3 Scientific Critique

3.1 Animal Research at Charing Cross & Westminster Medical School

Researchers at Charing Cross & Westminster Medical School (CXWMS) are using animal models to study a range of physiological and clinical problems. Recent projects include:

DEPT. OF ACADEMIC MEDICINE: Cutting the nerves to the hearts of dogs, then allowing three weeks for associated nerves to atrophy; finally removing tissues from the hearts to study the effects of denervation on heart metabolism[1].

DEPT. OF ANATOMY: Cutting the left optic nerve in 20 adult rats, and allowing the animals to survive for a month. After this time, the retinae of the eyes were dissected to see if cutting the optic nerve had damaged retinal cells as well[2].

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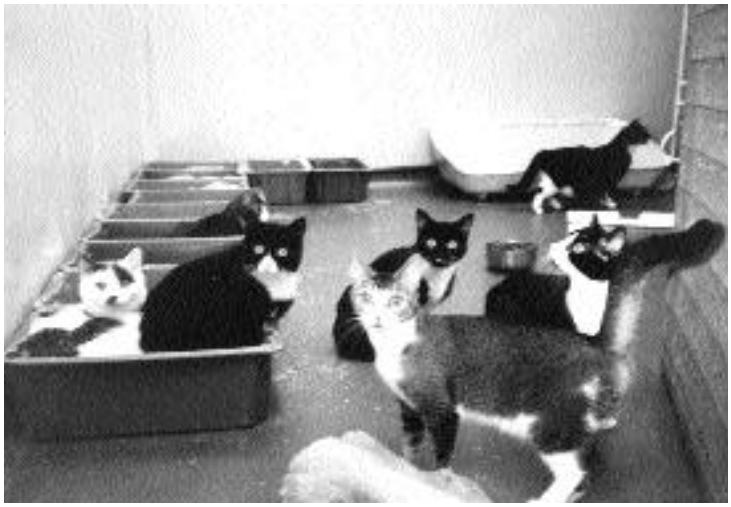
Rats with EAE, an attempt to mimic multiple sclerosis at CXWMS.

DEPT. OF ANATOMY: A study in rats with Experimental Allergic Encephalomyelitis (EAE), jointly funded by the Multiple Sclerosis Society of Great Britain and Northern Ireland, and the Wellcome Trust. This was a joint project with others at the University of Wales College of Medicine[3]. EAE is believed by some to be an animal model of multiple sclerosis.

DEPT. OF BIOCHEMISTRY: Destruction of part of the brain of anaesthetised rats before, eight hours later, dissecting out the brain for biochemical studies; funded by the pharmaceutical company, Bayer UK[4].

DEPT. OF PHYSIOLOGY: Study of acute and chronic changes in rat kidney function following removal of the other kidney, funded by the National Kidney Research Fund[5].

DEPT. OF PHYSIOLOGY: Decerebration of 23 anaesthetised adult cats and removal of part of the brain, for a study of discharge from the motor neurones;



Cats in the CXWMS cat colony.

funded by the US National Institutes of Health, the Wellcome Trust, and the Parkinson's Disease Society[6].

DEPTS. OF PHYSIOLOGY AND CHEMICAL PATHOLOGY: A study of renal reabsorption in anaesthetised rats infused with the diuretic drugs amiloride or furosemide (frusemide), funded by the Wellcome Trust[7].

DEPT. OF COMPARATIVE BIOLOGY: A study in rats of the local reactions caused by implants under the skin of Dacron™ tubing, as used in peritoneal dialysis equipment. This was a joint project with Khon Kaen University, Thailand[8].

Below: Beagle at CXWMS.

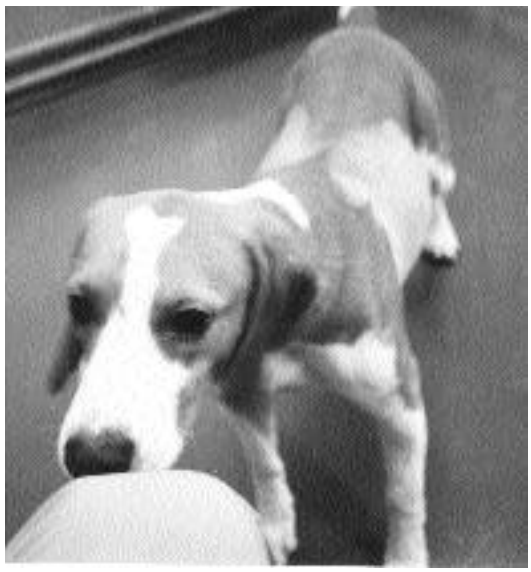
The brief survey above shows the range of ground covered at CXWMS. However, in this report we will concentrate on several apparently on-going projects.

3.1.1 ACADEMIC UNIT OF CARDIOVASCULAR MEDICINE

M.I.M.Noble is Professor in the Academic Unit of Cardiovascular Medicine[9]. He has no current projects listed in *Current Research In Britain*[10].

Nevertheless, various teams based around Noble have carried out studies of coronary thrombosis and blood clotting in dogs, reported in 1992, 1993 and 1994. They have also studied heart failure induced in dogs by pacing their hearts at high rate, reported in 1990 and 1992. Video evidence shows the latter project at least is continuing.

In the year-ending July 1993 Noble and colleagues were awarded cash for unspecified "clinical studies"; £15,912 from the BHF, £18,914 from the Garfield Weston Trust, and £250,000 from the EEC BIOMED I[11]. Noble had set up research networks within Europe to study coronary thrombosis, excitation-contraction coupling, and cardiac denervation, all funded by the EEC grant[9].



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3.1.1(a) Studies of Clot Busting Drugs

background - dissolving blood clots

A team in the Academic Unit of Cardiovascular Medicine produced two papers during 1992 and 1993, describing studies of the effects of certain drugs on the blood platelets and consequently on blood clotting[12,13].

The background discussion of clot-busting drugs which follows here is also relevant to work carried out at the second laboratory studied by the NAVS, i.e., the Institute of Neurology. Whereas at CXWMS the work is on heart attack, at the Institute of Neurology the work is on stroke.

Blood clots typically form in two ways. In the slow moving venous circulation, clots typically consist of a fibrin web enmeshed with platelets and red blood cells. In contrast, in the fast moving arterial circulation, clots typically consist of clumped platelets with little fibrin[14].

Since many people reading this report may be on routine treatment with anticoagulant drugs, to avoid any confusion it is important to differentiate between these and thrombolytic drugs.

anticoagulants

When clots have already caused a heart attack or stroke, drugs can help to prevent a recurrence; these are anticoagulants[15]. For the secondary prevention of fibrin-based clots, warfarin is commonly used, whilst for secondary prevention of platelet-based clots, low dose aspirin is widely used[14].

Warfarin, originally a rat poison, was introduced to clinical medicine as an anticoagulant after an American soldier used it in an attempt to commit suicide[16].

Aspirin was first suggested as an anticoagulant because of its well known ability to cause stomach bleeding in patients taking it for other purposes; subsequent studies confirmed this effect[17].

thrombolytics

Although warfarin and aspirin can prevent clotting, they do not effectively break up existing clots. That task requires a thrombolytic drug.

Under normal circumstances, the body produces its own natural clot-buster, called plasmin (or fibrinolysin). This works by dissolving the fibrin web[18] around which the clot has formed.

In 1933, researchers noticed that a filtrate of a particular streptococcal bacteria could break up blood clots in culture. They concluded that the filtrate acted directly on fibrinogen, and called it streptokinase[19,20].

Early studies of the way in which streptokinase works in other species were complicated by species differences[20], but it was eventually decided that in humans it really works by converting a proactivator in the plasma to an activator, which in turn reacts with plasminogen to form plasmin[21], which dissolves the fibrin web.

Almost 20 years after streptokinase's ability to dissolve fibrin clots was discovered, in 1952 it was found that it could also dissolve blood clots produced experimentally in rabbits; this was followed by experimental work in dogs, which confirmed the findings in rabbits[22]. However, as we show in the critique, these early animal studies raised doubts and concerns which did not materialize in clinical practice; the animal studies were a waste of time.

Meanwhile, another highly-active fibrin-dissolving substance had been discovered in human urine; this proved to be of considerable practical importance, and was called urokinase[20]. It was subsequently isolated from cultures of human foetal kidney tissue as well[19], and was found to be a plasminogen activator[20].

By the early 1960's, both streptokinase and urokinase were being used clinically to dissolve blood clots[20]. Even plasmin (fibrinolysin) was purified from human blood, and it too was being used clinically to break up blood clots[23].

During the late 1950's a researcher placed layers of fibrin on frozen sections of various tissues; he found that the fibrin dissolved in those areas that were close to blood vessels. The greatest activity was in those areas closest to veins, small veins, and the pulmonary (lung) arteries. It was soon found that the lining of the blood vessel (the endothelium) secreted the active substance, because it too dissolved clots which were placed in contact with it alone[20].

It was then discovered that when a clot forms in a blood vessel, the lining of the vessel secretes a substance known as tissue plasminogen activator (t-PA); this also activates plasminogen, which converts to plasmin, and dissolves the fibrin web[18].

T-PA was initially isolated *in vitro* (culture dish), from a melanoma (skin cancer) cell line, but can now be made by recombinant DNA technology, giving recombinant t-PA or rt-PA[19]. T-PA was finally introduced to medicine in 1987[24].

platelets - a problem not yet solved

In recent years, evidence has accumulated linking platelets to the deleterious effects of heart attack. Careful post mortem pathological studies have shown platelet clumps in the coronary vessels of people who have died suddenly from heart attack[25].

Readers with a particular interest in platelets and heart attack can also refer to an earlier NAVS report (Labs Unlocked, NAVS, 1994), which described and criticized related studies by other teams at St. Mary's Hospital Medical School in London. They were studying the role of another substance, Platelet Activating Factor, which stimulates platelet clumping[26].

At present there are still no effective drugs for breaking up platelet-rich clots. The CXWMS researchers are looking at this aspect of blood clotting, along with fibrin-dissolving drugs, and their relation to heart attack.

studies of serotonin antagonists

The CXWMS team points out that evidence is accumulating, from studies carried out elsewhere on dogs, that serotonin (5-HT) is an important contributor to platelet clumping. Consequently they, and researchers elsewhere, are studying the effects of serotonin-antagonist drugs on platelet clumping[12].

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Dogs awaiting vivisection at CXWMS.

For one experiment they anaesthetised seven beagle dogs and exposed their hearts. Venous and arterial cannulas were fitted for measurement of blood gases and blood pressure, and infusion of drugs. The coronary artery was crushed to damage its endothelium, and a ligature applied to close it off completely and simulate a heart attack. This resulted in the formation of platelet-rich blood clots, which the team used to study the effects of a serotonin ($5\text{-}HT_2$) antagonist drug, MDL 11,939, on platelet clotting[12].

The team cites research carried out elsewhere in 1988, and states that: "*Human platelet studies in vitro of platelets treated with 5-HT* [serotonin] *and ADP showed marked inhibition of aggregation by MDL 11,939 pretreatment*"[12]. So it was already known from clinical in vitro studies that the drug is likely to be useful in the treatment of patients.

They concluded: "*The fact that they* [the clots] *were dispersed by MDL 11,939 without physical assistance is supporting evidence for thrombolytic activity by this* drug"[12].

studies of calcium stabilizing drugs

In 1993 the team reported studies on another drug, trimetazidine, which stabilizes calcium in many types of cell. They wrote of clinical research, carried out by others twelve years earlier in 1981: "*Trimetazidine ... has been alleged to have effects on both in vitro and ex vivo platelet function. In patients with ischaemic heart disease given trimetazidine for 12 weeks, ADP and adrenaline-induced aggregation ex vivo at rest was claimed to be decreased; there was also a reported decrease in ADP- and adrenaline-induced aggregation* [of platelets] *after exercise*"[13].

Note that although this research had been carried out in vitro or using fresh human blood, according to the Charing Cross researchers the drug was only "*alleged*" or "*claimed*" to be beneficial.

To satisfy themselves they carried out studies on eight anaesthetised female beagle dogs, using similar techniques to those described above for their earlier experiment. A further six anaesthetised dogs were used for studies of bleeding time and blood coagulation in the mucosa of the mouth, and the effects of trimetazidine on this. This research was funded by the Garfield Weston Trust[13].

The team wrote: "*The main finding of this study is that trimetazidine was active in preventing platelet-rich thrombus* [blood clot] *formation in a dog model of coronary artery disease. A secondary finding was to confirm the clinical impression that the drug does not increase bleeding time or cause other clotting derangements*"[13].

They acknowledge that since the mid 1980's others have already used trimetazidine, to treat patients with angina; this is on account of its known effects on calcium distribution. Further, in 1992 yet another team had reported its use in patients undergoing heart surgery[13]. All before the CXWMS team reported its own study in dogs.

It is clear that the necessary evidence supporting the clinical use of this drug was already available, or potentially available, from clinical studies.

studies of tissue plasminogen activator

In 1994 the CXWMS team reported studies on the fibrin-clot-busting ability of recombinant tissue plasminogen activator (rt-PA), either alone or in conjunction with serotonin antagonists which break up platelet clumps. For this they used seven more dogs, anaesthetised and with the hearts exposed as in earlier experiments[27]. As the technique was similar to that already discussed, we will not cover the same ground again.

critique of clot busting studies in dogs

The clumping of platelets on blood-vessel walls has been studied experimentally for one hundred and twenty years, ever since 1875, and beginning with experiments on frogs[28].

Despite this, no effective platelet-clot busting drug has been discovered. Development of a drug to break up platelet clots will not come through animal studies; it will come through clinical observation, which is how the anticoagulant effects of aspirin and warfarin were first suggested. Recall too that the fibrinolytic effects of streptokinase and urokinase were discovered *in vitro* before they were tried in animals as clot-busters.

animal experiments not relevant

In fact, early studies of clot-busting in animals showed that the technique itself was unsafe. They showed that restoring the blood supply to heart tissue after having cut it off for a while led to the development of ventricular arrhythmias including fibrillation (which is usually fatal), temporary tissue damage, damage to small blood vessels, death of heart muscle cells, and bleeding[29].

These findings in animals led to early fears about thrombolytic treatment in patients, which have not been realised to any extent in clinical practice. One possible reason put forward to explain the discrepancy is that experimental reperfusion in animals is sudden, whereas clinical reperfusion is gradual[29].

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Beagles at CXWMS.

Some scientists engaged in thrombosis research question the use of animal models. Their relevance is complicated by variability in the causes of thrombosis in humans, and degree of stimuli which cause thrombosis in humans. The blood-vessel surfaces which thrombi form upon also differ between animal models and humans. Human coronary arteries, atherosclerotic and damaged by plaque fissure, and/or bleeding, probably differ substantially from the arterial surfaces seen in animal models[30].

