These data suggest that the bees were remembering the spatial relationships of the elements in each pattern. This alternative is consistent with the results of experiments H_1 and H_2, in which bees had essentially the same degree of difficulty learning to distinguish two pairs of spatially complex patterns, even though prominent parameters of the H_1 pair (vertical and horizontal versus diagonal boundaries) were quite different, whereas in H_2 they were the same. The difficulty that bees experience by learning to distinguish spatially complex patterns is not likely to be a consequence of their poor visual resolution: measurements from the distance at which bees appear to "study" and choose between the patterns in these experiments indicate that a minimum of 50 ommatidia in each eye would be directed at each segment of the most complex pattern (J) that bees were able to learn; even for patterns that bees cannot distinguish after 25 trials (those in experiment K, for instance), half that number would be involved. More likely, perhaps, is that the limiting factor is the resolution of the eidetic storage in the bee's brain.

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8. The training procedure used here is different in two important ways from that used in most other studies: the patterns were displayed vertically, and the S^- was presented simultaneously. In nature, of course, few flowers are horizontal, and S^- abounds.
9. A "choice" consisted of a bee flying into a funnel and landing. When a trained forager found no food, it almost always flew out (the normal behavior observed on nectar-depleted blossoms), inspected both flowers by hovering in front of them, and then landed again. When, as occasionally happened, a bee took off, hovered briefly in the same flower, and then landed again, the second landing was not counted as a choice since the bee had not examined the alternative pattern.
10. The effect of an early stopping rule like the one used here in experiments A through G is to increase the overall chance of a false positive. By means of a 20-million event Monte Carlo simulation, I determined that the net effect was an increase of 2.37. The summed probabilities in Fig. 1 were revised to compensate for this effect. The summed probabilities in all the experiments are based on the assumption, usual to work on bee learning, that each landing is an independent choice. The validity of this assumption was tested in two ways. First, four of the experiments (B_2, H_2, I_1, and J_1) were repeated with a testing protocol allowing bees to land only once on each return. The data from these tests were compared to the data obtained from the blocks-of-five protocol used here; no difference was noticeable. (For experiment B_2, the summed single-visit-protocol data were 58 S^+/2 S^- versus 56 S^+/4 S^- for the five-block-protocol data; for H_2 the results were 79 S^+/21 S^- versus 79 S^+/21 S^-; for I_1 the results were 77 S^+/23 S^- versus 74 S^+/26 S^-; for J_1 the results were 62 S^+/38 S^- versus 65 S^+/35 S^-.) Second, I compared the choice patterns within and between blocks of test for all 22 experiments reported here as well as for 66 similar experiments. I asked whether sequential choices of the same pattern within a block were either more or less common than would be expected on the basis of the overall choice ratio, whether the first choice in each block was more or less likely to be correct than the second, third, fourth, or fifth, and whether within-block variance was either larger or smaller than between-block variance. Each of these comparisons indicated that choices within a block were indistinguishable for choices on separate visits, and thus can be treated as essentially independent.
11. I thank C. G. Gould, K. M. Schenck, and A. Weisenberger for building the testing apparatus and G. Watson for statistical advice. Supported by NSF grant BNS 82-01004.

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Regional Myocardial Substrate Uptake in Hypertensive Rats: A Quantitative Autoradiographic Measurement

Abstract. *Severe hypertension causes global and regional changes in myocardial perfusion and substrate utilization. Regional perfusion and fatty acid utilization were evaluated by dual-tracer autoradiography in normotensive and hypertensive rats of the Dahl strain. The regional distributions of perfusion and fatty acid utilization were homogeneous in normotensive rats. Severe hypertension was associated with a homogeneous pattern of regional perfusion, but fatty acid utilization was focally decreased in the free wall of the left ventricle. The decrease in fatty acid uptake was associated with a concomitant increase in glucose utilization. These findings suggest that severe hypertension is associated with uniform myocardial perfusion and focal alterations in the substrates used for the performance of myocardial work.*

Increased myocardial work caused by severe hypertension leads initially to myocardial hypertrophy and, if untreated, to heart failure (*1*). Although the hypertrophic myocardium has a proportional increase in myocardial perfusion, capillary density is decreased and the myocardial perfusion reserve is abnormal (*2*, *3*). The energy requirements of the normal heart in the basal state are usually met by catabolism of free fatty acids (*4*), with minimal contributions by lactate and glucose. The substrates used to provide energy during persistent increased myocardial work, such as occurs in prolonged hypertension or aortic stenosis, are less well defined. When the left ventricle is called upon to perform increased pressure work, the myocardium may not respond uniformly. In this circumstance, the effects on endocardium versus epicardium and on the free wall versus the septum of the left ventricle may differ (*5*, *6*). We investigated the possibility that persistent increases in pressure work are associated with alterations in the catabolism of metabolic substrates in hypertensive rats. Using quantitative dual-tracer autoradiography, we evaluated the regional distribution of glucose and free fatty acid analogs and correlated the results with changes in regional perfusion.

