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Intracarotid Fluorescein Angiography:

A New Method for Examination of the Epicerebral Circulation in Man

WILLIAM FEINDEL, M.D., C.M., D.Phil., F.R.C.S.[C],*
Y. LUCAS YAMAMOTO, M.D., Ph.D.† and
CHARLES P. HODGE, R.B.P.,‡ *Montreal*

Patterns of blood flow were examined in the surface vessels of the surgically exposed brain by intracarotid injection of 1% fluorescein and rapid serial photographs timed by a photo-cell signal. Matching colour filters were used for black and white or Ektachrome film.

As developed in cats and monkeys, and applied in five patients during craniotomy, the technique gave a picture of flow patterns in the pial and cortical vascular bed, demonstrating "water-shed" areas bordering major arterial territories, laminar flow in veins, and, in particular, the details of filling and clearing in the fine pial vessels, the superficial cortical capillary bed and in the vascular beds of tumours.

Since these features are rendered in finer detail and sharper contrast than by standard x-ray angiography, the method affords a new means of more adequately examining the epicerebral circulation in man during craniotomy for a variety of lesions.

L'auteur a étudié la circulation cérébrale superficielle au cours d'interventions sur le cerveau mis à nu, grâce à l'injection de fluorescéine dans une carotide. Une cellule photo-électrique déclenchait la prise d'une série rapide de photographies. Des filtres de couleur appropriée étaient employés pour les films en noir et blanc ou pour les films Ektachrome.

Mise au point sur des chats et des singes et appliquée à cinq patients qui subissaient une craniotomie, cette technique a permis d'enregistrer le flot circulatoire dans les vaisseaux de la pie-mère et du cortex. On a ainsi pu mettre en évidence des zones d'inondation circonscrite à la périphérie des troncs artériels principaux, un flot laminaire dans des veines et en particulier le mode de remplissage et d'évacuation des fins vaisseaux de la pie-mère, du lit vasculaire superficiel du cortex et celui des tumeurs.

Les images obtenues sont plus nettes et mieux contrastées que celles que l'on peut obtenir par angiographie radiologique courante; cette technique permet donc d'examiner avec plus de précision la vascularisation cérébrale périphérique chez le patient qui subit une craniotomie pour une raison quelconque.

IT IS over 300 years since Willis and Lower first syringed India ink into the carotid arteries and discovered the function served by the arterial "circle" (depicted so elegantly for them by Wren) in maintaining collateral flow to the brain.[1, 20] Since then, a vast amount of anatomical and physiological information has become available to help in the clinical management of disorders of the cerebral circulation. Among the most significant approaches has been the widespread application of x-ray cervical and cranial angiography to define the anatomical sites of pathological changes in the arteries supplying the brain.[2]

More recently, serial radioactive brain scanning has offered a convenient way of studying vascular lesions of the brain, for example, by detecting the presence of angiomas[3] and by following the evolution of cerebral infarcts.[4] In addition, various methods for monitoring cranial and cerebral blood flow by radioisotopes[5-7] are

From the Department of Neurology and Neurosurgery, McGill University, and the Cone Laboratory for Neurosurgical Research, Montreal Neurological Institute, Montreal 2, Quebec.
*Cone Professor of Neurosurgery, McGill University.
†Research Associate, Cone Laboratory for Neurosurgical Research.
‡Neurophotographer, Montreal Neurological Institute.
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Reprint requests to: Dr. William Feindel, Montreal Neurological Institute, 3801 University Street, Montreal 2, Quebec.

beginning to provide some means of quantitative assessment of therapy directed toward improvement of the vascular irrigation of brain tissue. Thus it is possible to measure changes in cerebral blood flow before and after excision of arteriovenous angiomas and before and after carotid endarterectomy.[5, 7, 8] The vagaries of the natural clinical course in patients suffering from strokes or from intracranial hemorrhage are familiar enough to most physicians to emphasize the importance of applying such objective methods of evaluation.