Referring specifically to the study of platelet-rich thrombus in dogs, just two years ago researchers at the University of Michigan Medical School wrote that: *"It is recognized that the experimental model* [platelet-rich intravascular thrombus induced in the dog] *lacks the atherosclerotic lesions that are prominent in most, if not all, diseased human arteries in which thrombus formation occurs. The inability to replicate the entire human clinical pathophysiological state may suggest that prevention of thrombosis in a diseased human artery with atherosclerosis may not be prevented to the same degree as in the experimental animal"*[31]. In other words, the dog model is seriously flawed.

drug development flawed

So far as drug development is concerned, some drugs which prevent the clumping of platelets are highly selective for primate platelets, in comparison to platelets from other species. This differential sensitivity highlights the difficulties in interpreting results obtained on in vitro tissues from other species, or from animal models[32]. When exploring new drugs to break up platelet-clots, it is important to consider species differences, and to use primate, preferably human, tissue[33].

The first CXWMS project on platelets which we discussed, was a study of a 5-HT_2 (serotonin) antagonist drug in the beagle dog. This type of research in particular has severe limitations.

The different types of serotonin receptors differ in both structure and function in different animals, so caution must be taken in extrapolating conclusions from ani-

mals to other species, including humans[34]. In particular, in 1991 other researchers reported that the 5-HT_2 receptor subtype, the very type studied at CXWMS, shows apparent differences with some antagonist drugs which may be species-dependent[35].

No wonder that others recently wrote that: "*Understanding of the physiological role of 5-HT has been hampered by the multiplicity of receptor subtypes and the complexity of their pharmacology, discrepancies between binding studies and functional tests, and by species and tissue differences*"[36].

t-pa fails to impress

Studies of tissue plasminogen activator (t-PA) in dogs are also seriously compromised.

When t-PA was first suggested as a potentially useful clot-buster, it was evaluated elsewhere in dogs. The researchers involved produced arterial thrombosis in dogs by inserting a copper coil in their healthy blood vessels to induce fibrin-rich clots. They used less than a dozen dogs for this, before clinical testing in humans began. Speaking specifically of this, researchers at the pharmaceutical company Hoffman-La-Roche wrote in 1990 that: "*The ultimate model is humans and the ultimate evaluation is a well-defined population of patients*"[30].

Their caution was well justified, for in clinical use t-PA has not lived up to the promise shown by coronary thrombosis artificially induced in experimental dogs.

T-PA was introduced to clinical practice in 1987 and quickly became the darling of American cardiologists, even though it cost ten times more than streptokinase[24]. But in 1991 it was reported that: "*The largest heart attack study ever carried out has reported that a 30-year old drug* [streptokinase] *is as effective at preventing death from heart attack as* [the] *expensive modern agent t-PA - and it may even be safer*"[37].

T-PA caused significantly higher levels of stroke in treated patients. Worse, the strokes were caused by bleeding in the brain, rather than blockage of blood vessels in the brain[37]. Strokes caused by bleeding are generally more serious and disabling than those caused by blockage[38].

By 1992 the results of two large international and Italian trials (known by the acronyms ISIS-3 and GISSI-2 respectively) seemed to vindicate the use of streptokinase. Streptokinase works as well as t-PA, but is less likely than t-PA to cause strokes in treated patients[39].

Subsequently another international trial [known as GUSTO] appeared to show that t-PA was more effective than streptokinase; however, it still showed that 2 out of every 1,000 people treated with t-PA would have a stroke as a result of the treatment. The leader of the earlier ISIS-3 trial dismissed the GUSTO findings, saying that their significance may have been exaggerated in the statistical analysis; he was quoted as saying: "*What earlier studies suggested was that any difference between the two drugs is likely to be small. And that's what this new study found*"[40].

Nevertheless, the CXWMS team reasoned that t-PA and a serotonin antagonist used together might be better than t-PA alone. It was - and they consider their dog model to be better than the copper-coil model used in the initial tests of t-PA carried out elsewhere[27].

They concede that the clotting systems of dogs and humans are different, but write that: "*Despite these species differences, there was clear evidence of increased bleeding time and decreased fibrinogen levels during rt-PA administration*"[27].

We now await clinical trials in human patients.

3.1.1 (b) Dogs in Heart Failure Research

Studies involving rapid pacing of dogs' hearts have been carried out by a team at CXWMS since at least the mid 1980's. For example, one project reported in 1987 used 7 greyhounds[41]; a 1988 project used 7 mongrels[42]; another in 1988 used 10 greyhounds[43]; one in 1989 used 17 mongrel dogs[44]. See section 2, para 2.1.2, for details on dogs in this research.

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Dog at CXWMS with a recently implanted pacemaker.

Diary 18.7.94 (also 22.4.94): *R was upstairs with the dogs this morning. On the way home he said the one remaining labrador x was in a bad way, very bloated and with - drawn. It cowered in a corner, and when he touched it to try to encourage it to move, it yelped and spluttered. He was upset that the animal was suffering, and concluded: "It's not on, really""*

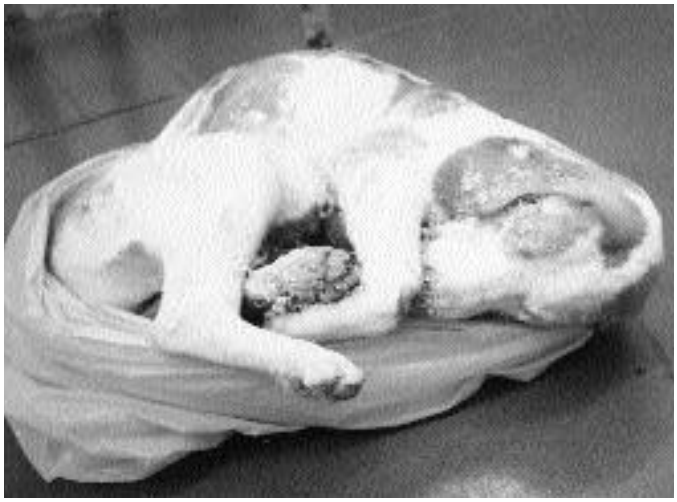
We do not know for certain who the Project Licence holder is for these experiments; however, Noble appears to be the senior academic on the team, and our investigator was told the experiments were being carried out by Noble and John Hynde.

As of June 1993, Hynde was described as "*research coordinator*", and funded by the Garfield Weston Trust[9]. In the two recent publications by Noble relating to pace-makers, Hynde was not mentioned as co-author; he is presumably new to this pro-ject.

background to heart failure

The pumping action of the heart normally maintains a balance between cardiac output and venous return. Congestive heart failure occurs when the pumping efficiency of the heart is impaired; it can affect the left or right sides of the heart individually, or both together[45].

Where the right side of the heart fails, blood collects within the tissues and organs. This stagnant blood prevents distribution of nutrients and oxygen, and prevents the disposal of wastes. The ankles and feet swell[45].



Frozen, dead beagle that had been used in a pacemaker experiment at CXWMS.

Where the left side of the heart fails, even though the right side is delivering blood to the lungs the left side of the heart can not cope with blood returning to it from the lungs. The lungs become congested with blood, pressure within them increases, and fluid leaks from the blood vessels into the air spaces to cause pulmonary oedema. It can result in suffocation[45].

Both sides of the heart can fail at the same time, or failure of one side can cause excess stress and lead to failure of the other as well. When both sides fail the result is collection of blood in the lungs and body tissues, and a dangerous damming up of blood (congestion) in the veins which return blood to the heart[45].

In humans congestive heart failure is known to be caused by three main factors.
(1) blockage of the coronary blood vessels (atherosclerosis), which impairs delivery of blood and oxygen delivery to the heart muscle. The heart becomes increasingly short of oxygen and contraction become inefficient.
(2) Persistently high blood pressure, which means that the heart muscle has to work harder to force open the aortic valve and pump blood around the body. The heart muscle enlarges with the effort, but becomes progressively weaker.
(3) A series of heart attacks will lead to reduced efficiency because dead heart cells are not replaced by new cells; they are replaced by scar tissue[45].

studies of pacing in dogs

We describe only the most recent published experiments; the results of earlier ones are mentioned in the subsequent critique.

In November 1990 Noble's team described the use of six anaesthetised dogs with artificially-induced heart block, their heart rates controlled by pacemaker. This was a study of how pacing affects the force of contraction of heart-muscle. It was funded by three organisations: one researcher from Canada was funded by the Heart and Stroke Foundation of Canada and the Alberta Heritage Foundation for Medical Research. Noble was funded by the Garfield-Weston Trust[46].

In 1992 the team described the use of rapid right-ventricular pacing to induce congestive heart failure in six mongrel dogs. This was to study the sequence of hormone changes caused by heart failure, and according to the team: "*All studies complied with the United Kingdom Home Office regulations governing the care and use of laboratory animals*"[47].

Pacemakers were implanted under anaesthesia, and the dogs allowed to recover. Their hearts were paced at 250 beats per minute until there were clinical signs of heart failure, on average after five weeks. Heart failure was indicated by rapid breathing, lung crackles, swelling of the paws, fluid in the abdomen, and loss of appetite. Blood samples were taken weekly for hormone analysis[47].

The experiment was funded by Charing Cross and Westminster Medical School and the Garfield-Weston Trust. The team concluded that: "*Further experiments appear warranted with this experimental method in order to try and elucidate more precisely the interrelationships between the changes demonstrated and fluid retention*"[47].

critique of pacing experiments in general

Recent clinical studies are showing that the interpretation of heart failure may not be as simple as once believed. Researchers at the University of Minnesota Medical School wrote in 1990: "*Insights gained in recent years have made it clear that heart failure is not a simple response to a depression of ventricular performance. Indeed, attempts to reproduce the syndrome by acutely damaging the myocardium in experimental animals have for the most part failed*"[48]. [Our emphasis].

This was hardly surprising; as long ago as 1967 it had been suggested that: "*Perhaps all cardiomegalies* [enlargements of the heart] *produced in experimental animals do not attain the degree of enlargement which could be observed in human pathology*"[49]. Since enlargement of the heart is a common feature of heart failure[45], this would indicate at least some differences between the human disease and animal models.

The enlarged heart in experimental animals is not subject to such large reduction in blood flow as the diseased human heart. Researchers at St.Mary's Hospital in London wrote in 1991: "*Abnormalities of coronary perfusion are present in animal models of left ventricular hypertrophy, although the impairment of flow reserve is usually considerably less than in clinical studies*"[50]. However, this could be due to yet another species difference between dog and human hearts.

Reduced blood supply to the heart muscle is a characteristic feature of naturally-occuring heart failure in humans[45]. Since dogs have a much better coronary circulation than humans to begin with, this too could cause difficulties in interpretation of heart failure and heart attack experiments.

In fact as recently as 1992, researchers at the National Heart and Lung Institute in London expressed the opinion that the rabbit would be a better model than the dog.

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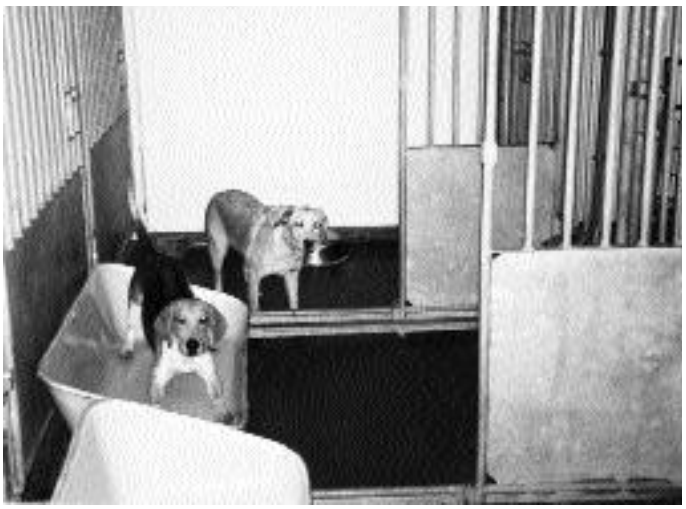
Speaking of heart attack in particular they wrote: "*Measurement of myocardial blood supply is of particular importance in canine models, which have been used for a large proportion of these studies* [of myocardial ischaemia]. *In this species, the myocardium has a variable, but often substantial, collateral blood supply. It is therefore of importance to measure the blood flow within the ischaemic region. In contrast, other animals, such as the rabbit, have virtually no collateral circulation, which is more akin to the situation in patients presenting with acute myocardial infarction in the absence of a long prior history of angina pectoris. Much of the controversy over interpretation of experimental findings from canine studies in vivo is due to dispute over the contribution made by collateral arteries*"[51].



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Beagle and mongrel at CXWMS - both have had pacemakers implanted.

Rapid pacing in dogs is just a way of inducing heart failure by ventricular overload. Many other techniques have been introduced over the years to achieve the same effect in dogs and cats, but all have their own particular difficulties, shortcomings and limitations[52]. This applies equally to rapid pacing.

Some key features of heart failure in humans are not reproduced in rapid-paced dogs. For example, writing in 1995, researchers at the National Heart and Lung Institute in London pointed out: "*There are points of similarity between the contractile responses of myocytes from the hearts of paced dogs and those from failing human heart, where the depression of contraction amplitude is also frequency dependent. However, the slowing of contraction and relaxation and the reduced sensitivity to thapsigargin, which are key features of human heart failure, are not reproduced in this [dog] model*"[53].

According to the CXWMS team's published report, although rapid ventricular pacing was first used by others to produce heart failure in dogs during 1962, it is only since 1982 that researchers have shown it to induce blood pressure and hormonal changes, as well as structural changes detectable by ultrasound, which are similar to those seen in human heart failure. The team cites just three references from the scientific literature to back up their assertion that the paced-dog model resembles human heart failure[47], so there is clearly not much evidence to support the suggestion. With greater experience, no doubt other shortcomings will become apparent; they already are.

As already outlined, in humans congestive heart failure is known to be caused by blockage of the coronary blood vessels, persistently high blood pressure, or a series of heart attacks[45]. In contrast, the CXWMS team says that the way in which rapid pacing causes congestive heart failure in dogs is not known[47]. Whatever the mechanism, it is unlikely to be the same one that produces heart failure in patients, who are never rapidly paced.

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For example, in a 1984 clinical study of patients suffering from rapid heart beat, the CXWMS team found that urine production (diuresis) in humans appears to be a result of reduction in levels of the circulating hormone, arginine vasopressin. In 1987 they described experiments in greyhounds rapidly paced for one hour, in which they had looked at the effect of pacing on urine production. The experiment in paced greyhounds was unable to confirm their earlier clinical findings: "*In a clinical study of an atrioventricular nodal re-entry tachycardia, [we] found that water diuresis occurred after reduction of plasma arginine vasopressin (AVP) concentration to half its control value, which was accompanied by the virtual disappearance of urinary AVP. The present study* [in electrically heart-paced dogs], *although suggestive, did not confirm inhibition of AVP secretion as the mechanism of the water diuresis in the tachycardia-polyuria syndrome*"[41].

They repeated the same message one year later in another publication, after more short-term rapid pacing experiments on greyhounds; their findings still appeared to contradict their clinical findings in patients with a rapid heart beat: "*These haemodynamic changes* [in electrically heart-paced dogs] *were associated with a substantial increase in free water clearance and a nonsignificant reduction in plasma arginine vasopressin (AVP) concentration. In a clinical study of an atrioventricular nodal re-entry tachycardia, [we] found that the tachycardia resulted in a large increase in left atrial pressure accompanied by water diuresis which occurred after reduction of plasma AVP concentration to half its control value*"[43].

