FOLLOWING THE INTRODUCTION of the intra-arterial $^{133}$Xenon injection technique for measuring blood flow in man, a number of investigations were published in which blood flow and the regulation of flow were studied in patients with brain tumors.1-7 There was general belief that there were disturbances of both flow and flow regulation, but the individual changes were not foreseeable, and there was no strict correlation with the type, the size and the localization of the tumor.1-6-8-10 The results obtained were apparently influenced by brain edema which is a frequent accompaniment to tumor development. However, the mutual interference between blood flow and edema development could not be clearly established because of the inherent problems in performing such studies in patients. Interest developed in experimental models in which the hemodynamics of cerebral edema could be investigated under controlled conditions. Since brain tumors were difficult to produce in animals, other pathological conditions associated with the development of edema such as cold injury or water intoxication, were used for this purpose.12-16 The results obtained indicated that edema leads to a local increase in tissue pressure, to microcirculatory compression and, consequently, to ischemia and disturbances of flow regulation.17 Although these findings seem to substantiate the clinical observations in patients with brain tumor, this relationship may be coincidental because neither flow nor vascular resistance changed in consequence of significant intracranial hypertension, even severe degrees of vasogenic peritumoral edema do not interfere with blood flow and flow regulation. This is in contrast to the cytotoxic type of edema, and indicates that microcirculatory compression by edema, when present, is the consequence of pericapillary glial hydrops and not of an accumulation of extravasated edema fluid.

SUMMARY In 27 pentobarbital-anesthetized cats cerebral blood flow and regulation of cerebral blood flow was measured one to 3 weeks following stereotactical xenotransplantation of a rat glioma clone into the internal capsule. Tumor growth was accompanied by severe vasogenic peritumoral edema in the white matter of the tumor-bearing hemisphere. White matter water content in the vicinity of the tumor increased from 69.1 ± 0.9 to 0.5 ± 0.7 ml/100 g wet weight (means ± se) which corresponds to an increase in tissue volume of about 60%. Intracranial pressure after 3 weeks was 12 ± 2.6 mm Hg. Blood flow in the peritumoral white matter decreased from 32.2 ± 5.6 to 16.6 ± 1.9 ml/100 g/min but it did not change in the peritumoral grey matter or the opposite hemisphere. The decrease in blood flow was due to the volume expansion of the swollen edematous tissue and not to a compression of the microcirculation because neither flow nor vascular resistance changed when referred to dry rather than to wet weight of the edematous tissue. Flow regulation in the peritumoral edematous white matter was not disturbed. CO$_2$ reactivity of blood flow was 5.4%/mm Hg change in pCO$_2$ (non-edematous contralateral white matter 6.4%/mm Hg), and the autoregulatory capacity between 40 and 170 mm Hg was 0.7%/mm Hg (non-edematous white matter 1.0%/mm Hg). It is concluded that in the absence of significant intracranial hypertension, even severe degrees of vasogenic peritumoral edema do not interfere with blood flow and flow regulation. This is in contrast to the cytotoxic type of edema, and indicates that microcirculatory compression by edema, when present, is the consequence of pericapillary glial hydrops and not of an accumulation of extravasated edema fluid.

Material and Methods

Tumor Implantation. Twenty seven cats were used. The animals were anesthetized with 30 mg/kg pento-barbital (intraperitoneal injection of Nembutal) and placed in a stereotaxic instrument. Under sterile conditions a skin incision and a small burrhole were made over the left hemisphere at Horsley-Clark coordinates anterior 18 mm and lateral 8 mm. Four million cells of the rat glioma clone RG2 (courtesy of Prof. Wechsler, Dusseldorf) were suspended in 40 $\mu$l 4% DMEM medium, and injected into the left internal capsule at Horsley-Clark coordinates anterior 18 mm, lateral 8 mm, height + 3 mm, using a microsyringe attached to a micromanipulator. The number of cells was determined in a cell counter (Coulter Electronics, Krefeld) and corrected for viability loss by estimating the percentage of vital cells with a phase microscope.