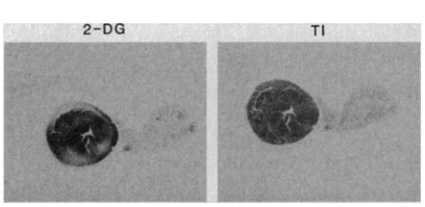

Fig. 1. Dual-tracer autoradiograph of a selected midventricular slice of myocardium from a hypertensive rat given injections of thallium-201 (Tl) and 2-deoxyglucose (2-DG) labeled with carbon-14. The thallium distribution is uniform, whereas glucose uptake is increased in the endocardium.

The Dahl strain of rats, derived from common Sprague-Dawley ancestors, have differing genetic predispositions to experimental hypertension (*7*). Originally, genetic separation was achieved on the basis of divergent blood pressure responses to ingested salt (NaCl). The hypertension-prone animals develop fatal hypertension from salt intakes to which hypertension-resistant animals respond mildly, if at all. Rats used in our study were weaned when they were 3 weeks old. Normotensive rats received 0.4 percent NaCl and hypertensive rats received 8 percent NaCl by weight in their food for 5 weeks. At age 11 weeks, the blood pressure of the rats was measured by tail cuff; the animals were weighed; and the regional myocardial utilization of substrate was determined. The mean systolic blood pressure of the hypertensive rats was 209 ± 13 mmHg, and their mean body weight was 228 ± 11 g. Normotensive rats had a mean

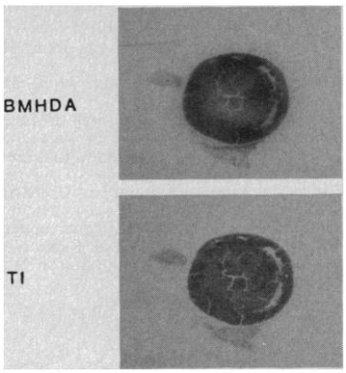

Fig. 2. Dual-tracer autoradiograph of a selected midventricular slice of myocardium from a hypertensive rat. Thallium-201 (Tl) was used to measure regional perfusion and carbon-14 was used as a label for β-methyl heptadecanoic acid (BMHDA) to measure the regional utilization of fatty acids. The thallium distribution is uniform, whereas the distribution of the fatty acid decreased in the endocardium and free wall.

systolic blood pressure of 131 ± 5 mmHg and a mean body weight of 227 ± 11 g.

Regional myocardial perfusion was assessed with ^{201}Tl-labeled thallium chloride. Regional glucose concentration was measured with 2-deoxy-D-[U-^{14}C]glucose ([^{14}C]DG) (*8*) or [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) (*9*). Regional fatty acid incorporation was studied with the use of the branched chain fatty acid β-methyl heptadecanoic acid labeled at the 1 position ([^{14}C]BMHDA) (*10*). The metabolic analogs enter catabolic pathways, proceed through a committed step, and remain in situ for an extended time. The regional concentrations of tracers in the myocardium were determined by quantitative dual-tracer autoradiography on 20-μm-thick frozen sections of the left ventricle cut in the direction perpendicular to the long axis of the left ventricle. The exact metabolic rate of glucose or fatty acid utilization was not measured in these experiments, but rather the regional concentration of each substrate in the tissue was quantified. The animals were given free access to a standard rat diet up to the time of the study. Three different experiments were conducted on a total of 23 rats.

1) Regional fatty acid utilization and regional myocardial perfusion were compared in eight rats (four normotensive and four hypertensive). The rats were first injected with 12.5 μCi of [^{14}C]BMHDA, then 10 minutes later with 500 μCi of ^{201}Tl. They were killed 5 minutes after the second injection.

2) Regional glucose utilization and regional perfusion were compared in ten rats (five normotensive and five hypertensive). The rats were first injected with 12.5 μCi of [^{14}C]DG, then 40 minutes later with 500 μCi of ^{201}Tl. The rats were killed 5 minutes after the second injection.