However, the 1988 publication was really about atrial natriuretic peptide, a circulating hormone believed to be responsible for salt excretion in the urine. Despite several published clinical reports (including one of their own) that increased urinary salt excretion occurs in patients with intermittent rapid heart beat, this did not occur in rapid-paced dogs, and the team speculated about why this should be so[43].

In 1989 the same CXWMS team, now joined by Noble, carried out more rapid pacing studies on levels of atrial natriuretic peptide in mongrel dogs. They expressed the

opinion that: "*Increases in atrial pressure give rise to complex changes in haemodynamics, circulating hormones, and renal function, which may differ according to the experimental [animal] model employed. ..... Care should therefore be taken in extrapolating the results from experiments employing one [animal] model to those using others, since some of these changes may be a direct consequence of the initial stimulus rather than acting via increased atrial pressure*"[44].

They continued: "*Pacing caused a rapid rise in plasma atrial natriuretic peptide (ANP) concentrations in both groups* [of dogs], *in agreement with our previous findings in normal dogs. In contrast, rapid atrial pacing in rabbits has been reported* [by others] *to produce a more gradual rise in plasma ANP, a difference that might be attributable to interspecies variation*"[44].

Yet, despite acknowledging in 1989 that species differences exist, in 1992 they were still reporting studies of atrial natriuretic factor in paced dogs[47].

Cris Illes/National Anti-Vivisection Society

critique of the pacing experiment described above

In the 1992 heart failure experiment described earlier, the team's measurement techniques are dubious; they had intended to assess the changes in fluid retention caused by developing heart failure, by measuring the dogs' weights. They were unable to achieve this, because the dogs stopped eating and this reduced their weights; it made it impossible to gauge the increase in weight caused by fluid retention. They pointed out that the same problem had faced other researchers before them[47], so we can only conclude that their falling into such a well-known trap is indicative of poor experimental planning.

The team eventually had to measure fluid retention by how much weight the dogs lost when pacing was finally stopped; as their hearts recovered over the next five days, body weight fell rapidly as the dogs passed increased amounts of urine[47].

The team didn't say whether the dogs began to eat again after pacing stopped and as the excess fluid was excreted; if they did, they would have put on weight again and the results would still be useless.

Some of their findings on hormone levels in dogs were similar to what was already known from clinical studies, by others, of human heart-failure patients. For example, the findings of increased levels of arginine vasopressin and noradrenaline in plasma, had already been reported by others from clinical studies of heart failure patients during the early 1980's. The findings of reduced levels of plasma atrial natriuretic factor, whilst not specifically shown in an earlier clinical study by others, had at least been suggested by it[47]; like the findings on vasopressin and noradrenaline, the precise effects on atrial natriuretic factor could also have been confirmed through clinical study.

Some of the team's findings, for example those on time to onset of heart failure, and on plasma levels of the hormone renin, contradicted findings by other researchers who had used beagle dogs. In the CXWMS team's study, plasma renin remained at normal levels until heart failure set in at five weeks, when it increased rapidly; the earlier beagle study had shown a gradual rise in plasma renin during pacing, with heart failure setting in at just two weeks. The CXWMS team speculated that this might be due to "*subspecies differences*"[47], an apparent extension of our normal criticisms based on species differences.

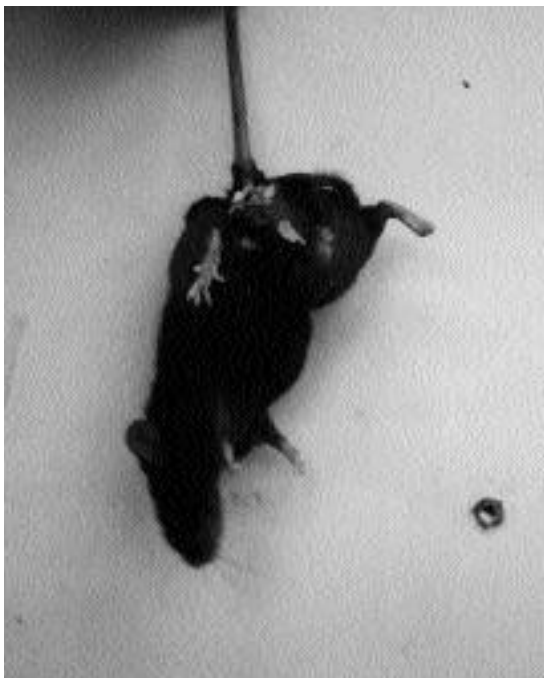
If they believe that mongrels and beagles are dissimilar, they can not possibly justify extrapolating their own research from mongrels to humans. Although some of their results have been similar to earlier clinical findings, they can not be predictably so. Since others are clearly carrying out clinical research in this field, their results will necessarily be more valid for human patients.

At least some researchers appear to doubt the value of studies of atrial natriuretic factor [peptide] in dogs. One from Ninewells Hospital and Medical School in Dundee wrote during 1994: "*10 years of natriuretic peptides research has raised several possible clinical uses both in helping diagnosis and in treating cardiorenal disease. The next ten years should establish whether any or all of these potential uses actually bears fruit. A final word of caution is that many of the potential therapeutic benefits have as yet been seen only in animals, and humans have the unfortunate habit of not benefiting from new drugs as much as animals. Only time will tell*"[54].

3.1.2 Depts of Anatomy, Biochemistry, and Histopathology: Muscular Dystrophy

The single most prolific source of research papers originating from CXWMS is the study of muscular dystrophy, mainly using the mdx mouse. It is a multi-departmental project.

Mdx mouse at CXWMS



Gris Iles/National Anti-Vivisection Society

Again we limit our discussion to the 1990's, although one researcher (T.A.Partridge) has been studying muscle cell transplants in mice since at least 1978, when he was in the Dept. of Experimental Pathology; he was being funded even then by the Muscular Dystrophy Society of Great Britain[55]. Partridge features in virtually all of the research into muscular dystrophy which we are about to discuss, but in May 1994 he moved to the Royal Postgraduate Medical School[56].

background: muscular dystrophy

Muscular dystrophy is a wide-ranging term, covering a number of inherited muscular disorders. The most common and devastating form is Duchenne muscular dystrophy; it afflicts boys only, appearing in 1 in 3,500 live male births. It usually appears by the age of 5 years, resulting in weakness, clumsiness or delay in walking, and apparent enlargement of the muscles. There is then

a relentless decline in muscular function, leaving the patient in a wheelchair by the age of 10 years, and dead from respiratory or heart failure by the early 20's[57].

There is a related form known as Becker muscular dystrophy, but this is milder; it appears later in life, is less incapacitating, and may have little effect on longevity[57].

For many years research has centred on the possibility of transplanting healthy muscle cells into the muscles of dystrophic patients. Like other researchers around the world, CXWMS researchers have been active in this field, carrying out experiments on animals which have as yet failed to translate to clinical benefit in patients.

Much of this work has been carried out on strains of mice with diseases which resemble muscular dystrophy in humans. The most popular mouse for this research is known as the strain mdx; like Duchenne muscular dystrophy in humans, this mouse model is caused by an x-chromosome-linked absence of the muscle protein dystrophin. This results in an initial extensive degeneration of skeletal muscles[58], although there the similarities end. We have more to say on this later.

The genes for Duchenne and Becker dystrophies were identified elsewhere during 1987, breaking a long period of stagnation in research into these diseases[57]. The isolation in 1987 of the gene responsible for Duchenne muscular dystrophy in humans raised hope, worldwide, that it might be possible to use muscle cells as vectors to carry normal genes into recipient muscle, thus providing a method of introducing missing genes into dystrophic muscle[59].

implanting myoblasts into nude mice

During the 1980's the CXWMS team successfully implanted immature muscle cells (myoblasts) into growing or regenerating muscles of both healthy and dystrophic mdx mice. They found that such implanted cells could establish themselves and continue to grow. However, existing methods for analysis of cellular and molecular events following muscle cell transfer were inadequate to answer a number of fundamental questions[60].

In 1991, in a project funded by Action Research and the Muscular Dystrophy Group of Great Britain, the team used athymic (nude) mice (reduced immune system) bred with the mdx gene as a mouse model. They found that the position of donor muscle cells growing in new host muscle could be located by the technique of in situ hybridisation, and remarked that this technique would be useful in the further analysis of muscle cell transfer experiments[60].

studies of immunological rejection in mice

Also in 1991, the team set about finding a way to prevent the immune rejection which would follow muscle cell transfers carried out in human muscular dystrophy patients; they regarded this as a "*major impediment*" to the procedure. In experimental animals, rejection could be overcome by various techniques which could not be used clinically. Human patients have to rely on immunosuppressive drugs, which have side effects[61].

Funded by the Muscular Dystrophy Group of Great Britain and Northern Ireland, they found that implanted muscle cells from unrelated animals could survive longer in recipient mouse muscle if they were injected as a suspension, rather than as

minced tissue as in previous experiments. The team speculated about the immunological reasons for this, but came to no firm conclusion. Still, they decided that: "*Whatever the nature of the failure to reject implanted donor precursor cells, it is clear that even the simple step of disaggregating muscle improves the survival of allogeneic muscle precursor cells and enhances the possibility of using them to introduce genes, and their products, into the muscle fibres of patients suffering from inherited myopathies*"[61].

implanting C2 cells into nude mice

There is available a cell line of muscle cells, established elsewhere in 1977 from strain C3H mice, and known as the "*C2 cell line*". These cells have the ability to fuse into muscle fibres when grown in tissue culture, and have been much used by researchers around the world to study the differentiation of muscle cells in vitro[62].

In 1991 researchers elsewhere injected C2 cells back into the muscles of adult C3H mice, the strain from which they had originated. The injected cells formed muscle fibres in live mice as well[62], confirming the in vitro findings.

In 1992 the joint Charing Cross / Guy's Hospital team decided to try similar experiments with nude (athymic) mice, animals which lack an immune system and so will not reject implanted foreign tissues[62]; rejection was not a problem with the earlier C3H experiment carried out elsewhere, because the recipient mice were of the same strain as those from which the cell line was originally established.

Funded by the Muscular Dystrophy Group of Great Britain, the team, along with colleagues from Guy's Hospital, tried implanting C2 cells into grafts of fresh or freeze-killed leg muscle tissue already removed from the same recipient animal (autografts). At periods up to 76 days after implantation, the grafts were removed and examined for the presence of C2 cells[62].

They found that when implanted into regenerating muscle of nude mice, C2 cells grew and fused with the host muscle cells to form mosaic fibres; however, when they were implanted into non-regenerating "inert" parts of the body, they simply produced muscle fibres of C2 origin alone. The C2 cells implanted along with freeze-killed muscle cells eventually became cancerous, and the researchers speculated that those implanted along with live muscle might also become cancerous if left long enough[62].

legislative compliance

The joint team concluded that this was a good way of growing large quantities of muscle cells for biochemical analysis, or for growing transgenic cells. They found that live mice were far more productive than tissue culture for this type of cell, and that: "*the amount of muscle obtained from a single graft, regularly between 20 and 30 mg, greatly exceeds that which could be produced in tissue culture at comparable cost, time and effort*"[62].

Researchers must fully evaluate the use of alternative methods before carrying out any piece of research; yet here we see British researchers, funded by the Muscular Dystrophy Group of Great Britain, suggesting the use of live animals simply because they are more productive than an alternative technique which does the same job[63]. Please refer back to our earlier section 1.3 Legislative Compliance.

biochemical studies of mdx muscle

Also in 1992, another CXWMS team carried out biochemical studies on muscle cells removed from an unspecified number of mdx mice. These animals had been grown in germ-free (SPF) conditions at CXWMS, and the project was part-funded by Action Research[58].

This team concluded, from in situ DNA polymerase labelling studies, that single-stranded DNA breaks are intimately associated in time and space with regeneration of dystrophic muscle in mdx mice. They expressed the opinion that such studies could be used to work out the mechanisms that regulate new gene expression during muscle regeneration in human muscular dystrophy patients[58].

In fact, they had already carried out a pilot study in human patients, using clinical biopsy material. These had given results "*reminiscent of*" those already seen in mdx mice, reinforcing the potential value of this type of investigation[58].

Yet if this type of experiment can be carried out on human biopsy material, there was no point in carrying out the initial studies on the wrong species - the mdx mouse.

implanting C2 cells into mdx mice

In 1993 D.J.Watt and J.Karasinski at Charing Cross enlisted the help of the Scanning Electron Microscope Unit at Leicester University, for further studies on mdx mice. This was funded by the Leverholme Trust[59].

This team extended the earlier work of implanting C2 muscle cells into athymic (nude) mice. This time C2 cells were implanted into the leg muscles of dystrophin-deficient mdx mice, to see if they would grow in that strain as well[59].

Three weeks after the C2 cells were implanted, the muscles were removed from the mice and examined for the presence of C2-based cells. They found C2 cells incorporated into the muscle into which they had been placed, and also into adjacent muscles; the implanted cells were producing dystrophin[59], the protein normally lacking in mdx mice and muscular dystrophy.

Even so, the researchers were not, apparently, over-optimistic: "*In contemplating myoblast transfer to humans, however, the question remains as to whether a muscle repopulated with precursor cells from an exogenous source would relinquish some of these implanted cells to adjacent muscles to allow their repopulation. Certainly in the mdx mouse, the present study and* [earlier studies at CXWMS] *suggests that this would occur, but distances to be migrated are minimal in comparison with the human and mdx fibres do not become surrounded by swathes of connective tissue, so characteristic of Duchenne muscular dystrophy*"[59].

fibroblast transfer

Healthy connective-tissue cells (fibroblasts) from skin carry the gene for dystrophin, although skin cells themselves contain little dystrophin; it is a muscle-specific protein. However, in theory this gene could be transferred to the muscle cell, enabling it to produce its own dystrophin. In 1989 it was reported from the USA that when developing dystrophic muscle cells and healthy skin fibroblasts were cultured togeth-

er in vitro, the skin cells fused with the muscle cells. Up to 28% of the muscle cells subsequently produced dystrophin[64].

The American researchers involved did not know if this would also happen in patients, or even in living animals, but wrote that: "*If fibroblasts can fuse with developing muscle in vivo, they might serve as a useful donor cell-type for the treatment of genetic diseases of muscle in humans*"[64].

Fibroblasts are easy to remove from the skin[65]. So, although the Americans used tissues from dystrophic mice for their experiment, there is no obvious reason why they could not have used human biopsy material. This would have saved time and a whole stage of research.

implanting fibroblasts into mdx mice

A CXWMS team decided to extend this research, but as usual they used the mdx mouse. Skin fibroblasts removed from healthy baby mice were implanted into the muscles of dystrophic mdx mice, and some of the muscle fibres were subsequently found to be producing dystrophin; it even appeared that some of the fibroblasts had converted to muscle cells[66].

Consequently, the team speculated that it might be possible to remove skin fibroblasts from muscular dystrophy patients, insert the missing dystrophin gene, then implant the patient's own fibroblasts back into his muscle. This would have the benefit of preventing any immune response and subsequent rejection[66].

critique of muscular dystrophy research

In 1991 Partridge published a review of muscular dystrophy research; he wrote that as recently as the mid 1980's, it was reasonable to say that researchers had learned very little from animal models of muscular dystrophy - or, indeed, from clinical studies of the disease[67].

