cally related, presumably both (B_2 and B_3) viruses need the same energy sources. Also, potassium may be necessary for penetration of virus into cells. This possibility was not investigated in the present study, but Levine *et al.* have evidence that potassium may be necessary for effective penetration of influenza (PR8) virus into chick cells and for intracellular synthesis of new virus. This evidence is lacking for the role of potassium in growth of Coxsackie B_2 virus.

Summary. Potassium ion augments the amount of infectious Coxsackie B_2 virus produced from MKC and MTC. Production of infectious Coxsackie B_2 virus was reduced 500 to 2,000 times in MKC and MTC by depletion of potassium from their medium. Similar results were not obtained with Coxsackie B_3 virus. Potassium was not required for virus attachment, but did stimulate glycolysis of tissue culture cells. Potassium could be replaced in a deficient medium after 12 hours of infection and still obtain maximal yields of infectious virus in MTC.

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Catecholamines in Homologous Heart Grafts.* (27262)

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Goodall and Kirschner(1) reported that cervicothoracic ganglionectomy in dogs results in a considerable decrease of the cardiac content of norepinephrine and epinephrine. However, since these catecholamines were not entirely depleted by this procedure, the possibility arose that a fraction of cardiac catecholamines could be stored in other than sympathetic structures, thus remaining unaffected by surgical denervation. Studies(2,3) on cardiac sensitivity to epinephrine after similar denervation yielded contradictory results, probably because complete sympathetic denervation is difficult to accomplish. More recently Cooper, Gilbert, Bloodwell and Crout (4) described a technic for ablation of all known autonomic structures in the mediastinum in dogs. They found a depletion of 50% of the catecholamine content in the denervated hearts within 1 to 2 days. Almost complete loss of ventricular catecholamines was noted on the third day after operation.

The present report concerns a study of the rate of catecholamine depletion in heart in which denervation was complete. This was accomplished by transplanting the heart into the neck of adult mongrel dogs and determining its catecholamine content at certain intervals after this operation. Wegmann and Kako(5) have reported that 74% of the catecholamines in the heart homogenates of puppies were found in the sediment obtained by subsequent low and high speed centrifugation. The present paper includes results on the possible influence of transplantation on distribution of catecholamines in the cell fractions of heart homogenates.

Technic. Twenty-seven puppy hearts were transplanted according to the technic previ-

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ously described(6). The aorta of the donor heart was anastomosed to the common carotid artery of the recipient, and the pulmonary artery of the donor heart was joined to the jugular vein of the recipient. The recipient dogs were sacrificed from 1 to 16 days after this procedure and the catecholamines were determined in the tissue of the left ventricle (8 to 12 g) of the heart grafts.

In 4 experiments, the catecholamine content of the heart grafts was determined in cell fractions obtained by differential centrifugation(5) 24 hours after transplantation. The tissue was homogenized in an Elvehjem-Potter homogenizer in ice-cold physiological sodium chloride solution. The homogenates were first centrifuged at 600-1000 g during 10 minutes. The supernatant fraction was free from microscopically visible cell particles and was submitted to centrifugation at 50,000-100,000 *g* in a Spinco-Ultracentrifuge during 60 minutes. The sediment resulting from this procedure contains catecholamines bound to submicroscopical cell structures ("granula") (5,6,7). The supernatant fraction obtained by high speed centrifugation contains catecholamines which are not bound to any known particulate cell fraction(6). The catecholamines of this soluble fraction are possibly released from their particulate store elements during the procedure of fractionation. To avoid loss of catecholamines in the particulate fraction due to influence of temperature, the whole procedure was carried out at 2-4°C(5,6,7). The results were plotted against repartition figures earlier reported(5) for 7 heart homogenates of untreated puppies.

In another 4 experiments, in which the effect of accelerated rejection of the graft on catecholamine content was studied, a splenic cell suspension of the donor animal was injected intraperitoneally into the prospective recipient dog 6 days prior to transplantation (8). Under these conditions the grafted heart usually does not survive more than 20 hours. The transplanted hearts were removed 16 to 20 hours after operation for catecholamine determination.