One of the more serious limitations in our present understanding of cerebral vascular disorders in general has been the lack of precise information on the flow and distribution of blood in the myriad small vessels of the brain. Those less than a half-millimetre in diameter are not visible on standard cerebral x-ray angiography, but it is precisely in this microvascular bed that the main business of nourishing nerve cells takes place. The patient who has been rendered hemiparetic from cerebral ischemia but in whom the cerebral arteries and veins appear normal on angiography presents an example of this limitation in x-ray definition of these small vessels.

In previous reports[8, 9] we have described how blood flow in cerebral vessels of this small size can be examined on the surface of the brain during craniotomy under special conditions. This can be done by injecting Coomassie blue, a non-toxic, densely coloured dye, into the internal carotid artery. With serial stroboscopic colour photography, it is then possible to record the passage of this dye through the vascular plexus on the cortical surface of the surgically exposed brain. In this way, vessels down to the smallest branches perforating the cortex have been examined and the patterns of filling and clearing of the cortical capillary bed have been defined.[10] These dye pictures can then be compared with radioisotopic flow curves, measured directly by miniature gamma detectors placed on the pial surface of the brain during operation. This quantitates changes in local blood flow before and after excision of focal cerebral lesions which are associated with alterations in the cerebral circulation.

From the neurosurgical point of view, the application of this method has been of practical value in allowing a more systematic and controlled excision of angiomas, aneurysms and vascular tumours. The technique is of more general interest in regard to the study of cerebral vascular disorders since it has brought under direct analysis an important part of the cerebral circulation in man which so far has been little examined *in vivo*. In particular, the method makes it possible to study the cerebral microcirculation anatomically as well as physiologically and to relate these findings to the neurological condition of the patient.

The purpose of this report is to describe a modification of this radioisotopic dye method involving the use of fluorescein, which has been shown to provide such excellent pictures of the retinal circulation.[11-13] We found that intracarotid fluorescein gave a brilliant anatomical display of the surface circulation of the brain in cats and monkeys. Compared to Coomassie blue, there was a sharper contrast and finer detail, especially in the capillary and venous phases. This new technique as developed in experimental animals and the initial results of its use in patients were reported earlier.[14] Since then Russell and Simcock[21] have independently shown a similar technique to be of value in experimental studies of the leptomeningeal circulation in rabbits.

It is convenient to refer to the surface vessels of the brain—the pial arteries and veins, their perforating branches and the superficial cortical capillary bed—as the epicerebral circulation. We wish to describe here the main features of fluorescein angiography of these epicerebral vessels in experimental animals and in man. To illustrate the usefulnes of the technique, we note a patient whose clinical course presented as a stroke, although the cause of this as shown by fluorescein angiography proved to be occlusion of vessels in a cerebral glioma and adjacent brain.

METHOD

The technique was developed in cats and monkeys. A wide craniotomy was carried out in the animals to expose the lateral surface of one hemisphere. Through a fine polyethylene catheter placed retrograde in the superior thyroid artery, 0.2 to 1.0 ml. of 1% sodium fluorescein* was directed into the common carotid artery. Serial photographs of the dye passage through the surface vessels of the hemisphere were taken at intervals of 0.4 second, or longer when indicated, with an electric motor-driven Nikon camera. Intervals between frames were timed by a cadmium photo-cell connected to a Sanborn paper-strip recorder, which signalled the flash of a stroboscopic light having a duration of 115 microseconds. When using Kodak Tri-X Pan film, the light was covered with a Wratten 47 filter and the camera lens by a Wratten 58 filter.

*The fluorescein was prepared by Moore Kirk Laboratories, East Woodcock, Connecticut, U.S.A.