But he felt that in 1991 this no longer held true: "*On the contrary, animal models of Duchenne muscular dystrophy have played a crucial role in shaping our current view of this disease and are destined to form the major route to elucidation of its pathogenesis. The development of ideas in relation to animal models of muscular dystrophies offers a valuable insight into the values and limitations of animal models of human disease*"[67].

animal models are different

Yet of the most commonly used animal model, the mdx mouse, he conceded: "*Initial excitement, aroused by the possible genetic homology of mdx with Duchenne muscular dystrophy and Becker muscular dystrophy, was soon quenched by reports that overall clinical and pathological characteristics of the mdx mouse bore little resemblance to any human dystrophy. The animal was not noticeably weak or clinically compromised. In fact, after an early transient phase of slight weakness, its muscles grow larger and stronger than normal and the animals remain healthy and active throughout a more or less normal lifespan*"[67].

Numerous published reports from many laboratories stress the differences between Duchenne muscular dystrophy and that in the mdx mouse[68,69,70]. Further, it is acknowledged that all of the other existing animal models also differ in some ways from Duchenne-type muscular dystrophy in humans[71].

muscle cell transfer

Despite these contradictions between human and mdx mouse dystrophies, Partridge thought: "*Most obviously, identification of dystrophin as the missing protein in both Duchenne muscular dystrophy boys and mdx mice, raises the prospect of replacing this protein and, at the same time, provides a model system for testing the feasibility of replacement therapy. The long-standing idea of introducing the missing protein into dystrophic muscle by means of grafts of normal myogenic cells has been examined [by us] and been shown not only to be practicable but is also probably beneficial in mouse models*"[67].

Despite the known limitations, the discovery of several animal models of Duchenne muscular dystrophy boosted research into muscle cell transplants[72].

Whilst others were using mice, several North American teams opted for direct studies in humans instead, to assess the benefits of transferring muscle cells[72]. The donor cells were taken from close relatives of the patient[65].

One such attempt, by P.K.Law, followed 23 years of animal research[73]. In 1989 Law initiated trials on human subjects[74], but although he claimed some clinical success, his results were "*seriously questioned by his peers at the First International Congress of the Cell Transplant Society*". Medical journal *The Lancet*, reporting on the conference, decided that there was "*undue optimism over human myoblast transfer*"[75]. Many muscular dystrophy researchers contend that Law's study was seriously flawed, mainly because he didn't even have a control group of patients to test whether the apparent benefits were illusory[74].

Later clinical experiments by others could not confirm Law's results, and didn't show much hope that muscle cell transfer can be a successful treatment for Duchenne muscular dystrophy[76]. *Scientific American* reported that "*the results of these experiments have been underwhelming*"[74].

One such American clinical experiment, conducted by H.Blau and colleagues after their own animal experiments, was reported in the British science journal *Nature* in April 1992. Blau had injected cultured muscle cells into up to 100 injection sites in the leg muscles of eight patients with Duchenne dystrophy[77]. *Nature*, on its "*contents*" page, described Blau's experiment as a "*transplant success*"[78], but in fact it was an unequivocal clinical failure[72].

cxwms researchers think mdx is a good model

In 1992 Partridge and his colleague Jennifer Morgan published a review of cell transplantation in the mouse mdx model of muscular dystrophy. These studies had shown that muscle cell transfer was possible, giving rise to dystrophin production at the desired site, although it was still not clear whether there was an associated improvement in muscle function. Further: "*It should be emphasized that in these experimental studies, especially those concerned with myoblast transplantation, the best results have been achieved under idealised conditions which could not be realized in the same way in man*"[79].

In 1993, long established Partridge, by now Reader in Experimental Pathology at CXWMS, published another review paper in which he once again discussed animal

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Mdx mouse at CXWMS.

models of Duchenne muscular dystrophy. By now he regarded the mdx mouse as a "genuine animal model" of the human disease, but conceded: "*The mouse remains active and healthy, enjoying a more-or-less normal lifespan; muscle regeneration is so effective that there is real hypertrophy* [overdevelopment], *while fibrous and fatty changes are minor*"[57].

Earlier in the same article he had described how, in humans, Duchenne muscular dystrophy causes muscular weakness and, although the muscle appears to overdevelop, this is due to gross overdevelopment of fibrous and fatty tissue in the muscle fibres. Death follows by the very young age of age of twenty years[57].

These features are all in stark contrast to the benign disease in mdx mice. To us at the NAVS, the mdx mouse does not sound like a genuine animal model of the human disease. At best, the mdx mouse in its early stages has a few characteristics that resemble the human condition.

more clinical failures

Whilst researchers at CXWMS were experimenting with muscle cell grafts into mice, others elsewhere were still attempting to adapt the techniques to human Duchenne muscular dystrophy patients.

Another trial looked at the efficacy of muscle cell transfer, again after promising results in animal studies. However, the trial showed little or no persistence of injected muscle cells in 11 of the 12 boys treated, and there was no improvement in any of the patients over the six months of the trial[80].

In 1994 J.E.Morgan at CXWMS admitted that: "*Experiments in mice have supported the idea of treating Duchenne muscular dystrophy (DMD) by implanting normal muscle precursor cells into dystrophin-deficient muscles. However, similar experiments on DMD patients have had little success*"[76].

Despite the extremely positive results of the animal studies, including their own, in 1995 CXWMS researchers were again forced to concede: "*The results of such* [clini-

cal] *trial have been disappointing, since, following implantation, extremely few fibres expressed the donor gene product and did not provide sufficient numbers of dystrophin-positive fibres to alleviate the myopathy*"[66]. It seems that the human experiments were beset by difficulties that were relatively easy to circumvent or avoid in mouse experiments[76].

the technique can't be transferred

Which makes us wonder why all this research is still going on. After all, Partridge himself was quoted in 1989 as saying of experimental muscle cell transplants in mice: "*A mouse is 3,000 times smaller than man, and you can't transfer a technique across that sort of size difference*"[81]. That was before ANY of the research described in this section.

fibroblast implants

Which brings us to the implantation of fibroblasts (skin cells) rather than muscle cells. It is too early to give a balanced opinion on the likely outcome of this very recent work, but if muscle cell transfer is anything to go by there is a rough road ahead. If it does succeed, it is reassuring to know that the technique was initially developed in vitro.

As of September 1995 the CXWMS team was fairly hopeful about fibroblast implantation, but still concerned that the implanted cells might only multiply at the site of injection, and not spread. They were about to begin experiments - on mice of course - to see whether the implanted cells would reach distant muscles. They were warning against overoptimism at this stage, saying: "*If all goes well, we might think about starting clinical trials within five years. But its a big if*"[65].

gene therapy flops as well

The trend at present is towards gene therapy, rather than muscle cell transfer[76]. Yet in 1991 a professor of biochemistry at the University of Utah poured cold water on the concept of gene therapy. He wrote that, despite numerous press conferences suggesting otherwise, discovery of the map position or nucleotide sequence of the gene that causes muscular dystrophy had not resulted in any effective treatment. Knowing the nucleotide sequence of the gene had not resulted in a single therapy[82].

Blau, whose clinical muscle-cell transplant had flopped, concurred two years later in 1993. She was reviewing a newly reported piece of research on gene therapy, carried out by other workers, and wrote that: "*Since the cloning of the dystrophin gene, one of the largest isolated to date, Duchenne muscular dystrophy (DMD) has been of great interest as a target for gene therapy. ... Although the cloning of the gene has revolutionised diagnosis, the only treatment available to DMD patients is palliative*"[83].

Blau wrote of the mdx mouse used in that latest gene therapy research: "*Unlike man, this animal is only transiently affected by the lack of dystrophin it experiences throughout life*". She continued: "*A major question is whether the beneficial effects reported ... in the mdx mouse will also pertain to DMD patients*"[83]. Her doubts were justified.

In fact, although gene therapy has cured dystrophic mice, in which replacing a single gene can prevent the destruction of muscle, some geneticists question the relevance of the work for treating humans. It does not tackle the crucial question of how to insert the gene into every muscle[84].

The creation of genetically-modified animal models for human genetic diseases such as muscular dystrophy involves inactivation of the target gene, the so-called '*gene-knockout*' procedure. This has been proven to be a successful methodology in experimental animals, but although the embryonic stem cell has been of crucial importance for the development of the mouse models, it still isn't known if there is an equivalent counterpart in the human[85].

To most people, it would seem logical to resolve this problem before wasting any more time on animal studies.

Partridge's and Morgan's 1992 review, cited earlier, also dealt with gene therapy in the mouse mdx model of muscular dystrophy. Their conclusion applied equally to myoblast transfer and gene therapy: "*It should be emphasized that in these experimental studies, especially those concerned with myoblast transplantation, the best results have been achieved under idealised conditions which could not be realized in the same way in man*"[79].

In fact, a muscular dystrophy researcher at the University of Wisconsin was quoted, recently, as saying that although mdx mice had helped him gauge the efficiency of his own gene transfer method, they "*are virtually worthless for testing treatment efficacy*"[86].

Yet in 1994 J.E.Morgan at CXWMS wrote that: "*Gene therapy for Duchenne muscular dystrophy, by introducing dystrophin constructs via retroviral or adenoviral vectors, has been shown to be possible in the mouse, but the efficiency and safety aspects of this technique will have to be carefully examined before similar experiments can be attempted in man*"[76].

Morgan may well test the efficiency of gene transfer, only to conclude, like others before, that the model is virtually useless for testing treatment efficacy.

A news item in *New Scientist*, December 1995, makes the point that all of the attempts at clinical gene therapy carried out over the last few years have failed. Not just for muscular dystrophy, but for all other gene-related diseases as well. Apparently there have been, in total, 100 clinical attempts at gene therapy around the world, and not one of them has resulted in a cure. In fact, the Director of the US National Institutes of Health wants researchers to do less clinical research and more "*basic science*". Also, to be more circumspect when talking to the media; he feels that if gene therapy is overhyped, the public will feel let down when it sees no immediate benefits[87].

Quite why he should think more basic science will make a difference is not clear. After all, animal researchers can already cure muscular dystrophy in mice; its just humans that are the problem.

At least one researcher, at the University of Chicago, is quoted as saying in October 1995 that gene therapy is already grounded in a set of solid scientific principles[80]. It seems that the experts can't agree about what they already do or don't know.

3.1.3 DEPARTMENT OF BIOCHEMISTRY

background - ky mice for kyphoscoliosis research

Kyky mice at CXWMS.

Cris Illes/National Anti-Vivisection Society

Kyphoscoliosis in the mouse is a degenerative muscle disease, which results in chronic deformation of the spinal column[88]. These mice are used by G.Coulton.

G.R.Coulton is a lecturer in the Department of Biochemistry[89]. We have already discussed his use of SPF-bred mdx mice (See earlier, 'Biochemical Studies of MDX Muscle'), a project which appears to be going on at the same time as his kyphoscoliosis research.

Since 1987 he has been working on the molecular genetics and ultrastructural characterization of a "*murine model for human spinal muscular atrophy*"[90]. From 1993 to 1996 he has been funded by the Medical Research Council for the positional cloning of the gene encoding kyphoscoliosis, described as a "*new neuromuscular mutation in the mouse*"[90] [i.e., the ky mouse]. As of June 1993 Coulton had just been awarded an MRC grant for £242,633, period and project unspecified, just described as "*preclinical studies*"[91].

Coulton is collaborating with others at the Ludwig Institute for Cancer Research in London, St.Mary's Hospital Medical School in London, the Institute of Child Health in London, the Universite Catholique de Louvain in Brussels, the Institut Pasteur in Paris, CIBA-Geigy in Basle, the Alfred I. Dupont Research Institute in USA, and Washington University in USA. They write: "*Research is focused on three main areas: (1) a molecular, genetic and physiological investigation of an inherited neuromuscular disease in the kyphoscoliotic mouse. (2) an investigation of the role of myogenic transcription factors in the regulation of muscle regeneration in inherited muscle disease. (3) investigation of the relationship between structural rearrangements of chromatin, gene transcription and cell differentiation in vivo and in vitro. Our general aim*

is to understand the molecular genetic and cellular processes which determine the fate of skeletal muscle following degenerative muscle disease. Funded by the MRC and Royal Society"[92].

studies of ky mice

In 1992 Coulton and colleagues killed ky mice at ages ranging from birth to 210 days, to examine their tissues. They decided that hereditary kyphoscoliosis in the mouse has a neuromuscular basis, and proposed that it may be a useful model for human muscle disease and scoliosis[93].

Cris Iles/National Anti-Vivisection Society

Kyky mice at CXWMS.

In 1995 they published two more papers on ky mice.

The first was a joint study with the Brussels group, of the structure and mechanical power relationships of two muscles as isolated preparations. These were removed from ky/ky mice held in an SPF colony at Charing Cross Medical School, which had been killed with ether. All funding was Belgian[94], so conceivably the research was done in Brussels.

The second was the mapping of the ky locus to a small region of chromosome 9; this was funded by the Medical Research Council, the Science and Engineering Research Council, and pharmaceutical company Merck Sharpe Dohme Ltd[88].

critique of kyphoscoliosis research

The mouse model of kyphoscoliosis was first described in 1973, by other researchers at the ARC Animal Breeding Research Organisation in Edinburgh; it had arisen as a spontaneous mutation, and they called it ky[95].

The ky/ky mouse is relatively new, and little is known about it at this stage in comparison to the mdx mouse model of muscular dystrophy.

However, Coulton believes that mouse mutants with muscular dystrophies are important models for a variety of human muscular diseases, and are invaluable tools for the study of pathology and the underlying defects involved with neuromuscular dysfunction. He extends this belief in particular to the mdx mouse[88].

There is no doubt that when researchers have a better understanding of the ky/ky mouse it will be found to be just as inapplicable to human patients as is the mdx mouse.

3.1.4 DEPARTMENT OF MEDICAL ONCOLOGY

3.1.4 (a) Studies of Breast Cancer In Rats

Our investigator has video of rats inoculated with cancer; he says they were bought-in from an outside supplier in this state, by a researcher called Coombes; publications by Coombes (cited below) confirm that the Ludwig/Wistar rats used have NMU-induced mammary tumours [NMU is nitrosomethylurea], and are supplied in that state by Harlan-Olac in Oxfordshire.

R.C.Coombes is Professor and Head of Department of Medical Oncology, also Director of the Cancer Research Campaign Laboratories at CXWMS[96]. At the year ending July 31st 1993, he and colleague L.Bulwela had been awarded a grant of £32,952 by the MRC, for unspecified "*preclinical studies*". However, it is clear that the vast majority of his funding is for "*clinical studies*", with grants totalling over

C. Iles/National Anti-Vivisection Society

Rat cages at CXWMS.

£1,144,739 from seven sources (mainly CRC and MRC)[11].

background to breast cancer research

Breast cancer is 100 times more common in women than in men[97]. One in eight, or about 28,000 women, develop it in Britain each year[98,99], and 13,000 die from it[100].

During the 1980's, several research groups elsewhere found that the active hormonal form of vitamin D can inhibit the growth of human cancer cell lines in vitro; these include breast cancer, melanoma, colon cancer and leukaemia cells[101].

Also during the 1980's a synthetic analogue of vitamin D, calcipotriol, was found to be useful in treating the skin complaint psoriasis. It was introduced to the British National Formulary for this purpose in 1991[102].

