium.

After 8 days acceleration of swelling of vascular endothelium and granulomatous lesions were observed and necrosis of the myocardium was prominent.
Endothelial hyperplasia occurred at 14 days. In the accelerated reaction these changes were intensified and necrosis began as early as 4 hours after grafting.

Histochemical changes of DPNH diaphorase, lactic, malic, and succinic dehydrogenase showed only significant diminution of malic dehydrogenase in the cardiac muscle which was concurrent with the increase of this enzyme in the serum.

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**EXPLANATION OF PLATES**

**PLATE 70**

**Fig. 1.** Biopsy of homografted heart at 3 hours, demonstrating lymphocytic peri-
vascular infiltrate. Hematoxylin and eosin. × 235.

**Fig. 2.** Biopsy of homografted heart at 5 hours, demonstrating a polar distribution
of the perivascular infiltrate which consists of lymphocytes, plasma cells, macro-
phages, and histiocytes. Hematoxylin and eosin. × 235.

**Fig. 3.** Biopsy of homografted heart at 19 hours, demonstrating a perivascular cellular
infiltrate which consists of lymphocytes, plasma cells, macrophages, histio-
cytes, Aschoff giant cells, and Anitschkow cells. Hematoxylin and eosin. × 135.

**Fig. 4.** Higher magnification of biopsy of homografted heart at 19 hours, demon-
strating the perivascular islands of cells which have been enumerated in the explana-
tion for Fig. 3. Hematoxylin and eosin. × 400.
(Chiba et al.: Transplanted heart)
PLATE 71

Fig. 5. Higher magnification of biopsy of homografted heart at 19 hours, demonstrating the Anitschkow-like cells in the cellular infiltrate in the interstitial portions of the myocardium. These cells have a distinctive nuclear chromatin pattern. The chromatin is aggregated in the center of the oval nucleus of the cells. Hematoxylin and eosin. × 400.

Fig. 6. Section of homografted heart at 14 days, demonstrating endothelial hyperplasia of the arterioles. Granulomatous myocarditis exists both perivascularly and interstitially. Necrosis of the myocardium is present focally. Hematoxylin and eosin. × 235.

Fig. 7. Section of the homografted heart, demonstrating focal areas of necrosis of the myocardium which begins at 8 days. Granulomatous myocarditis coexists with the focal areas of necrosis. Hematoxylin and eosin. × 235.

Fig. 8. Section of the homografted heart which sustained accelerated rejection. Marked necrosis with polymorphonuclear neutrophilic cellular infiltration is present as early as 4 hours after grafting. Hematoxylin and eosin. × 135.
(Chiba et al.: Transplanted heart)
PLATE 72

Fig. 9. Section of the homografted heart which sustained accelerated rejection. A large branch of a coronary artery shows marked intimal proliferation. Perivascular granulomatous cellular infiltration and necrosis of the myocardium with polymorphonuclear neutrophilic cellular infiltration exists adjacent to the coronary artery. Hematoxylin and eosin. X 135.
(Chiba et al.; Transplanted heart)