The method was further modified for use in the operating theatre. Sodium fluorescein (1 to 2 ml. of 1%) was rapidly injected into a PE-160 polyethylene catheter placed in the internal carotid artery. A telephoto lens (300 mm., F 4.5), placed a metre from the surgical field, was focussed on the brain through an overhead mirror (Fig. 3). The lens was covered by a Wratten barrier filter No. 9 plus a Wratten 2B ultra-violet absorbing filter. With this combination of filters, high-speed 35 mm. Ektachrome daylight film was used for the rapid-sequence photography. This film was then developed as a negative transparency by a technique used for fluorescence photomicrography[15] which utilizes a C-22 Kodak kit to give an increase in contrast and to extend the film speed above ASA 1500. Colour or black-and-white prints were made from this negative for detailed examination.

RESULTS

Experimental Studies

The main features of the fluorescein angiogram of the surface vessels are illustrated by three sample photographs from a sequence of 36 frames taken of the monkey brain. The first photograph, Fig. 1a, from the second frame taken at 0.7 second after injection, shows the later arterial and early capillary filling. Large and small pial arteries filled with fluorescein appear white and are easily distinguished from the veins which remain dark. The superficial vascular plexus over the hemisphere is uniformly filled with fluorescein except in the region of the posterior cerebral artery at the tip of the occipital lobe (to the left).

Fig. 1b, the fourth frame taken at 1.5 seconds after injection, shows that the territory of the anterior cerebral artery clears more rapidly than that of the middle cerebral artery. The "watershed" between zones supplied by these two major arteries is well defined. At this stage also the larger pial veins show fine laminar streaks which originate from