Details of the biological preparation of the tumor clone and the preparation of cell suspensions have been published.16 Following tumor cell implantation,

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the animals were allowed to recover, and kept for 1 to 3 weeks.

Analytical Methods. One to 3 weeks after tumor implantation, the animals were again anesthetized with 30 mg/kg pentobarbital, immobilized with gallamine triethiodide (Flaxedil) and placed under mechanical ventilation with room air. Blood pressure, blood gases and body temperature were maintained within physiological limits. Catheters were inserted into a femoral vein, a femoral artery and into the left ventricle of the heart via the brachial artery. The correct position of the cardiac catheter was controlled by recording intraventricular pressure. A needle was also placed into the cisterna magna for recording of intracranial pressure. Blood flow was measured using the intracardiac microsphere injection technique. Microspheres, 1.5 to 3 million, labelled with "Scandium, Cerium and Ruthenium, respectively, were suspended in 2 ml 10% dextran using a sonifier, and injected in 10 sec into the left ventricle of the heart. Starting 10 sec before injection of the microspheres and continuing for 1 min, arterial blood was withdrawn from the femoral artery at a calibrated speed of 3 ml/min. Radioactivity of the blood sample was used to calculate tissue blood flow (see below).

In 6 animals surviving 3 weeks after the implantation, regulation of blood flow was studied. CO2 reactivity was tested by adding 6% CO2 to the respiration air, and autoregulation by increasing or decreasing blood pressure with norfenefrin (Novadral) or camphor sulfonate (Arfonad), respectively. Flow measurements in these animals were made before, during and after changing aPco2 or blood pressure, using differently labelled microspheres. During each flow measurement arterial blood was sampled for determination of blood gases, and cerebral perfusion pressure was calculated by subtracting mean cisternal pressure from mean arterial blood pressure.

Fifteen min before the end of the experiment, 1 ml/kg 2% Evans blue was injected intravenously for assessment of blood-brain barrier permeability. Animals were subsequently killed by air embolism, the brains were quickly removed and tissue samples weighing 150 to 250 mg were dissected in a humid chamber from the tumor, grey and white matter adjacent and distant to the tumor, and from homotopic regions of the contralateral hemisphere. The samples were dried at 100°C for determination of water content, and subsequently assessed for microsphere radioactivity, using a 3-channel scintillation counter (Biogamma II, Beckman, Fullerton, CA). When multiple blood flow measurements were performed, interchannel spillover was corrected by standard cross-over techniques. Regional blood flow (rCBF) was calculated from the equation

$$rCBF = \frac{R_t \times F_b \times 100}{W_t \times R_b} \text{ ml/100 g/min}$$

where $R_t$ is tissue radioactivity, $W_t$ tissue weight, $F_b$ the flow rate of blood sampling (ml/min) and $R_b$ blood radioactivity.

Results

In 22 of 27 animals, xenotransplantation of the rat glioma clone RG2 into the cat brain resulted in the growth of a solid tumor which, within 3 weeks, reached a diameter of about 10 mm (fig. 1). Microscopically, the tumor cells had a uniform appearance and resembled anaplastic astrocytes with numerous mitoses. The tumor stained deeply with Evans blue, indicating that the tumor vessels did not present a barrier for circulating macromolecules.

In the peritumoral white matter, severe brain edema developed. As has been described before, brain edema was of the vasogenic type and was mainly due to extravasation of plasma ultrafiltrate which spread from the tumor vessels into the intercellular spaces of the adjacent brain tissue. In contrast to the tumor, the blood-brain barrier was not severed in the edematous white matter, as evidenced by the absence of staining with Evans blue.

Water content in the white matter increased during the observation period of 3 weeks from 69.1 ± 0.9 to 80.5 ± 0.7 ml/100 g wet weight, corresponding to a volume increase of about 60%. In the more distant parts of the ipsilateral hemisphere, the increase was less pronounced and reached a maximum value of only 76 ml/100 g wet weight within 3 weeks. In the contralateral hemisphere and in the grey matter of both the ipsilateral and the contralateral hemisphere water content did not change significantly (fig. 2).