Coombes' team acknowledges that others had already shown calcipotriol to be as effective as vitamin D on cells grown in vitro. By 1991 they also knew, from their own clinical studies of several hundred psoriasis patients, that unlike vitamin D calcipotriol does not cause dangerously high levels of calcium in the body, at least when applied to the skin of psoriasis patients[103].

So in 1991, funded by the Cancer Research Campaign, they described the use of calcipotriol ointment to treat breast cancer nodules in the skin of 14 patients. Treated nodules reduced in size in three of the patients, and the team suggested that calcipotriol merited further investigation[103]. At this stage there was no mention of animal experiments.

studies of vitamin D analogues in rats

However, following these clinical studies, in 1992 they described the effects of calcipotriol and another vitamin D analogue on human breast cancer cells in vitro. Having once again confirmed its efficacy, they tried using it by intraperitoneal injection to treat NMU-induced mammary tumours in rats; it was also effective against these cancers. During these experiments, rats whose tumours ulcerated or grew to greater than 10% of the body weight were killed by terminal anaesthesia. The project was funded by the Cancer Research Campaign[101].

Later in 1992, funded by the Cancer Research Campaign, Coombes' team joined forces with others at St.Georges Hospital for a similar study of other vitamin D analogues[104].

studies of ovariectomy and ducorubicin in rats

In 1993 Coombes' team again experimented with rats bearing mammary tumours induced by injection of NMU, and supplied by Olac in Oxfordshire. They treated them either by removing the ovaries or by giving the drug doxorubicin - both techniques are used clinically in patients. They wanted to study the growth factors which developed in serum from rats treated with the different procedures. The project was funded by the Cancer Research Campaign[105].

studies of limonene in rats

In May 1994 they published studies of treatment with limonene, a substance extracted from orange peel. Researchers elsewhere had already found it useful in the prevention and treatment of chemically-induced rodent cancers when given in the diet. The CXWMS team decided to see if limonene would work better when given with another drug, and used rats with tumours "*between 10 and 20 mm in diameter*". They found that both substances given together worked better than either given alone, and suggested that the combination be tried against other cancer types. There is no mention of who funded the project, but it was a joint one with a researcher from Celltech Ltd., Slough, and another from the University of Wisconsin[106].

critique of rodent models of breast cancer

For these animal studies, the CXWMS team is concentrating on chemically (NMU) induced breast cancers in rats.

Racks of rat cages at CXWMS.

Cris Iles/National Anti-Vivisection Society

Yet although breast cancers are common in humans and rodents, their pathology differs. The only similarity is in well-differentiated carcinomas, which are rare in women but common in rodents[107]. Researchers have been unable to find a useful animal model comparable to infiltrating breast carcinoma in women[108].

One standard rat cancer is that induced by the chemical DMBA. Yet it has important differences from human cancers. Unlike the human cancer, the rat cancer is dependent on the hormone prolactin for growth and it has a low ability to spread[109]. No doubt the NMU-induced cancer used at CXWMS will also eventually be found to quite different from human breast cancer.

Cancers in animals differ from cancers in humans[107], and the differences are vast[110]. Generally, transplanted and spontaneous cancers in animals do not spread, in contrast to cancers in humans which nearly always spread[111] and there are well known cases where animal cancers do not predict the human situation[112].

Experimental rodent cancers often have genetic mutations which are scarce in human cancers[113], and the significance of this is not known; even in experimental cancers the results are ambiguous[114].

We note that in particular, one of the CXWMS experiments on cancer therapy involved a combination of ovariectomy [removing the ovaries] and dosing with the anti-cancer drug doxorubicin[105]. The very act of removing the ovaries from rats is subject to species differences which could confound such an experiment.

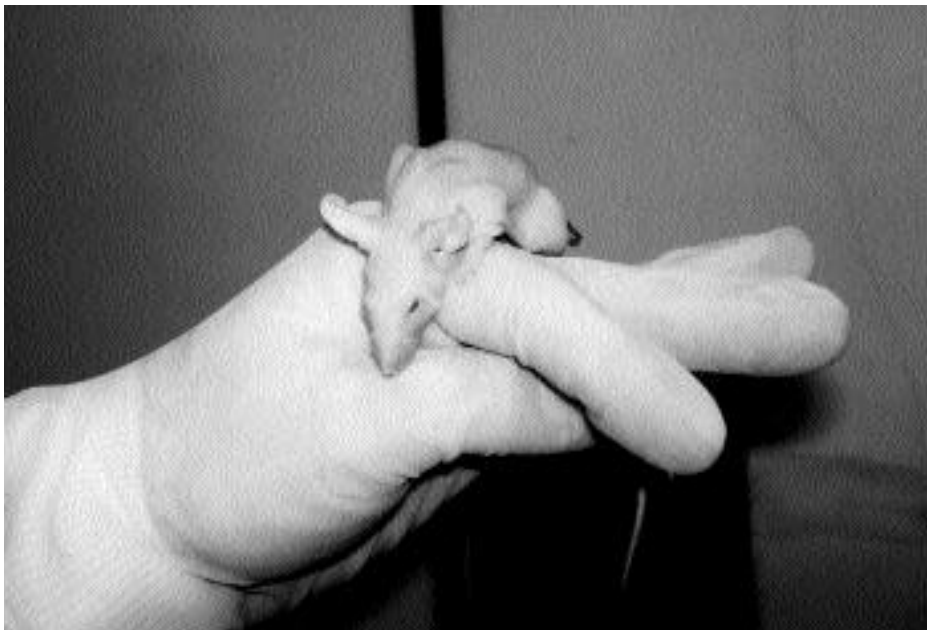
According to researchers at Heidelberg University, writing in 1991: "*Different strains of rat may respond differently to surgical procedures, particularly with regard to ovariectomy. ... In fact, preliminary data indicate that this is indeed the case*". They were referring to the effects of ovariectomy on blood cells, including those of the immune system; it affects the total leukocyte count, and also affects the relative levels of T and B lymphocytes[115], which could possibly adversely affect experimental results in a cancer treatment study.

Cancers would be expected to be antigenic in the animals in which they first arise and should stimulate an immune response. In fact a state of immunity often does exist even in animals which carry growing cancers; a state of "*concomitant immunity*" develops in which animals have developed immunity against their own cancer but the cancers continue to grow. Evidently, the immune response can only deal efficiently with a small number of cancer cells and is inadequate to reject a large cancer load[116]. Reducing the immune response by modifying leukocyte and lymphocyte production must affect the outcome to some extent.

3.1.4 (b) STUDIES OF HUMAN CANCERS IN NUDE MICE:

background to immunological carriers

Some cancer cells produce antigens, or markers on the cell wall. In healthy cells these markers are less abundant or even absent. Where the antigen is normally present in healthy cells, but its production is far greater in cancerous cells, it is called a "*tumour associated antigen*". If the alteration is more drastic and the antigen is completely new to the cancer cell, it is called a "*tumour specific antigen*"[117].



Cris Iles/National Anti-Vivisection Society

Nude mouse at CXWMS with implanted cancer.

Some of these markers may be secreted into the bloodstream, where they can be detected by sensitive immunological techniques[116]. They can also be targeted for attack by monoclonal antibodies, usually raised in animals, and usually in mice.

This is a topic of particular interest at present. Appropriate monoclonals would be expected to kill cancer cells in living animals or humans, but a more promising application is said to be the targeting of drugs or other cell-killing agents; the monoclonal is attached to a drug, enzyme or toxin subunit, and used as carrier to specifically direct the agent onto the cancer cell. In a word, the monoclonal antibody is given a "*warhead*" to destroy any cancer cell to which it binds[116].

In the Department of Medical Oncology, J.A.Boden is using nude mice to grow transplanted human colon cancers (adenocarcinomas). These are then being used in studies of monoclonal antibody targeting of radiation or drugs.

Boden is based at the Royal Free Hospital. However, he is part of a group studying Antibody Directed Enzyme Prodrug Therapy, acronym ADEPT. During 1993 Boden was working on the ADEPT project with the CXWMS team, in collaboration with others at ZENECA and the PHLS laboratory at Porton Down[118]. Our investigator confirms that as of early 1995 Boden was working at both CXWMS and the Royal Free.

Another member of this team is S.Wedge who, as of 1993, was a Research Fellow at the Medical Oncology Unit[119]. Together they were studying the use of monoclonal antibody-targeted enzyme-based drugs to treat cancer. As of 1993 they had compared a range of drugs *in vitro* and *in vivo*; ongoing work was said to include the design, synthesis and evaluation of new prodrugs, together with pharmacokinetic studies and in vitro studies of drug-cell interactions. This was funded by the Cancer Research Campaign[118].

A search shows that Boden has published a series of research papers with CXWMS personnel. All of his publications since early 1994 have been published from the Royal Free; prior to that they were published from CXWMS.

This also shows that at both places he was working on the treatment of human cancers grown in nude mice, and specifically on the use of monoclonal antibodies as a drug or radiation carriers. This is in accord with the stated aims of the ADEPT project.

The Royal Free Hospital publications on this work are all published by the Cancer Research Campaign Targeting and Imaging Group.

studies on radioimmunotherapy

In June 1994, from the Royal Free, Boden's team described studies of how radioimmunotherapy could be made more effective[120]. Radioimmunotherapy is the use of radiation attached to monoclonal antibodies, which target specific substances or "*markers*" and so, theoretically at least, will home in on cancer cells.

They wrote that although radioimmunotherapy has appeared promising in animal studies in many laboratories, clinical results have been disappointing. So they had tried to make it more effective with drugs which reduce blood flow through cancers. Their model was a human colon cancer (adenocarcinoma) cell line being grown in nude mice by subcutaneous implantation in the flank[120].

The drug selected was FAA, which had already been shown by others to be effective against solid rodent cancers and against some human cancers grown in nude mice. However, the beneficial effects which the others had seen in mice had not been seen when they tried FAA in humans. The team wrote of this: "*The relevance of these studies to human disease needs to be established, because although levels that have proved therapeutic in model systems have been achieved in patients, only limited responses have so far been observed in the clinic. The vasculature of human tumours is likely to be different from that of subcutaneous tumours in mice, and there is also evidence of site-specific action of the drug*"[120].

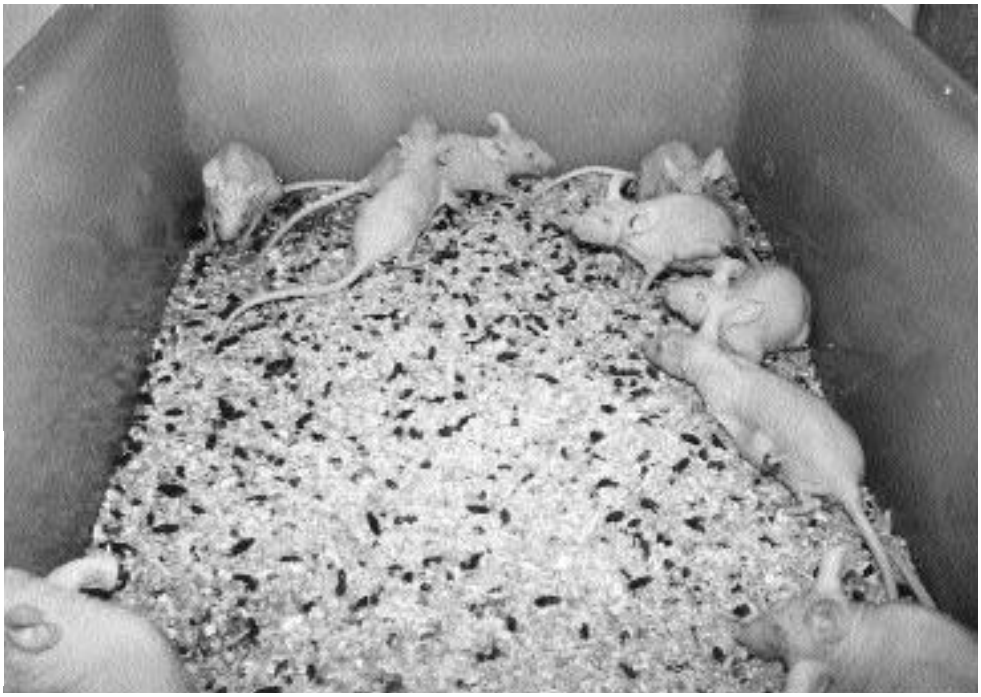
Despite this, because FAA was known to be effective in mice, they used mice for their own project. It was entirely funded by the Cancer Research Campaign[120].

studies of drug targeting

In December 1994, again from the Royal Free, they described more experiments in nude mice growing human colon adenocarcinomas. It was a study of how monoclonal antibody-targeting of drugs could be made more precise, and was funded in part by the Cancer Research Campaign[121].

studies of antibody clearance

The most recent publication was in January 1995, again published from the Royal Free Hospital. The team had grown a transplanted human colon adenocarcinoma in nude mice, and studied the clearance of a radio-labelled antibody used to treat the cancer; since persistence of radiolabelled antibody is a major limitation of radioimmunotherapy, they were looking for ways of removing the excess circulating antibody/radiation complex. The project was funded by the Cancer Research Campaign[122].



Cris Iles/National Anti-Vivisection Society

Nude mice with cancer implants at CXWMS.

critique of targeting studies in nude mice

Human colon adenocarcinomas were the first human cancers to be transplanted into nude mice, in 1969. Since then, the use of nude mice to grow human cancers has continually increased; they have been used for studies of cancer growth, development, spread and treatment[123].

So far as studies of cancer spread are concerned, human cancers grown in immunodeficient mice show differences from human cancers in their natural state. Human cancers grown in mice seldom spread, and when the host animal dies it may not even be due to spread of the cancer[124].

The doubling (growth) times of human cancers transplanted into nude mice are about one-fifth of the values seen in human cancer patients. Hence it must be expected that this will lead to exaggerated responsiveness of the cancer to chemotherapy and fractionated radiotherapy[125]. This leads to difficulties and over-optimism in the study of potential new treatments.

Although for some drugs and types of cancers the results seen in nude mice have resembled those already seen in clinical experience, differences between mice and humans with respect to drug pharmacokinetics and pharmacodynamics may lead to either overpredictionor underprediction for clinical utility[124].

There are some striking examples of this. For example, procarbazine was the most effective drug in treating human small-cell lung cancer transplanted into mice, and etoposide gave poor results in human testicular teratomas transplanted into mice[125]; both of these results are the opposite of clinical experience. In clinical practice, procarbazine is only useful for the treatment of Hodgkin's disease[126], and etoposide is a very useful drug for the treatment of germ-cell cancers[127] like teratoma. In the latter case, reliance on the nude-mouse model would have led to the potential value of etoposide being missed.

The dose limiting toxicities of drugs may also differ between species, or metabolic processes may go on at different rates; despite this, some researchers still believe nude mice are a valuable tool for drug evaluation programmes[124].

Others say that human cancers transplanted into mice are unable to predict the clinical activity of drugs according to cancer type; comparison of animal and clinical results shows no correlation between the activity of drugs against human cancers grown in animals and those generally obtained in the clinical setting for the corresponding cancer type. Further, the clinical value of any new drugs discovered by this technique remains to be seen[123].