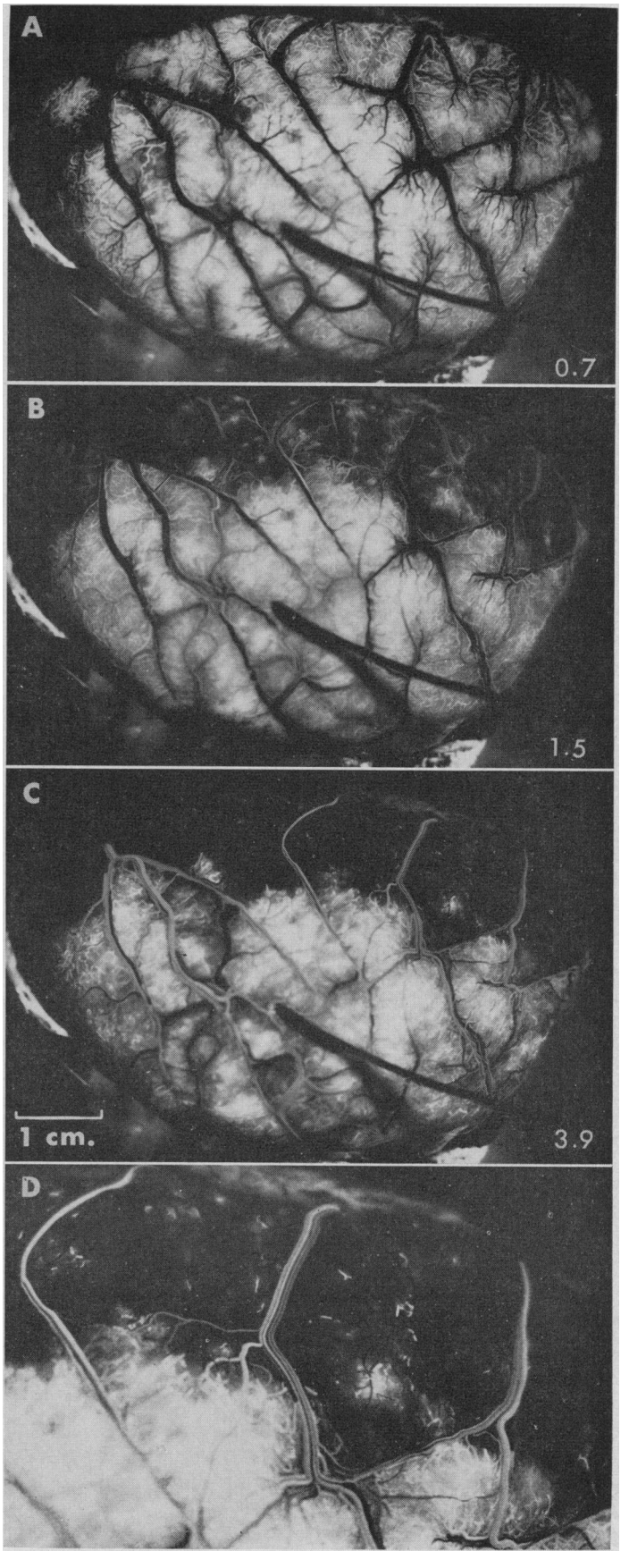

Fig. 1.—Samples of serial photographs from a 36-frame series taken of a fluorescein angiogram of the monkey brain. The frontal region is to the right, the occipital to the left. The photographs were taken at 0.7, 1.5 and 3.9 seconds after intracarotid injection of 1% fluorescein. The lower photograph is an enlarged view of the pial veins showing the laminar flow patterns.

small venous tributaries and remain remarkably intact, sometimes for a distance of several centimetres, after their embouchement into larger collecting veins. This is clearly shown in Fig. 1c, the tenth frame at 3.9 seconds after injection, and in an enlarged section of this (Fig. 1d), where the zone of the anterior cerebral artery has almost cleared and is bridged by veins draining the middle cerebral territory. Multiple laminar streams were visible even up to the point where the veins enter the superior sagittal sinus. Each successive entering stream displaced more centrally those already coursing in the vein. The background fluorescein in the middle cerebral territory has become reduced in area with patchy clearance of the capillary bed. (A dissecting instrument used as a marker is seen in the first three pictures.)

Studies in Man

In May and June, 1966, the epicerebral circulation was examined by fluorescein angiography in five patients during therapeutic craniotomy. In each instance, the anatomical details of the surface vessels were superior to those obtained on rapid serial x-ray angiography before operation. In two patients with neoplasms the routine six-film serial x-ray angiograms showed little or no vascular flush in the tumour. But on fluorescein angiography an abnormal vascular bed demarcated these tumours clearly from the normal brain, a feature of some practical value during excision.

In a patient with an occipital lobe angioma, selection of pial arteries for division during the surgical removal was more easily made by demonstrating the surface vessels with fluorescein.

In a fifth patient, it was of interest that an area of atrophic cortex giving rise to severe electrographic epileptogenic abnormality was partly bounded by the "water-shed" between the anterior and middle cerebral arterial zones, a finding which merits further study.

The value of fluorescein angiography in providing a picture of the cerebral vascular bed which can be correlated with clinical findings is illustrated in detail by the observations on a patient (L.N.) with a glioma in the left parietal region. Six weeks before admission, this patient had sudden onset of speech difficulty which simulated clinically a stroke, though after that her gradually worsening condition strongly suggested a tumour. Arteriography showed a rounded mass of tumour vessels in the inferior parietal region, but the preoperative diagnosis was undecided between a malignant glioma and meningioma. In the arterial phase of the x-ray angiogram large veins from arteriovenous shunting through the tumour bed are well seen (Fig. 2a). In the venous phase (Fig. 2b) normal veins on the anterior surface of the hemisphere were

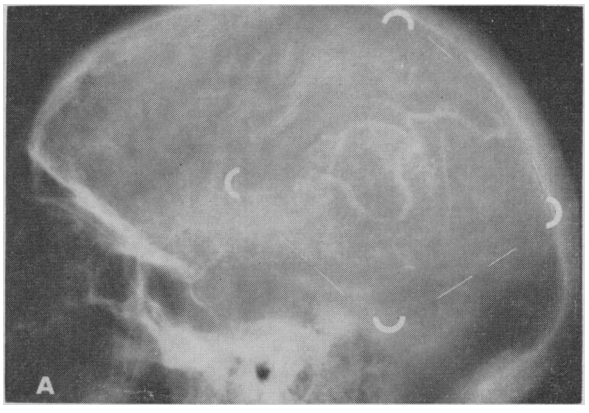

Fig. 2a.—Arterial phase of the x-ray angiogram (Case L.N.) showing the rounded mass of abnormal vessels with early venous filling. The outline of the site of the craniotomy is indicated in white.