Although considerable, brain swelling during the observation period did not cause a significant increase in intracranial pressure. After 3 weeks cisternal pressure was only 12 ± 2.6 mm Hg, indicating that within this period edema was not complicated by intracranial hypertension.

Blood flow in the edematous tissue nevertheless markedly decreased. Within 3 weeks it fell from 32.2 ± 5.6 to 18.6 ± 1.9 ml/100 g/min in the adjacent and to 21.8 ± 2.8 ml/100 g/min in the more distant regions of the ipsilateral white matter. In the non-
ischemic contralateral hemisphere and in the grey matter changes were not significant, indicating that the decrease in flow was actually due to edema development and not to general changes of cerebral perfusion pressure (fig. 2).

This was confirmed by plotting the water content of the white matter against the regional flow rate, which revealed a significant inverse relationship between the two parameters (fig. 3). Water content also correlated with vascular resistance but there was a considerable scatter despite the fact that blood flow and water content were measured in the same tissue sample.

These findings suggest a compression by vasogenic edema of the microcirculation, as has been suggested before.\(^6,7,10,13\) However, the changes in flow and vascular resistance were much less dramatic, when they were expressed per unit of dry weight (fig. 3). Assuming that during edema development the dry weight of the brain tissue does not decrease, and that there is no formation of new vessels, this would imply that both the changes in blood flow and vascular resistance were due to the volume expansion of the (swollen) edematous tissue and not to an obstruction of blood flow. The apparent decrease in flow, in consequence, was not "ischemia" in the proper sense of this term.

This finding was further substantiated by the fact that regulation of blood flow was not significantly altered. When arterial PCO\(_2\) was increased from 30 to about 60 mm Hg, the average change of blood flow in the non-edematous white and grey matter was 6.4 and 16.5%/mm Hg, respectively (fig. 4). These values are very similar to the change in the tumor-bearing hemisphere in which blood flow increased by 5.4%/mm Hg in the edematous white matter and by 17.8%/mm Hg in the non-edematous grey matter, respectively. CO\(_2\) reactivity, in consequence, was not influenced by edema development (fig. 4).

Autoregulation in the grey matter of both the ipsilateral and contralateral hemisphere was present over a pressure range between 40 and 170 mm Hg. At higher perfusion pressure, a breakthrough of the autoregulation occurred in both hemispheres (fig. 5). In the white matter, the autoregulatory capacity was less efficient than in the grey matter. In the vicinity of the tumor, blood flow increased by 0.7%/mm Hg pressure rise. However, this increase was less than in the opposite (non-edematous) white matter, where an average increase of 1.0%/mm Hg pressure increment was seen. Moreover, calculation of vascular resistance revealed that there was a gradual rise over the whole pressure range, indicating that autoregulation was not

FIGURE 2. Changes in water content and blood flow in the white matter of the cat brain one to three weeks after xenotransplantation of a rat glioma clone into the internal capsula. Water content and blood flow were measured in the same regions using a tissue sampling technique. Values are means ± SE.
abolished (fig. 5). The observed deficiency of the autoregulatory capacity in the white matter, in consequence, was relatively mild and — more important — not specific for the accumulation of edema fluid.

Measurements performed in the tumor were much less consistent than in the peritumoral edema. The water content of the tumor varied between 74.5 and 83.5 ml/100 g wet weight (mean 80.0 ml/100 g/w.w.),
and blood flow between 13 and 72 ml/100 g/min (mean 29 ml/100 g/min). Autoregulation was absent in 2 out of 3 cats, but there was a distinct CO₂ reactivity in 3 other animals. The reason for this variability, presumably, was the occurrence of regional tissue necrosis, the degree of which could not be adequately estimated by the present tissue sampling technique. The results, therefore, were not further evaluated in the present investigation.

Discussion

In contrast to human patients, peritumoral edema has only occasionally been investigated in animal experiments. There are a few reports about the morphology and biochemistry of peritumoral edema following implantation of Walker 256 metastatic tumor in rats, ependymoblastoma or methylcholangthrene in mice, choriocarcinoma in monkeys and Brown-Pearce carcinomas in rabbits. The only previous investigations on regional blood flow in experimental brain tumors, to our knowledge, are 2 short communications in which autoradiographic studies have been reported in the rat following Walker 256 carcinoma and glioma cell implantation, respectively. In these studies a decrease in cerebral blood flow was noted in the vicinity of the tumor but this was more likely due to local compression by the tumor mass than the consequence of edema because the small amount of white substance in the rat brain restricts the accumulation and spread of edema fluid.