3) Regional glucose and fatty acid utilization were compared in five rats (two normotensive and three hypertensive). The rats were first injected with 3 mCi of [^{18}F]FDG, then 30 minutes later with 12.5 μCi of [^{14}C]BMHDA. The animals were killed 15 minutes after the second injection. All of the injections in the three experiments were given intravenously.

Immediately after the animals were killed, their hearts and lungs were removed, frozen in liquid nitrogen, and processed for quantitative dual-tracer autoradiography as described earlier (*11*). The tissue sections and graded standards were placed on x-ray film (*12*) for exposure.

The initial autoradiographic exposures showed the short-lived tracers (exposure times: for ^{201}Tl, 6 hours; for [^{18}F]FDG, 3 hours). Imaging of the second (longer-lived) tracer was initiated when ten half-lives of the short-lived tracer had elapsed. The ^{14}C-labeled tracers required approximately 2 weeks for adequate film exposure.

The autoradiographs were digitized and quantified with a videodensitometry film analysis system (*13, 14*). The center of the left ventricle was identified on each slice. The endocardial region was delineated as the inner half of the myocardium defined along a series of radial segments. The results for segments involving the septal region and the free wall were summed for quantitative comparisons.

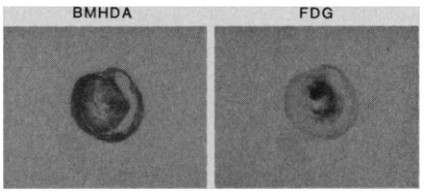

Fig. 3. Dual-tracer autoradiograph of a selected midventricular slice of myocardium from a hypertensive animal. β-Methyl heptadecanoic acid (BMHDA) labeled with carbon-14 was used to measure regional fatty acid utilization and 2-fluorodeoxyglucose (FDG) labeled with carbon-14 was used to measure regional glucose utilization. The areas of decreased fatty acid deposition correspond to the zones of increased glucose utilization.

Dual-tracer autoradiographs comparing myocardial perfusion and substrate (thallium-glucose, thallium–fatty acid, and glucose–fatty acid) in hypertensive rats are shown in Figs. 1 to 3. Myocardial perfusion, as indicated by ^{201}Tl distribution, was homogeneous, whereas accumulation of the glucose analog [^{14}C]DG was heterogeneous in the myocardium of hypertensive rats, with greater uptake in the endocardium and left ventricular free wall (Fig. 1). In contrast, the fatty acid analog [^{14}C]BMHDA showed decreased uptake in the endo-

Table 1. Quantitative autoradiographic data from three experiments on 17 rats (three normotensive and three hypertensive rats in experiment 1; three normotensive and three hypertensive rats in experiment 2; and two normotensive and three hypertensive rats in experiment 3). Values are means ± standard error except in experiment 3, for which only mean values are given. LV and RV refer to left and right ventricles, respectively.

Region	Uptake of label in			
	Normotensives	Hypertensives	Normotensives	Hypertensives
	Experiment 1			
	[^{14}C]*BMHDA (nCi/g)*		^{201}*Tl (ratio to LV epicardium)*	
RV	473 ± 89	320 ± 15	0.83 ± 0.08	1.04 ± 0.03
Septum	558 ± 79	378 ± 21	1.22 ± 0.04	1.09 ± 0.005*
LV Endocardium	570 ± 105	278 ± 5*	1.12 ± 0.04	0.97 ± 0.03
LV Epicardium	548 ± 91	329 ± 2*		
	Experiment 2			
	[^{14}C]*DG (nCi/g)*		^{201}*Tl (ratio to LV epicardium)*	
RV	60 ± 23	263 ± 53*	1.00 ± 0.06	0.90 ± 0.08
Septum	135 ± 63	633 ± 122*	1.22 ± 0.08	1.22 ± 0.04
LV Endocardium	112 ± 36	649 ± 146*	1.15 ± 0.02	1.11 ± 0.04
LV Epicardium	57 ± 17	411 ± 143*		
	Experiment 3			
	[^{14}C]*BMHDA (nCi/g)*		[^{18}*F]FDG (ratio to LV epicardium)*	
RV	580	211	1.14	0.93
Septum	651	206	1.07	3.37
LV Endocardium	581	203	1.05	1.67
LV Epicardium	622	270	1	1

*$P < 0.05$ compared to values for normotensive rats.

cardium and free wall of the myocardium of hypertensive animals, although myocardial perfusion was homogeneous (Fig. 2). Dual-tracer studies with the glucose analog [^{18}F]FDG and the fatty acid analog [^{14}C]BMHDA showed a complementary distribution of the two agents in the myocardium of hypertensives (Fig. 3). These data suggest that zones of decreased fatty acid uptake are associated with increased glucose uptake.