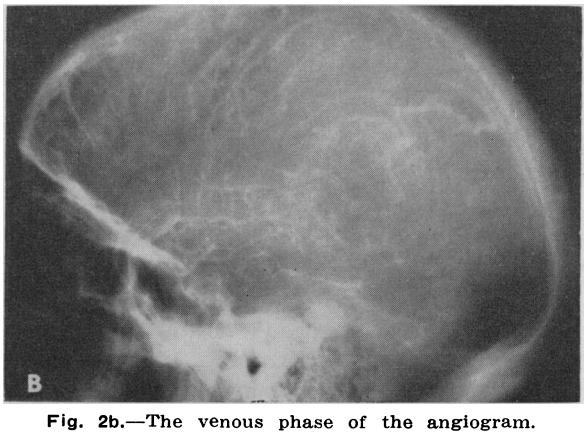

Fig. 2b.—The venous phase of the angiogram.

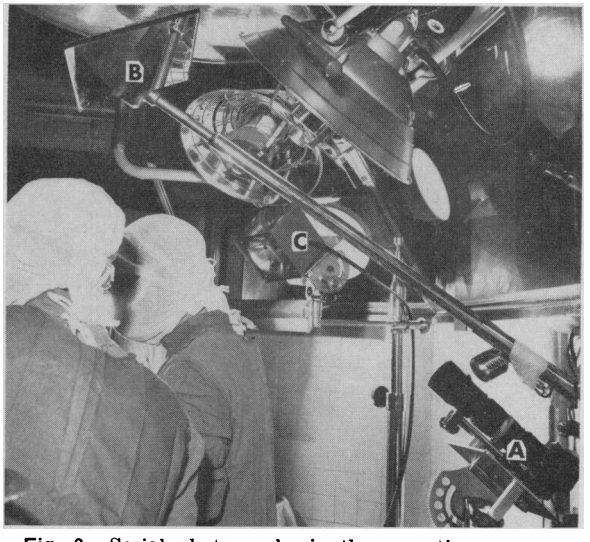

Fig. 3.—Serial photographs in the operating room are taken with a 300-mm. telephoto lens on a motor-driven Nikon camera (A) which views the exposed brain through over head mirror (B). (C) is the stroboscopic light.

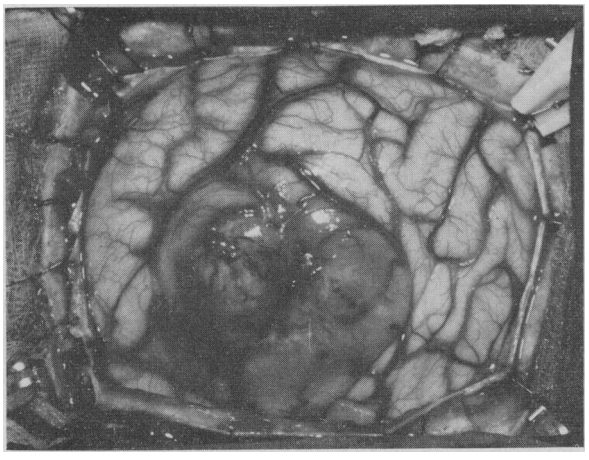

Fig. 4.—Photograph of tumour and exposed brain to compare with fluorescein angiography.

filled and the vascular flush of the tumour was still evident.