Several researchers have noted that although the structure of human cancers grown in nude mice is similar to that of the natural cancer, the framework on which the cancer grows is derived from mouse tissue. This framework (stroma) is finer and less well developed than usual, possibly reflecting the changed growth rate[125].

Some researchers have found that human cancers growing in animals develop blood supplies structurally similar to those of the original cancer[128]. Despite this, others believe that with any treatment studies in which the blood supply plays an important role, caution needs to be taken in assuming that the transplanted cancer is a valid model for the human cancer, and more evidence is needed about this[125].

After one of their own experiments, even Boden's team was forced to concede that: "*The vasculature of human tumours is likely to be different from that of subcutaneous tumours in mice, and there is also evidence of site-specific action of the drug [FAA]*"[120].

Ever since Cesar Milstein and Georges Kohler in Cambridge described the first monoclonal antibodies in 1976, their potential value in the targeting of anti-cancer drugs has been stressed[129]. By the early 1980's monoclonals were being used in attempts to treat cancer patients[130], yet 20 years after they were introduced, monoclonals still do not work well in patients.

The use of monoclonal antibodies in treatment always had predictable difficulties, mostly because rodent monoclonals cause an immune response in the patient, and the route of administration is inconvenient. The use of so-called '*humanised*' monoclonals, modified to resemble human antibodies, may allow administration of repeat courses but still isn't an ideal solution. Further, there is no good preclinical [animal or in vitro] model for studying monoclonal therapy. Whether in vitro systems can predict for effects in life is largely unknown, and antibody types have different functions in rodents and humans[131].

It seems unlikely in principle that targeting with antibodies could be wholly effective in eradicating cancers. One predictable problem is that if just a few of the cells in the cancer failed to express the specific antigen they would survive the attack and the cancer would grow back[116].

To make matters worse, even the best of monoclonals are not very selective. In 1992 this was suggested as a reason for the poor results from treating patients with drugs attached to monoclonals[132]. And, as recently as 1994, Boden's team conceded that radiation attached to monoclonals had also appeared promising in animal studies in many laboratories, but clinical results had been disappointing[120].

The technique as developed in rodents simply does not work well in humans; it is unlikely to work in the future unless researchers bypass the rodent studies and concentrate on immunotherapy in human patients instead.

an *in vitro* alternative

There is an in vitro system which models reasonably closely some of the essential and important properties of cancers. In this system, the cancer cells grow as a round ball-shaped aggregate, and are subject to gradients in the concentrations of oxygen and nutrients that simulate conditions in many naturally growing cancers. The *in vitro* system is now very popular, although some researchers still chose to grow the "*spheroids*" in the abdominal cavity of mice[133].

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3.2 ANIMAL RESEARCH AT THE INSTITUTE OF NEUROLOGY

The Institute of Neurology, as its name implies, concentrates on research into the nervous system and brain. A wide range of projects has been carried out in recent years, including:

DEPT. OF NEUROCHEMISTRY: Studies of the epilepsy drug vigabatrin, in rats. Joint project with the School of Pharmacy. Funded by the Medical Research Council[1].

DEPT. NOT KNOWN: Studies of an animal model of epilepsy, the so-called kindling model in rats, funded by Action Research[2].

SOBELL DEPT. OF NEUROPHYSIOLOGY: Cutting or crushing some of the spinal nerves in old cats (up to 15 years old), at the point where they leave the spinal cord, to see if they would regenerate or atrophy; the project used 12 cats, and was funded by the Medical Research Council[3].

Cats at the Institute of Neurology.

Cats Illes/National Anti-Vivisection Society

SOBELL DEPT. OF NEUROPHYSIOLOGY: Studies of nerve connections between two adjacent parts of the spinal cord, in a project using 21 adult cats. Joint project with another researcher in Germany[4,5].

SOBELL DEPT. OF NEUROPHYSIOLOGY: Study of the effects of respiration on nerve impulses in the spinal cords of 13 adult cats, funded by the Wellcome Trust[6].

SOBELL DEPT. OF NEUROPHYSIOLOGY: Damaging various nerves with diphtheria toxin to study the effect on nerve junctions[7], and the way the nerves regenerate after damage[8,9]. These published reports since 1992 discuss experiments on at least 26 cats (including controls), variously and jointly funded by the Medical Research Council, the Wellcome Trust, and the Motor Neurone Disease Association

MULTIPLE SCLEROSIS RESEARCH GROUP: Joint project with others at Queen Mary and Westfield Hospital and Hammersmith Hospital. A study of experimental allergic encephalomyelitis in guinea pigs, funded by the Multiple Sclerosis Society of Great Britain and Northern Ireland, and the Medical Research Council[10,11].

However, we will concentrate on several apparently on-going projects.

3.2.1 DEPT. OF NEUROCHEMISTRY

Researchers in the Dept. of Neurochemistry are using animals to study the drug dexfenfluramine, and related molecules. We discuss three of their experiments published in 1993 and 1994[12,13,14].

Cris Iles/National Anti-Vivisection Society

Rat with dialysis probes permanently implanted in its head.

background: dexfenfluramine

In 1937 it was noticed that depressive patients treated with amphetamines tended to lose weight; this was studied further in humans, and also in animals, and found to be due to a voluntary reduction in food intake. Subsequently, amphetamines were used to suppress appetite, and as antidepressants. However, their side effects and risks of addiction are such that they should nowadays rarely be prescribed for either purpose[15].

Dexfenfluramine (d-fenfluramine) is closely related to the amphetamine drugs, from which it is derived by the addition of a CF_3 [trifluoromethyl] group. This slight chemical change has altered the pharmacological characteristics of the compound, leaving it free from central nervous system stimulation and with a low risk of abuse or dependency[16]. It causes the release of serotonin in the brain, instead of noradrenaline[15]. D exfenfluramine has been used clinically as an appetite suppressant for about ten years; during this period it has been taken by more than 5 million patients. Dexfenfluramine is derived from a parent compound [fenfluramine] which has itself been taken by more than 20 million people over the last 30 years[17] in more than 100 countries[18]. During this time there have been no clinical reports of neurotoxicity[19].

It follows that the manner in which humans metabolize dexfenfluramine, and its effects on the mind and body, are, or should be, well known from clinical study.

The researchers at the Institute of Neurology concede that dexfenfluramine has also been used extensively in animal experiments [worldwide] on the neurochemical control of feeding. Despite this, they say that exactly how fenfluramine suppresses feeding is still "*the subject of much controversy*", although it is believed by some to increase the extracellular levels of a brain neurotransmitter, serotonin (5-HT)[12].

When brain tissue is mashed up in a blender, small membranous vesicles containing neurotransmitters are released from the disrupted synapses; these vesicles are known as synaptosomes and are the basis for the serotonin theory of dexfenfluramine's activity.

Synaptosomes are regarded as a model of the functional nerve ending, complete with neurotransmitters; they retain many of the functional and morphological properties of the intact nerve ending[20]. It has been known since 1985, from in vitro studies carried out elsewhere, that dexfenfluramine and its metabolite dexnorfenfluramine both release serotonin from synaptosomes and then inhibit its reuptake[12], hence increasing the level of dexfenfluramine in the surrounding medium.

It is hardly surprising that the team consider the role of serotonin in loss of appetite to be controversial. They cite a number of research projects carried out elsewhere on rats, all of which have given totally contradictory results; these include studies with dexfenfluramine-induced loss of appetite and the effects upon this of nerve poisons known to reduce serotonin levels. Depletion of brain serotonin had been variously: "*reported to attenuate, not to affect, or enhance dl-fenfluramine induced* [loss of appetite], *while depletion by prior administration of* [a serotonin-synthesis inhibitor] *was found either not to affect or to enhance the* [loss of appetite]"[12].

studies of serotonin displacement

They tried to throw some light on the matter by carrying out yet more animal experiments, investigating whether the loss of appetite induced by dexfenfluramine and its metabolite dexnorfenfluramine were affected by depletion of serotonin or by drugs which displace serotonin at serotonin receptors in the brain. Hence, various combinations of serotonin-depleting drugs were used to deplete or displace serotonin in the brains of rats[12].

Levels of the metabolite, dexnorfenfluramine, built up in the rats' brains. However, the team decided that this did not cause the loss of appetite, because dexnorfenfluramine doesn't build up in humans like it does in rats; species differences mean that the half life of the drug in rats is more than twice that in humans[12].

The outcome of this project was that: "*Species differences are likely to be influential due to the variation in pharmacokinetics with species. Nevertheless, the present findings both underline the importance of actions at [serotonin] receptors in the pharmacological control of feeding and suggest the need to continue to investigate how fenfluramine inhibits feeding*"[12].

Which, of course, they did.

studies of sex differences

It has been known since the mid 1980's, from research done elsewhere, that a brief period of stress causes loss of appetite in rats of both sexes. If the stress is prolonged, males begin to eat again, with only the females having persistent loss of appetite[13].

The Institute of Neurology team thought this clinically relevant, since "*Women are more vulnerable than men to disorders of feeding, these disorders may well involve [serotonin] abnormalities, and there are many reports of sex differences of [serotonin] function*"[13].

Yet almost all of the animal work on fenfluramine had hitherto been carried out on male rats only; so they: "*systematically compared the effects of dexfenfluramine on feeding and on hypothalamic concentrations of the drug, its metabolite dexnorfenfluramine and [serotonin] and dopamine in male and female rats*"[13].

They found clear sex and age differences in the levels of dexfenfluramine and dexnorfenfluramine which built up in the animals' brains. 30 day-old females built up substantially higher levels of dexfenfluramine, whilst concentrations of the metabolite dexnorfenfluramine remained similar in both sexes; 100 day-old females also had higher dexfenfluramine levels than males, but significantly lower levels of the metabolite dexnorfenfluramine than in males[13].

The team concluded that: *"Although rats and humans show major quantitative differences in the hypophagic [reduced eating] potency, metabolism and distribution of dexfenfluramine, the above findings indicate the importance of further study of its effects in both sexes*"[13].

And yes, they did carry out further studies.

interaction with serotonin-synthesis inhibitors

In 1994 they reported on fenfluramine's interaction, with serotonin-synthesis inhibitor drugs, on extracellular serotonin levels in the hypothalamus, and their combined effects on feeding activity. This time, microdialysis probes were implanted into the rats' brains to determine precisely the levels of serotonin at various times and points[14]. Despite the previous results having indicated the need to study both sexes, this time they once again studied males only.

The results this time suggested that although fenfluramine significantly increased the extracellular levels of serotonin in the brain, this was not responsible for the fenfluramine-induced loss of appetite[14].

This would appear to contradict the long held theory that loss of appetite is caused by increased extracellular serotonin in the brain.

critique of dexfenfluramine experiments

According to some American researchers, mice are the best animals to use, because of similarities in drug metabolism between mice and humans[21]. Hence, it is not clear why the Institute of Neurology team is persisting with experiments in rats.

Despite this supposed similarity between mice and humans, there is currently a great deal of controversy over the safety of dexfenfluramine; all of it caused by the contradictory results of animal experiments, a direct result of species differences and equally applicable to non-toxicological studies[22].

Cris Iles/National Anti-Vivisection Society

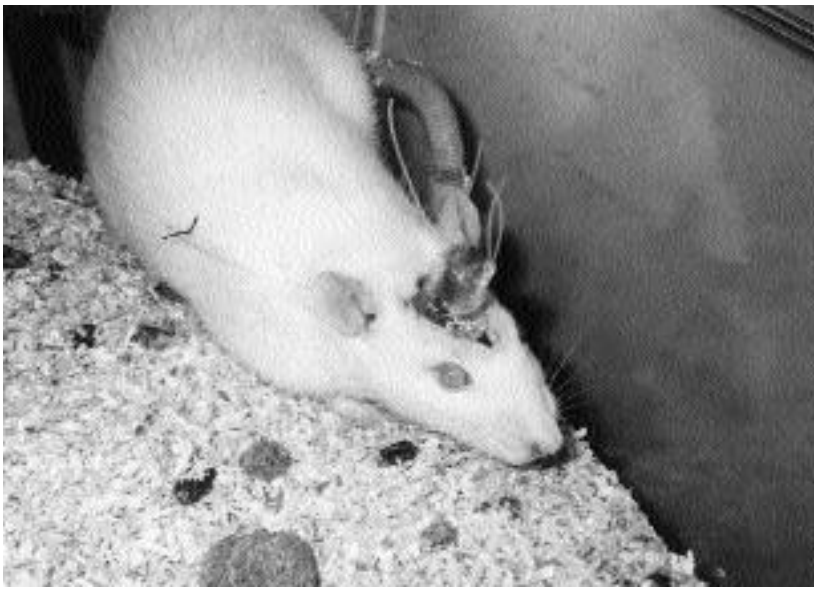
contradictory results in mice and monkeys

Researchers at the prestigious Johns Hopkins School of Medicine had studied fenfluramine in squirrel monkeys and mice. They found that in both species it caused serotonin deficits in the brain, which persisted long after fenfluramine treatment was stopped[21].

However, in 1994 the US Environmental Protection Agency disagreed with this result. They found no long-term serotonin deficit in mice; in fact, of all the amphetamine-related drugs tested, fenfluramine was the only one which didn't cause persistent changes in the brain[23].

In short, their results in mice were the exact opposite of those obtained in mice at Johns Hopkins. Since the results seen in mice were contradictory, the tests were a waste of time; and remember that according to some researchers, mice are supposed to be the best model of dexfenfluramine metabolism in the human!

rat results inapplicable to humans



Cris Iles/National Anti-Vivisection Society

The Johns Hopkins tests prompted a researcher at the University of Leeds to comment that: "*This compound is very actively metabolised in man but inefficiently so in the rat and squirrel monkey, which leads to much higher brain concentrations in these animals than would be found in man. For these reasons, the changes seen in the squirrel monkey are unlikely to be of any greater relevance for man than were the earlier speculations from rat data*"[17].

He continued: "*Allegations arising from inappropriate high-dose studies in rats (fenfluramine) and squirrel monkeys (dexfenfluramine) are not backed up by any serotonergically mediated central nervous system pathology in man, yet dexfenfluramine has been used by more than 5 million patients for 6 years, and the parent compound by more than 20 million patients for 26 years*"[17].

In 1991 researchers at the pharmaceutical company Wyeth, studied fenfluramine in mice and rats; they added rats to their research because they had already studied fenfluramine in mice and had "*noted deviations from the expected results*" in that species. They wrote: "*Fenfluramine, which is considered to act via serotonergic mechanisms is a potent anorectic at doses which do not affect behaviour in the rat, while in the mouse there is no separation between anorectic and sedative doses. These data illustrate further*

differences in serotonin pharmacology between the two species"[24].

Cris Iles/National Anti-Vivisection Society

Metabolism of dexfenfluramine varies not only between species, but even between different strains of a species. Taking the rat as an example, in 1992 researchers at the University of Alberta reported that dexfenfluramine caused a diversion of glucose to form lactate in male Wistar rats, but not in JCR:LA-corpulent rats. The latter strain was most susceptible to dexfenfluramine, and the team proposed it as a model of human obesity for future dexfenfluramine studies[25].

Researchers, at the University of Hertfordshire, recently wrote that even the most up to date animal testing procedures could not always provide all the neurotoxicity information required in a risk assessment for humans, because of the complexity of the nervous system and the species differences which exist. They continued: "*In the case of relatively new prescribed drugs such as the appetite suppressant dexfenfluramine, more information is required on species differences in respect of metabolism and neural cell sensitivity to this compound*"[26].