The present model of experimental glioma cell clone implantation in the cat provides for the first time the opportunity to investigate under standardized conditions edema associated with a primary brain tumor in a larger animal. Using this model, we had found previously that edema was purely of the vasogenic type, and that the mode of edema formation was very similar to that observed in human brain tumors. We also noted that blood flow in the edematous peritumoral tissue decreased, but this decrease was less than could be expected on the basis of the volume expansion of the swollen brain tissue. The present study corroborates this notion. Vascular resistance, in fact, did not decrease even in the presence of extreme degrees of edema, and neither autoregulation nor CO₂ reactivity were disturbed.

This conclusion is in contrast to previous observations in human patients and also in experimental studies using other forms of brain edema, such as triethyl tin poisoning, ischemic-anoxic brain swelling, cryolesion of the cortex, water intoxication or x-irradiation. In these studies the decrease of flow in the edematous tissue was attributed to local compression of the microcirculation by extravasated edema fluid.

There are 2 explanations for this discrepancy. In some of the cited studies, the decrease in flow was ap-
parently due to volume expansion of the swollen tissue, as in our experimental situation. Hadjidimos et al., for example, published a numerical relationship between cerebral blood flow and water content on the basis of which the changes in tissue volume and blood flow can be calculated. According to this equation, an increase in white matter water content from 70 to 85 ml/100 g wet weight — which corresponds to an increase of tissue volume by 100% — resulted in a decrease of flow to 54%, i.e. the flow decrease was almost entirely due to tissue swelling.

In other experiments, disturbance of blood flow may have been due to cytotoxic brain swelling rather than to vasogenic edema. The distinction between these two forms of edema has been made by Klatzo® in regard to the permeability of the blood-brain barrier which is severely impaired in vasogenic but not in cytotoxic brain edema. Water increase in vasogenic edema is mainly due to extravasation of plasma ultrafiltrate across the leaky blood-brain barrier whereas in the cytotoxic type of edema it is the consequence of a (metabolically induced) change in tissue osmolality and a disturbance in cell membrane polarization. The latter is associated with an equilibration of transmembrane ion concentration gradients, and an influx of fluid from the extracellular space into the intracellular compartment.® 9 Electron microscopic observations have revealed that cell swelling preferentially involves the perivascular glial cells which, in turn, may cause a compression of the microcirculation at the capillary level.®

A sensitive indicator of the cytotoxic type of edema is a loss of tissue potassium content.® This is different from pure vasogenic edema, as in the present experimental situation, in which potassium content slightly increases because cell membrane polarization is preserved.® 14 In several of the above cited experiments, however, brain swelling was associated with a decrease in potassium content which indicates that edema was, in fact, at least partially of the cytotoxic type.® 3,® 7

In conclusion, our experiments suggest that disturbance of flow regulation or vascular resistance in cerebral edema is an indication of a complicating cytotoxic component, most likely due to intracranial hypertension and compression ischemia.® 3 Microcirculatory disturbances, when present, therefore, would be the consequence of (cytotoxic) perivascular glial swelling rather than of an accumulation of (vasogenic) edema fluid. This observation may be of interest for the therapy of edema. It is well known that corticosteroids have an ameliorating effect in the vasogenic® 23 but not in the cytotoxic, particularly the ischemic, type of edema.® 4 Treatment of the latter form is by osmotherapy and improvement of blood flow to restore cell metabolism.® 5,® 6 Testing flow regulation, therefore, may be a useful tool for differentiating between the 2 types of edema and for establishing a therapeutic protocol for the optimal clinical treatment of brain edema.

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Blood flow and regulation of blood flow in experimental peritumoral edema.
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doi: 10.1161/01.STR.12.2.211

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/12/2/211

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