Craniotomy was carried out on May 15, 1966, as outlined in Fig. 2a. A biopsy disclosed that the tumour was a malignant glioma, and a limited removal was made. The tumour measured 6 cm. in diameter and presented on the surface (Fig. 4).

The fluorescein angiogram at operation is illustrated by nine samples taken from 36 serial frames (Fig. 5). The initial picture at 0.3 second after injection disclosed a halo of arterial vessels on the tumour margin which at once made it distinct from the rest of the exposed brain. In the second frame, at 0.8 second, this vascular zone was even more evident. After that, it became gradually confluent with the normal vascular bed of the surrounding brain. As evident from referring to Fig. 2a and b, much more detail of the superficial vascular bed of the tumour and of the epicerebral vessels of the exposed brain was seen with fluorescein. For

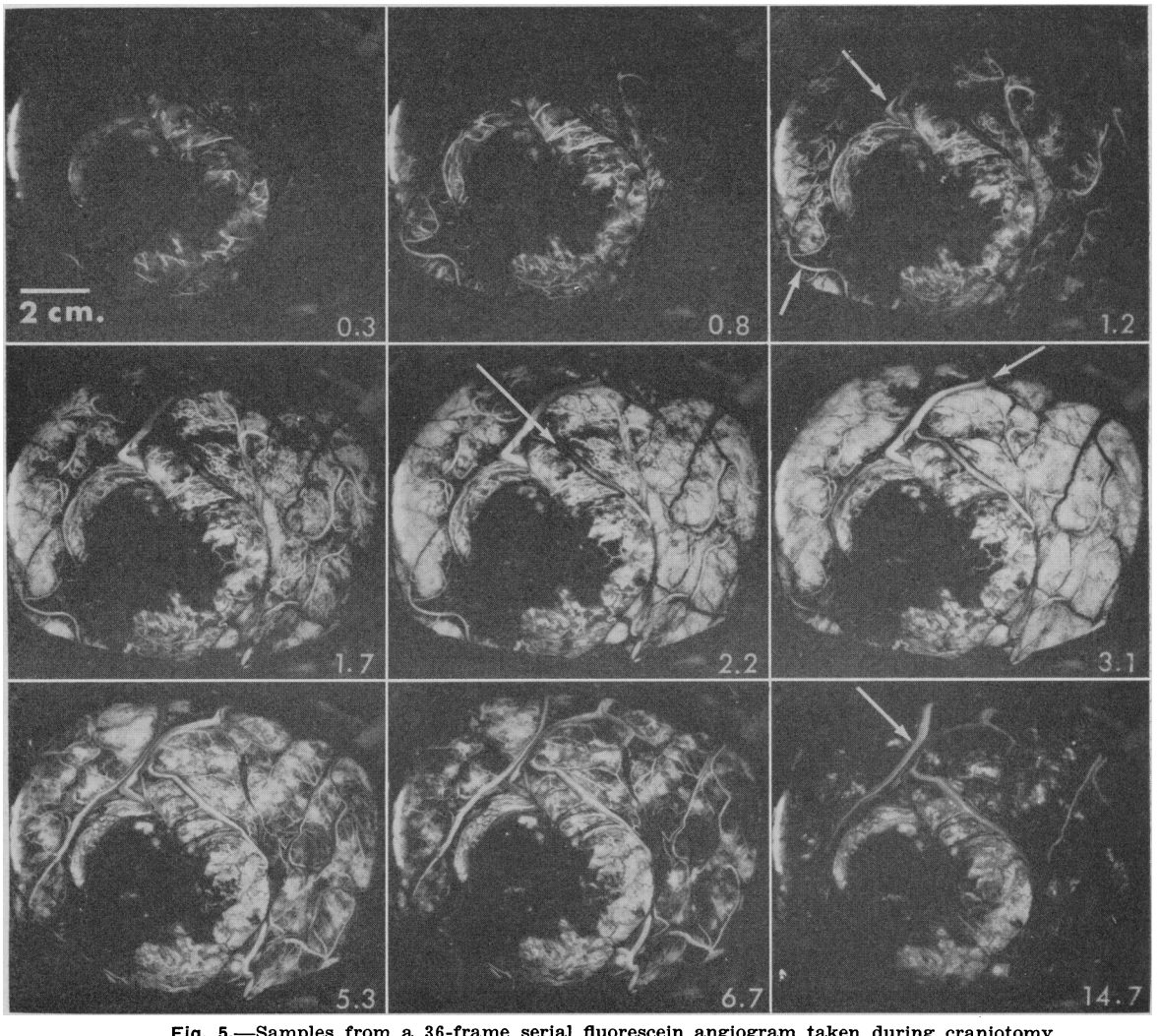

Fig. 5.—Samples from a 36-frame serial fluorescein angiogram taken during craniotomy for the tumour illustrated in Figs. 2 and 4. The series runs from left to right as indicated from the timing in seconds on each frame. See text.

example, in the third frame at 1.2 seconds after injection, the posterior temporal branch of the middle cerebral artery could be made out (lower left arrow) and early filling of one of the red veins draining the shunted blood from the tumour became evident (upper arrow) in the same photograph. In addition, throughout the fluorescein series a discrete central zone of the tumour extending forward to the posterior temporal artery remained clear of the dye. During surgical removal of the tumour the tissue in this area showed several small thrombosed vessels. It seemed probable that vascular occlusion in this superficial part of the tumour and adjacent cortex was related to the stroke-like onset of the patient's symptoms.