An international team of researchers writing from Universities in Germany, Italy, and the USA recently stated that: "*Man is unusual in efficiently metabolising fenfluramine and norfenfluramine by deamination to inactive compounds excreted into the urine. ... In the rat differences in kinetics, saturation, route of administration, and brain uptake mean that the smallest brain level exposure which produces apparent swellings in neurons is 50-100 times that expected for man*"[27].

Even the Johns Hopkins team wrote in 1991: "*The basis for fenfluramine-induced 5-HT deficits has remained controversial, but recent anatomical findings support the hypothesis that fenfluramine damages serotonergic axons. Whether 5-HT neurotoxicity in rodents can be extrapolated to man is uncertain. Rats metabolise dexfenfluramine differently from man, and could also differ in their sensitivity to the neurotoxic effects of this drug*"[28].

It is clear that because of the already-known species differences, even many of the researchers involved in this field are questioning the relevance of the animal data for man. That includes the drug's manufacturers.

Dexfenfluramine was developed by the pharmaceutical company Servier, but in the USA is licensed to Interneuron and sub-licenced to Lederle. According to Interneuron, results of dexfenfluramine studies in animals appear to be irrelevant to humans, because of pharmacokinetic differences between animals and humans[21].

the alternative

Dexfenfluramine is widely used, for the treatment of millions of people; humans respond to it quite differently to other species.Consequently, the only way to obtain data of relevance to humans is through clinical studies or in vitro studies with human tissues. That is how all progress to date has been achieved.

For example, even though the effects of fenfluramine and dexfenfluramine have been investigated in many feeding models in experimental animals, the relevance of the animal models to humans is still uncertain[29].

Further, the therapeutic effect of fenfluramine in autism and its potential value in reducing suicidal intent in humans couldn't have been predicted by existing animal models. Some autonomic and cardiovascular effects are also difficult to extrapolate from one species to another, because of the complexity of the physiological controlling systems involved[29].

Fenfluramine has a variety of actions on cardiovascular models in animals, but animal experiments didn't predict its mild but clinically valuable antihypertensive action, nor the reduction in plasma noradrenaline levels which it causes in humans. These discoveries resemble the unexpected discovery of the antihypertensive effect of the beta-blockers, which also were not detected in animal studies, but had to await careful clinical observation during trials in angina patients[29].

3.2.2 DEPTS. OF NEUROLOGICAL SURGERY AND NEUROCHEMISTRY

One of the most intensive ongoing studies at the Institute of Neurology is that of stroke (cerebral ischaemia). Since 1992 this has resulted in several publications on gerbils from the Dept. of Neurochemistry[20,30] and baboons from the Gough-Cooper Dept. of Neurological Surgery[31,32,33,34].

background: stroke

Stroke is the third most common cause of death in the UK, and the prime cause of severe physical disability. It affects around 100,000 patients each year. Risk of stroke increases exponentially with age, with half of all first strokes being in people over the age of 70 years[35].

There are three main causes of stroke. *Ischaemic strokes* are a result of localised loss of blood flow in the brain; this can be caused by a blood clot blocking a blood vessel in the brain itself, or, more commonly, by narrowing and obstruction of atherosclrotic blood vessels in the brain. In contrast, *haemorrhagic* (bleeding) strokes are caused by rupture of a blood vessel in the brain[36]. Haemorrhagic strokes are generally more disabling than ischaemic strokes caused by blockage of blood vessels[37].

At present there is no effective treatment for stroke which really improves the outcome. A number of drugs and blood-dilution techniques have been tested, but there is no clear evidence that they are worthwhile[35]; we discuss some of these later.

As in heart attack (already discussed for Charing Cross), aspirin started a week or two after a minor ischaemic stroke definitely aids long-term secondary prevention[35].

However, aspirin does not break up existing clots, and so researchers worldwide are looking for drugs which will safely break up a blood clot in the brain. Although, to be of any benefit, such a drug would need to be given extremely quickly; perhaps impossibly quickly.

Researchers have spent many years on basic research, and on looking for potential drugs, in animal models of stroke. Worldwide, many animal species are used for this purpose, including cats, dogs, rabbits, non-human primates, and rodents. There are two main techniques in use[38].

Global cerebral ischaemia involves ligaturing the common carotid arteries, to cut off blood flow to the whole forebrain. This technique has been used since about the 1960's[38].

Focal cerebral ischaemia is believed by some to be more representative of clinical stroke in patients. It involves ligaturing smaller blood vessels, to cause localised loss of blood flow in parts of the brain; commonly the middle cerebral artery is tied off. The technique came into use during the late 1970's[38].

At the Institute of Neurology, basic research into the physiology, pathology and treatment of stroke is carried out. The Dept. of Neurochemistry team is using the global ischaemia model in gerbils, whilst the team in the Gough-Cooper Dept. of Neurological Surgery uses the focal ischaemia model in baboons.

studies of sodium distribution in the gerbil brain

In 1994 a Dept. of Neurochemistry researcher reported studies of global ischaemia in gerbils, in conjunction with others at the Institute of Child Health, London, and at Queen Mary and Westfield College, London[30]. Queen Mary and Westfield College supplied the NMR imaging equipment[39], and as this tends not to be portable we assume the actual work was carried out at Queen Mary and Westfield College.

The team point out that the study of sodium in biological systems is critical for an understanding of physiology and pathophysiology. Hence, they regard spectroscopy and imaging of the sodium isotope ^{23}Na to have great potential for the study of living systems. ^{23}Na occurs naturally in the body; it is an almost pure form of sodium, and after the proton is the second most abundant NMR-observable nucleus found in living tissues[30].

They write that clinically, ^{23}Na magnetic resonance imaging (^{23}Na MRI) has already been used on patients to study well-differentiated lesions such as tumours, but little has been reported on its use to study stroke[30].

Consequently they decided to use (^{23}Na MRI) to study stroke, to see if it was sensitive enough to follow the time course and spatial distribution of sodium changes in the initial stages of stroke and reperfusion. Instead of using this established clinical technique to look at patients, they experimented on gerbils[30].

Six gerbils were anaesthetised and the common carotid arteries ligatured with nylon snares. Sodium distribution throughout the brain before, during and after the induced stroke was then studied by MRI scanning[30,39].

The team found that during a stroke there is a shift in the position of sodium, from extracellular to intracellular. This eliminates the concentration difference across the nerve-cell wall which is necessary for normal nerve-cell function. When the snares are released and blood supply is returned to normal, there is a delay of about four minutes before the sodium gradient is re-established[30,39].

The team concluded that: "*Despite its inherent insensitivity, ^{23}Na MRI is possible in a small animal model of stroke. ... When allied with cerebral blood-flow determinations, ^{1}H and ^{31}P NMR spectroscopy measurements and diffusion weighted ^{1}H imaging, we believe the technique will contribute further to our understanding of the pathophysiology of cerebral ischaemia*"[30].

The project was jointly funded by Action Research, The Brain Research Trust, and the Wellcome Trust[30].

studies of brain mitochondria in gerbils

Mitochondria are found in all cells of the body. They are, quite literally, the cell's powerhouse; they store energy released from the breakdown of glucose, in the form of a chemical called ATP [adenosine triphosphate][40].

In 1995 a Dept. of Neurochemistry team reported studies of the effects of stroke, on the metabolism of mitochondria and synaptosomes in 20 Mongolian gerbils. This project was funded by the Medical Research Council, the Wellcome Trust, the Brain Research Trust, and the Institute of Neurology itself[20].

The rationale for carrying out the experiment was that: "*Changes in mitochondrial structure and function, seen after complete interruption of blood supply, play a crucial role in cell survival or in cell death, because intact mitochondria and ATP production are essential for normal brain function and integrity. Hence, it is important to study the relationship between cerebral blood flow and mitochondrial function, to establish the cerebral blood flow at which brain mitochondrial function is impaired*"[20].

Again they used snares to tie off both carotid arteries, inducing global ischaemia; by varying the tightness of the snares they were able to inflict a graded level of brain ischaemia. Their aim was: "*To establish the relationship between cerebral blood flow and mitochondrial and synaptosomal energy status as judged by substrate oxidation and mitochondrial respiratory chain complex activity*"[20].

Different degrees of stroke were inflicted on the anaesthetised gerbils, and maintained for 30 minutes. After this the animals were killed by decapitation and the brains removed for biochemical study. They found that the greater the degree of stroke inflicted, the more the mitochondrial respiration was impaired, but that this was not simply due to lack of oxygen; other parts of the respiratory chain were also affected[20].

The team decided that: "*Further clarification is therefore needed of the relationship between the activity of the various respiratory chain complexes and ATP production, and in particular at what level of deficiency a particular complex decreases ATP production in a biologically (clinically) significant way*"[20].

In other words, much more work on gerbils is needed; and is no doubt being done.

studies of focal ischaemia in baboons

Meanwhile, another team in the Gough-Cooper Dept. of Neurological Surgery was studying stroke in baboons.

This team expresses its opinion that several reliable animal models of focal cerebral ischaemia have been developed in primates, using occlusion of the basal cerebral arteries; further, that ligation of the middle cerebral artery, branches from the posterior choroid arteries, and the proximal basilar artery are the best simulations of a clinical stroke in patients[31].

Even so, none of these models can simulate the functional disability which results from low blood flow in the region of the middle surface of the hemispheres and corpus callosum[31]. There are two hemispheres, forming the uppermost region of the brain and by far its largest section; the corpus callosum is a bridge of material which interconnects the two hemispheres, enabling corresponding areas in each hemisphere to function together as a coordinated whole[41].

So, to devise a model which they think will be better, the team set about producing: "*Acute focal ischaemia in the territory of the anterior cerebral artery in the experimental primate by clipping the proximal segment of the common anterior cerebral artery*"[31].

In 1992 they described the surgical technique and gave brief data from the results. Nine baboons were anaesthetised and the right eye removed, followed by part of the skull. The common anterior cerebral artery was located and clipped for 30 minutes, after which the brain was perfused, through the aorta, with a saline and formalin mixture to fix it. Some of the arteries were selectively injected with dyes, to study the routes of blood flow after the stroke. This project was funded by the Brain Research Trust[31].

studies of the effect of stroke on nervous impulses in baboons

At about the same time, again funded by the Brain Research Trust, they used another seven baboons to study the effects of stroke on the passage of nervous impulses from motor areas of the brain to the corresponding hand or foot. Some members of the team had already reported related clinical studies on patients under anaesthesia for: "*the development of clinical and intra-operative monitoring of cerebral ischaemia using transcranial stimulation of the motor cortex". The present study in baboons was designed to complement that clinical study*"[32].

The baboons were anaesthetised and measuring electrodes fitted to the spinal cord in the neck, and also attached below the skin of the hands or feet to measure electrical activity of the muscle (electromyelograms or EMG's). Stimulating electrodes were placed in contact with the cortex of the brain, and fixed in place with acrylic glue. The response to cortical stimulation was measured with the electrodes attached to the spine and limbs, before the common anterior cerebral artery was clipped to simulate a stroke. The effects of varying degrees of stroke on impulse conduction were measured[32].

From these baboon experiments the team concluded that: "*monitoring of the EMG has clinical potential in the assessment of cortical ischaemia*"[32].

They then concede that: "I*n our clinical experience, a change in EMG amplitude after temporary occlusion of a major cerebral artery during intracranial vascular surgery has proved a sensitive indicator of cortical ischaemia*"[32].

Their findings in the baboon were already known from clinical studies, carried out by themselves on patients undergoing routine brain surgery. In fact, as they wrote one year later: "I*n cerebral vascular surgery, either global or focal changes of cerebral perfusion commonly occur due to deliberate arterial hypotension or temporary clipping of the parent vessels of lesion*s"[33].

So there is clearly great scope for intelligent clinical observation of this sort.

studies of brain transmission in baboons

Nevertheless, still funded by the Brain Research Trust, in 1993 they reported an experiment on eight anaesthetised baboons, in which they had measured transmission of an electrical impulse across the surface of the brain cortex before and after clipping the common anterior cerebral artery. They found that passage of the impulse across the brain was impeded by the stroke they had inflicted between the stimulating and measuring electrodes[33].

effect of thinning the blood in baboons

In 1994, funded by the Medical Research Council and Hoechst UK, they reported a study on fifteen baboons in which they had tried a potential method of treating stroke. Laws of physics dictate that if blood is diluted below its normal viscosity it will be better able to penetrate partially blocked blood vessels; however, diluting blood also reduces its oxygen-carrying capacity, so a balance would have to be struck for such treatment to be effective[34].

This was not a new idea. The team said that the technique had already been tried by at least three other groups, either clinically on patients, or experimentally on dogs or cats, but the results had been contradictory and had: "*added weight to the arguments for and against haemodilution therapy of cerebral ischaemia*". The team thought these contradictions had arisen because the earlier researchers had looked at the outcome of the procedure, rather than directly examining its effects on the circulation[34].

Stroke patients might argue that it is the outcome which matters above all else, this being far more important than esoteric changes to the circulation.

Still, the team once again measured impulse transmission across the baboon brain, before clipping the common anterior cerebral artery for 80 minutes. The effect of diluting the animal's blood with dextran solution [a plasma substitute in regular clinical use], to allow increased blood flow into the stroke area, was then noted[34].

They found that diluting the blood could improve blood flow to areas of brain affected by blockage in a vessel, so long as other blood vessels were still open. Reduction in number of the oxygen-carrying red blood cells, to just one quarter of its normal level, does not impair and may even improve recovery of brain function. However, if carbon dioxide levels increase too much as a result of diluting the blood, the improvement may be negated[34].

They concluded that: "These observations indicate that isovolaemic haemodilution is still

a viable candidate for the treatment of focal cerebral ischaemia, but that any degree of [increased carbon dioxide level] *should be avoided during such therapy*"[34].

critique of the stroke experiments in animals

Studies of stroke in animals have many variables including, amongst others, species and even strain of animal used, age, sex, diet, choice of global or focal model, and whether the inflicted stroke is transient or permanent[42].

gerbils - a very common model

Gerbils are often selected for experiments precisely because the blood supply to their brain differs from other species. In other species there are arteries at the rear which interconnect the carotid and other arterial systems; these are absent in gerbils. Consequently, gerbils have a natural tendency to develop severe neurological deficits and unilateral infarction on the same side as the ligated common carotid artery[38].

Gerbils are said to have one chief advantage - the surgical procedure is simple and convenient. But they also have several disadvantages. Their small size ensures that physiological monitoring procedures become technically daunting for the researchers; perhaps more important for the outcome of the experiment, such monitoring procedures are physiologically destabilizing to the animal itself. Hence, experiments in gerbils are often physiologically unmonitored, and so are of little value in interpreting stroke pathology[38]. However, this last criticism would not apply to the Dept. of Neurochemistry research; their animals are extensively monitored, albeit raising the possibility of physiological destabilization.

Another major complication of the gerbil is that the species is unusually susceptible to seizures anyway. This introduces a confusing experimental variable into the assessment of outcome[38,42]. All of these disadvantages apply equally to all gerbil models, including bilateral common carotid occlusion38 as used by the Dept. of Neurochemistry team.