Catecholamines were determined according to the fluorimetric method of Euler and Lishajko(9). In this study norepinephrine and epinephrine were not determined separately. For fluorescence measurements 2 filters with light transmission at 412 mμ and 508 mμ respectively were used in a Farrand photofluorometer. Since it had been found(10) in this laboratory that the cardiac catecholamine concentration varies inversely with the heart weight, the results from this series were plotted against values previously obtained in the hearts of 7 puppies of the same weight range (3-5 kg).

TABLE I. Catecholamine Content of Heart after Homologous Transplantation. (Units = μg/g tissue.)

	Days after transplantation			
	0	1	2	3
Normal hearts	.78 ±.25 n = 7	—	—	—
Transplanted hearts	—	.87 ±.07 n = 7	.12 ±.19 n = 5	<.03 n = 5
Idem, accel. immun. R.	—	.78 ±.44 n = 4	—	—

Results. Changes in the catecholamine content of homologous heart grafts are shown in Table I. No loss of catecholamine content was detected in the grafts 24 hours after transplantation. However, 48 hours after transplantation the catecholamine content of the grafted hearts was sharply reduced. On and after the third day following operation no catecholamines could be detected in the grafts.

Analysis of catecholamines in the fractions obtained by subsequent low and high speed centrifugation of heart homogenates 24 hours after transplantation showed no changes ($p < .001$) as compared with the values found in the fractions of tissue homogenates of hearts *in situ* (Table II).

Table I illustrates that accelerated rejection of the homograft did not result in diminution of the catecholamine content of the heart from 16 to 20 hours following transplantation. These hearts showed severe cellular damage in histologic and biochemical studies(8).

Discussion. The results reported here illustrate that catecholamine content of the completely denervated transplanted puppy heart remains unchanged during the first 24

TABLE II. Catecholamines in Cellular Fractions of Puppy Hearts.

	Fraction I Supernatant (800 × *g*)	Fraction II Sediment (75,000 × *g*)	
	μg/g homog.	μg/g homog.	% of I
Normal hearts *in situ** n = 6	.46 ± .11	.34 ± .06	74
Transplanted hearts n = 4	.41 ± .12	.29 ± .05	71

* Wegmann, A., Kako, K.(5).

hours after operation. Intracellular distribution of catecholamines remains unchanged during the first 24 hours after operation. Intracellular distribution of catecholamines remains equally unaffected. After 24 hours however the cardiac catecholamines disappear and none can be detected after 72 hours.

These results are in accord with the findings of Cooper, Gilbert, Bloodwell and Crout (4) who noted depletion of catecholamines 72 hours after regional ablation of the autonomic structures in the mediastinum of dogs. It is probable that previous failure to induce a complete loss of cardiac catecholamines by sympathetic ganglionectomy(1) has been due to incomplete sympathetic denervation of the heart.

The question whether the rapid loss of catecholamines in the graft after the first 24 hours following transplantation could have been influenced by the rejection phenomena was answered as follows: In 4 experiments accelerated rejection of the transplant was produced by prior injection of homogenate of spleen cells from the donor (Table I). This invariably resulted in widespread necrosis of the myocardium which became apparent as early as 8 hours following transplantation; as a result these transplants did not survive longer than one day. The catecholamine concentration of these hearts rejected at an accelerated rate was approximately the same as that of the control or of the one-day transplants which were not subjected to accelerated rejection (Table I). The results, therefore, demonstrate that accelerated rejection *per se* does not result in diminution of catecholamine concentration in the transplanted heart.

Since it has been shown by Wegmann and Kako(5) by differential centrifugation that catecholamines in dog hearts are stored in submicroscopical structures it seemed possible that, within the first 24 hours and prior to depletion of catecholamines, these substances could have been released from their particle bound storage sites without having been metabolized or released from the graft. However, 24 hours after transplantation, determination of the catecholamine content in the ultracentrifuged fractions of 2 cardiac graft homogenates did not demonstrate any change in distribution of the amines as compared with the partition found in normal puppy heart homogenates(5).

Summary. Complete denervation of puppy hearts was accomplished by homologous transplantation. Cardiac content and intracellular distribution of catecholamines remained unaffected the first 24 hours following transplantation. Within this time period accelerated rejection did not influence the catecholamine content of the heart graft. After 72 hours no catecholamines could be detected in transplanted heart.

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