In the fourth, fifth and seventh frames, taken at 1.7, 2.2 and 3.1 seconds after injection, the pial and cortical vascular bed became gradually filled in a patchy distribution. A second large shunting vein visible in the frame at 2.2 seconds (arrow) joined the earlier one to run toward the sagittal sinus at 3.1 seconds (arrow). These veins filled rapidly during the arterial phase before the microvascular bed was shown and are distinct from the normal pale veins which at this stage remained dark. The filling of the capillary bed reached a maximum at 3.1 seconds. After this, the sequence of clearing of the dye became slower as noted in three additional sample frames selected at much longer photographic intervals. A post-central vein which had a normal filling time is first seen in the twelfth frame at 5.3 seconds and stands out clearly at 14.7 seconds (arrow) from the partly cleared pial and cortical vascular bed. Even at 14.7 seconds the abnormal vessels of the tumour still retain some dye. All these times were recorded with the patient under hypothermia at 30.5° C. rectal temperature.

Clearance curves of intracarotid xenon-133, recorded by gamma-detecting probes at four sites on the exposed brain, indicated a reduced blood flow through the part of the tumour with the absence of vessels in its superficial layer, as compared to the rest of the tumour and the adjacent cortex.

Transit time curves were then measured by intracarotid mercury-197–chlormerodrin (Neohydrin) (Fig. 6). A characteristic curve with the early peak at 1.3 seconds was recorded from the zone near the posterior branch of the middle cerebral artery (Fig. 5, 1.2 seconds, lower arrow). Each curve from the two shunting veins, previously noted, had a slower initial slope resembling the arterial curve, and a slower decline. A probe over the normal vein, seen from 5.3 seconds onward in the fluorescein series, recorded a second peak at 5.2 seconds. In previous studies we have found this delayed peak typical of the venous phase.