Quantitative data were available in 17 animals (Table 1). As suggested by visual inspection of the images, the distribution of perfusion was homogeneous in the myocardium of both the normotensive and hypertensive animals. The [^{14}C]DG uptake (expressed as nanocuries per gram, after normalizing for the administered dose) was much lower than the fatty acid uptake in the myocardium of normotensive animals. The ratio of fatty acid to glucose was 9.4 in the right ventricle, 5.0 in the septum, 5.5 in the endocardial region of the left ventricle, and 10 in the epicardial region of the left ventricle. In contrast, glucose uptake was higher than fatty acid uptake in the same regions of myocardium of hypertensive animals. The ratio of fatty acid to glucose was 1.2 in the right ventricular free wall, 0.5 in the septum, 0.40 in the endocardium of the left ventricle, and 0.78 in the epicardium of the left ventricle.

Since the animals were fed the same diet, it is unlikely that a difference in circulating glucose or fatty acid concentration could account for these differences. These data suggest that substrate use is altered in prolonged severe hypertension before ischemia occurs. It is uncertain whether this is the result of a decrease in the ratio of capillaries to sarcomeres or of a defect in membrane transport, energy production, or energy utilization.

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Alterations in L-Glutamate Binding in Alzheimer's and Huntington's Diseases

Abstract. *Brain sections from patients who had died with senile dementia of the Alzheimer's type (SDAT), Huntington's disease (HD), or no neurologic disease were studied by autoradiography to measure sodium-independent L-[^{3}H]glutamate binding. In brain sections from SDAT patients, glutamate binding was normal in the caudate, putamen, and claustrum but was lower than normal in the cortex. The decreased cortical binding represented a reduction in numbers of binding sites, not a change in binding affinity, and appeared to be the result of a specific decrease in numbers of the low-affinity quisqualate binding site. No significant changes in cortical binding of other ligands were observed. In brains from Huntington's disease patients, glutamate binding was lower in the caudate and putamen than in the same regions of brains from control and SDAT patients but was normal in the cortex. It is possible that development of positron-emitting probes for glutamate receptors may permit diagnosis of SDAT in vivo by means of positron emission tomographic scanning.*

Senile dementia of the Alzheimer's type (SDAT) and Huntington's disease (HD) are among the chronic degenerative neurologic disorders that affect memory. The symptoms of SDAT, which is a relatively common disease, often resemble those seen in cortical disconnection syndromes; clinical signs of cortical dysfunction, the agnosias and apraxias, occur frequently (*1*). Pathologically, the SDAT brain is atrophic; the characteristic histological findings are numerous neuritic plaques and neurofibrillary tangles in the cortex and hippocampus (*1*). In the cerebral cortex of the SDAT brain, there are decreases in cholinergic markers (*2*), catecholamines, and somatostatin (*3*). In contrast to SDAT, HD appears to be a subcortical dementia (*4*). Pathologically, there is a loss of neurons in the caudate and putamen, and many neurotransmitter systems appear to be affected (*5*).

Glutamate is the putative neurotransmitter of both intracortical association fibers and cortical efferents to many subcortical structures, including the caudate and putamen (*6*). Because glutamate and some of its analogs are neurotoxic, it has been proposed that abnormalities in glutamate neurotransmitter function may play a causal role in neurodegenerative disorders such as HD and olivopontocerebellar atrophy (*7*). By autoradiography, we examined one aspect of the glutamatergic system—namely, the glutamate receptor (*8*)—in sections of human brains obtained post-mortem.

The brains from a series of patients who had died with SDAT, HD, or no

Table 1. Affinity constants and numbers of high- and low-affinity quisqualate binding sites (picomoles per milligram of protein) in brains from control and SDAT patients. Measurements were made by autoradiography as described (*8*); values are means ± standard error of mean; $n = 5$ for both groups.

Donor	Affinity constant		Binding sites	
	High affinity (n*M*)	Low affinity (μ*M*)	High affinity	Low affinity
Control	21 ± 11	148 ± 52	1.82 ± 0.37	3.39 ± 0.50
SDAT	75 ± 29	277 ± 144	1.38 ± 0.21	1.46 ± 0.19*

*$P < 0.01$ (independent t-test).