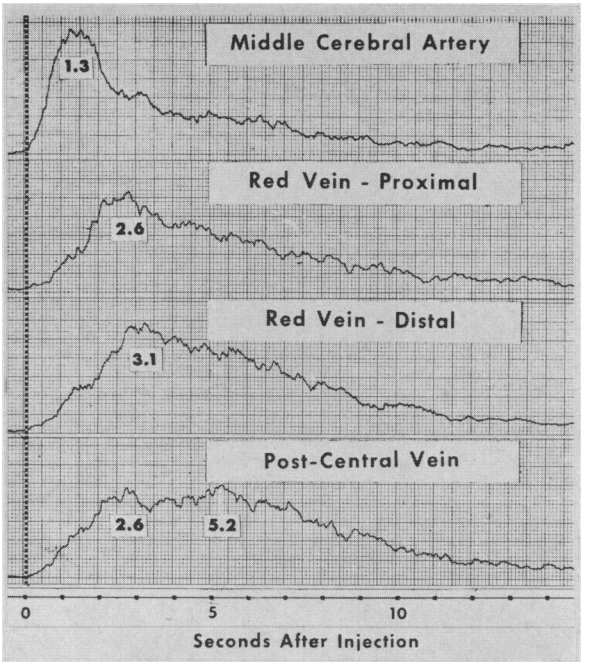

Fig. 6.—Radioisotopic flow curves recorded from the exposed brain after injection of 250 microcuries of ^{197}Hg into the internal carotid artery. Proximal and distal indicates the relation of veins to the vascular bed of the tumour.

DISCUSSION

Standard x-ray angiography, quite properly termed cerebral arteriography and phlebography by its initiator, Moniz,[2] does not make visible any of the smallest pial vessels or the dense capillary bed of brain tissue. Fine vessels on the surface of the brain have occasionally been examined by incident-light microscopy during craniotomy.[16, 17] But this technique is necessarily restricted to a small sampling of the total circulatory bed in the surgical field.

Fluorescein angiography, on the other hand, demonstrates vessels of all sizes on the exposed brain. In particular, it provides a picture *in vivo* of the finest surface vessels and of the cortical capillary bed.

Under conditions outlined here the placement of a catheter in the internal carotid artery has presented no problems or complications. Sodium fluorescein itself is a dye of low toxicity and has been widely used in the study of the peripheral circulation and recently the retinal circulation. Most techniques involve larger doses of dye than the 20-mg. injections used here for intracarotid studies.

In experimental studies, the technique has been of value in examining the problem of vas-

cular occlusion in the monkey, with a view to determining the degree of venous and capillary reflux which may occur. This feature has already been well analyzed by the fluorescein technique in the retinal circulation by Dollery *et al.*[13] The distinction between the smaller arterial and venous vessels in the pial plexus is ordinarily not always obvious, though this is an essential step in any attempt to study the surface circulation. This differentiation is possible with Coomassie blue, but the contrast between arterial and venous vessels is even more clearly shown on fluorescein angiography.

In addition to its use in examining the anatomical aspects of the epicerebral circulation, the fluorescein method has been of value in the surgical management of angiomas and aneurysms, in the same way as Coomassie blue. Identification of the feeding arteries to an arteriovenous malformation allows a planned excision to be made. The integrity of cortical flow can be assessed following surgical manipulation of aneurysms or of major cerebral arteries. The enhanced detail of the vascular pattern in brain tumours aids their identification. The combination of fluorescein angiography with quantitative radioisotopic flow analysis offers a means of investigating structural and metabolic intracranial arteriovenous shunts.[18]

These physiopathological findings on the epicerebral circulation *in vivo* are being compared with anatomical studies of the cerebral microcirculation by x-ray projection microscopy of fixed specimens of brain, in which the vascular bed has been injected with radiopaque materials.[19]

SUMMARY

Intracarotid injection of sodium fluorescein with rapid-sequence stroboscopic photography during craniotomy affords a new method for examining the surface vessels of the brain in experimental animals and in man. Features of the epicerebral circulation not visible on x-ray angiography are shown with sharp contrast and detail by fluorescein. When combined with quantitative radioisotopic blood flow curves from the exposed brain, fluorescein epicerebral angiography makes possible the more effective investigation and surgical management of lesions involving the cerebral circulation.

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