Writing in 1990 Brian Meldrum, an animal researcher at the Institute of Psychiatry in London, wrote of such global ischaemia models: "*A compromise between precise modelling and experimental reproducibility is often necessary. In global ischaemia models, complete ischaemia will usually give more exact results than incomplete ischaemia (because the latter is heterogeneous between animals and within the brain). Incomplete ischaemia may, however, better reflect the clinical situation*"[42].

There is no point in obtaining "*more exact*" results if they are less applicable to patients.

all animal models of stroke are clinically irrelevant

Yet the baboon models used at the Institute of Neurology are no better; several baboon models exist already, with different arteries being clipped or tied off in each of them. Even tying off one of the two main blood supplies to the brain causes substantially different outcomes in experimental animals and many human neurosurgical patients. As one researcher wrote in 1961: "*Animal experiments may be misleading. It is generally admitted that no laboratory animal suffers cerebral damage from unilateral carotid ligation. In man, on the other hand, this is quite common, and in 1914 Hunt reviewed 1159 cases and reported an incidence of permanent cerebral damage of*

8.7% after unilateral common-carotid ligation. A figure of the same order would be expected now; indeed many neurosurgeons would consider it an underestimate"[43].

However, most laboratory procedures involve tying off smaller arteries, in an attempt to simulate more realistic clinical strokes. The attempts are all in vain.

Ligation of the middle cerebral artery is said to be one the best simulations of a clinical stroke in patients[31]. This technique has been used worldwide on cats, dogs and primates for twenty years or more[44]. Yet even this, the version believed by some to most closely represent human strokes, has produced no clinically useful benefits.

Although large animals like these are convenient for the surgery involved in stroke research, the experimental outcome shows considerable variability. This is partly due to anatomical differences in the blood supply of the brain[42]. They all fail to reproduce stroke in humans.

Animal brain and vascular anatomy is different from that in humans. The brain circulation in rats, rabbits, gerbils, and cats is quite different from humans, and we know from comparative anatomy that the brains of these animals are different from each other and from human brains[45].

Even larger animals like dogs are different: "*Studies of basilar arterial function in canine as well as in other species are limited because of the marked dissimilarity of the formation of the intracranial circulation from the extracranial circulation as compared to that of the human being*"[46].

Monkeys too have important differences: "*Only in the monkey ... is the origin of the intracranial circulation closely akin to that of man. In spite of the similarity of formation of the cerebral circulation in man and monkey, the completeness of the circle of Willis and the singleness of the anterior cerebral arterial system in the monkey point up significant differences within the cerebral circulation itself as between the human being and the subhuman primate*"[46].

As long ago as 1976, researchers at the National Institute of Neurological and Communicative Disorders and Stroke, in Bethesda USA, wrote that: "*Many [animal research] methods have temporarily prospered only to fall into disuse or disrepute. Nowhere is this more evident than in the field of experimental cerebrovascular disease. .... It is somewhat illogical to assume that species which are anatomically diverse from each other and from man should function in physiological and biochemical patterns similar to man*"[47].

Studies of focal (localised) or global (whole-brain) cerebral ischaemia in animals are unlikely to yield clinically useful information which is applicable to patients suffering from stroke. Drugs can give quite different effects in similar experiments carried out in different animal species. Ultimately, no drug or technique developed in animals has proven clinically useful to treat stroke in patients. A basic problem is that artificial strokes inflicted in animals are not accurate copies of the disease in humans.

Even the use of anaesthetics can render an experiment pointless. In 1990 it was stated that: "*In man, cerebral ischaemia occurs almost invariably in conscious individuals, whereas in animals, general anaesthesia is a common feature in animal models of cere-*

bral ischaemia. Anaesthesia can modify cerebrovascular reactivity and the demands for substrate (oxygen and glucose) in discrete central nervous system regions. The possible interaction between any anaesthetic and the anti-ischaemic drug (synergistic or antago - nistic) in relation to observed neuroprotection is always of concern"[44].

Put simply, many of the animal models which were developed to explore the pathophysiology of stroke are simply not suited for assessing drugs for clinical use[44].

Variability in outcome, between animals, is a feature of many models of cerebral ischaemia. Further, most human strokes are caused by blood clots, whereas those in animals are caused by ligatures or clips[42,44]. Such lesions are completely unlike human thromboembolic strokes and even unlike the cerebral blood-deprivation caused by clinical heart attack. Most induced experimental lesions are made instantly, whereas clinical insults are often more gradual[45].

Animal models of stroke produced by blood clots are seldom used in the evaluation of new drugs, because of intrinsic problems of reproducibility[42,44].

The animals chosen usually do not replicate human stroke victims; many stroke patients have multiple lesions in the blood vessels of the brain, as well as past and present illnesses and risks such as hypertension, smoking, diabetes, and high blood-lipid levels; these may all have affected the circulatory system before the stroke, and will greatly modify the outcome and response to treatment[45].

Many of the important effects of stroke are not testable in animals. Vision, sensation, speech and memory are examples of deficits that are not testable; in animals, results can only be defined in structural or biochemical terms[45].

The timing of various treatments used in animal models often does not reflect that possible in stroke patients. Treatments are usually given to animals before, during, or shortly after the stroke is inflicted; in contrast, many stroke patients do not seek medical care quickly, and when they do there are usually long delays before they are evaluated by specialists experienced and knowledgeable in stroke treatment[45]; we will return to this point later.

So, animal models do not replicate the situation in stroke patients. As a result, treatments that work in animal models have not proven effective in humans[45]. This confirms that animal models are unsuitable for either drug-related or basic research, and calls into question the whole basis of the Institute of Neurology studies.

chaos in anti-ischaemic drug studies

Some researchers are trying to develop anti-ischaemic drugs, with the intention of reducing the tissue-damage that results from localised oxygen deprivation in the brain after a stroke. There are many examples of the chaos resulting from studies in animals.

For example, in gerbils the opioid receptor antagonist drug naltrexone has no significant protective effect. This contradicts its effects in cats, in which naltrexone reduces brain damage and prolongs survival time[48].

Another drug, the calcium antagonist nimodipine, gave inconclusive results in models of focal ischaemia produced by tying off the middle cerebral artery. In rats the

drug wasn't consistently effective, but it gave favourable effects in rabbits and baboons[49].

In spite of the wide variety of animal models used, and the range of survival times obtained, one particular class of drug has always been found to be effective. This is the NMDA (*N-methyl-D-aspartate*) antagonists, which give a consistent pattern of protection in animals, although by some as yet unknown means[42].

Despite this, NMDA antagonists have failed to improve the condition of human stroke victims[50]. Yet, as a result of the consistent findings with NMDA in animals, the focal ischaemia model is becoming increasingly dominant in anti-ischaemic drug development[44]; never mind that the drugs it produces don't work for stroke patients.

Other anti-ischaemic drugs developed in animals have clearly failed to make any clinical impact either. In an eight-page review of drug therapies for stroke, published in 1995, Brian Meldrum at the Institute of Psychiatry stated: "*Most impressive is the increasing diversity of compounds that offer protection in animal models of focal and global ischaemia. Five years ago there were only three classes of compounds that merited serious consideration: calcium antagonists, glutamate antagonists, and free radical scav - engers. Now there are so many that it is not feasible to survey them all in a review of this length*"[51].

Writing at about the same time in 1995, other researchers at the Syntex Research Centre in Edinburgh expressed their own opinion of all these new compounds: "*Considerable effort has been expended in recent years in the search for pharmacological interventions which might help to improve the clinical outcome following stroke. This search has led to a wide variety of chemically diverse agents which appear to exert neuro - protective effects in animal models of brain ischaemia, including N-methyl-D-aspartate receptor/channel antagonists, inhibitors of glutamate release, 21-aminosteroids, ganglio - sides, nitric oxide synthase inhibitors, enhancers of GABA-ergic and adenosinergic neuro - transmission and calcium entry blockers. Despite this variety, there is still no clinically proven treatment for acute stroke*"[50].

Whilst researchers continue to screen new anti-ischaemic drugs for stroke, the animals they use continue to give inapplicable results. As others from Syntex Research also pointed out in 1995: "*The identification of neuroprotective agents that may be of potential benefit in the clinical management of stroke is ultimately based on efficacy in in vivo animal models of cerebral ischaemia. However, these models invariably blur potentially important structure-activity relationships between compounds due to their dif - ferent fates in the complex in vivo environment (brain uptake, metabolism, plasma pro - tein binding, and so on)*"[52]. All of which are made worse by the metabolic and pharmacokinetic differences between different species of animal.

This Syntex team apparently agreed with their colleagues, continuing: "*A wide vari - ety of pharmacological agents have been shown to reduce the neuronal damage produced by cerebral ischaemia in animal models. These agents include NMDA antagonists, non-NMDA excitatory amino-acid receptor antagonists, calcium and sodium channel inhibitors and inhibitors of lipid peroxidation. However, despite the promise held out by animal experiments, none of the agents which has so far reached the stage of clinical test - ing has yet been shown unequivocally to be of benefit in the management of stroke. This is undoubtedly due partly to the post-stroke intervention time and partly to the fact that*

stroke is not a homogeneous entity, producing ischaemic lesions of widely varying size and severity"[52].

In 1995 others, at the Astra Neuroscience Research Unit in London (a department of the Institute of Neurology), wrote of the global ischaemia model in gerbils: "*At the present time no neuroprotective compounds have been shown to be effective in the treatment of ischaemic stroke in man and therefore the predictive value of these models is unknown*"[53]. We would argue that since the technique has been in use since the 1960's and there is still no cure for human stroke, global ischaemia in gerbils is known to be non-predictive for humans.

As another researcher put it so aptly in 1990: "*The credence that clinicians place on anti-ischaemic drug efficacy in gerbils is minimal*"[44].

thrombolytic drug studies

Although the use of anti-ischaemic drugs has so far proved unsuccessful in treating stroke, there is another potential way of dealing with the problem. As in heart-attack, it should theoretically be possible to open-up blocked blood vessels in the brain by means of clot-busting drugs.

We have already discussed the introduction of the clot-busting drugs streptokinase, urokinase and tissue plasminogen activator (t-PA) in our review of heart research at Charing Cross. We will not repeat that here, but instead will look specifically at their use to treat stroke.

The idea of using clot-busting drugs to treat ischaemic strokes (i.e., strokes caused by blood clot in the brain) is not new. The technique was first suggested in the 1950's[54].

During the 1950's, several neurosurgeons reported the beneficial effects of surgically removing blood clots from the brains of stroke patients. In 1958 two American neurosurgeons suggested that the poor results sometimes seen in such operations were due to local damage caused by the procedure itself, and perhaps more important, to the delay in operating. They wrote: "*This time is ordinarily consumed in the obligatory delay required for consultation between family physician and specialist, transportation, diagnostic angiography, and preparation for surgery. What has been needed is an agent, known to be effective in reversing thrombosis, which can be administered immediately on the appearance of symptoms by the first physician to arrive on the scene*"[55].

Human plasmin had been purified by this stage, and had even been used clinically by others; but this American team was the first to suggest, and to use plasmin, clinically for the treatment of ischaemic stroke. Without any mention of animal experiments, they treated three stroke patients. In one of them the blood supply to the affected part of the brain was restored, but the other two were less successful. The successful case had been treated "*within six hours*" of her stroke[55].

During the early 1960's streptokinase was tried clinically in a small number of stroke patients, but the results were not encouraging. The drug gave an increased risk of secondary strokes caused by bleeding in the brain[56]. Such strokes are generally more disabling than strokes caused by blockage of blood vessels[37].

After these clinical studies streptokinase was believed to be toodangerous to use. It

was even thought to be too dangerous to use in those heart attack patients who showed any signs of brain lesions or past strokes[56].

In 1976 another American neurosurgical team tried treating stroke patients with urokinase instead[57,58]. They tried it clinically, and were scathing about animal experiments: "*While cerebral infarction is readily produced in the experimental animal, the animal model differs in many respects from that of the human counterpart. Consequently, animal data have not provided a definitive answer as to whether increase of blood supply to the ischaemic cerebral areas, some hours after the [stroke], is likely to be of therapeutic benefit*"[57].

Unfortunately, they found that like streptokinase, urokinase also carried an increased risk of secondary strokes[58].

Clinical experience had shown secondary strokes to be a potentially serious limitation of the clot-busting approach to stroke treatment, and it fell into disuse[54]. However, during the 1980's the successful use of clot-busting drugs to treat heart attack revived interest in these drugs as a method of treating stroke. Streptokinase and urokinase were tried again, as was the new drug, Tissue Plasminogen Activator, t-PA[56].

The now widespread availability of brain imaging techniques, and the success of t-PA in treating heart attack, both contributed to a revival of the use of clot-busting drugs for stroke[54].

Before being used clinically to treat stroke, in 1985 t-PA was first tried in rabbits with experimental stroke; by this stage it had already been tried clinically on several heart attack patients. Nevertheless, blood clots were injected into the carotid circulation (which supplies the brain) of rabbits to cause strokes, then t-PA was injected intravenously to study the results[59]. Blood-clots are seldom used in the experimental evaluation of new drugs, because of intrinsic problems of reproducibility[42,44].

Nevertheless, these rabbit experiments appeared to show that t-PA was effective and safe. Yet the researchers still lacked confidence in their animal model: "*Successful treatment was accomplished with doses that did not appear to cause important cerebral haemorrhagic complications. The histological patterns of damage were similar in both control and t-PA-treated animals. ... The apparent safety of the drug in these experimental conditions does not prove that t-PA will be safe in cases where larger emboli lodge in the cerebral circulation and haemorrhagic complications may arise after the onset of ischaemia. Until such preliminary studies are completed, trials with human patients will be premature*"[59].

In 1987 t-PA was introduced for general clinical use[60], so the authorities must have been happy with the results of animal studies and the early clinical trials.

However, by 1992 t-PA was shown by two large clinical trials (ISIS-3 and GISSI-2), to significantly increase the risk of stroke when used to treat heart attack patients[61,62]; this despite its apparently being safe in the early rabbit studies of stroke. In fact, animal experiments with rt-PA as a treatment for stroke had given contradictory results about its benefits as well as its safety.

Thus in 1992 it was stated that: "There has been no recent important advance in the

treatment of thromboembolic cerebrovascular disease [stroke]. There have, however, been reports of the benefit of t-PA given early to patients with thrombotic cerebral infarction. Studies in animals have demonstrated increased recanalisation of affected vessels after t-PA administration. Although some of these investigations have also shown a reduction in neurological deficits, others have not"[63]. [our emphasis].

Yet clinicians were still studying t-PA in stroke patients. They found that rt-PA given to patients within eight hours of an ischaemic stroke could partially or completely reopen blocked blood vessels in about one third of them. Unfortunately, t-PA treatment for ischaemic stroke still caused secondary strokes through bleeding in the brains of up to 47% of patients; in 6% of these the bleeding was serious, and in another 10% it was fatal[64]. Strokes caused by bleeding in the brain are generally more disabling than strokes caused by blockage of blood vessels[37].

It seems that so far as the old drug streptokinase is concerned, doctors still can't decide amongst themselves as to its value in treating ischaemic stroke.

Streptokinase has been shown to be beneficial in treating heart attack, and when used for that purpose carries less risk of causing secondary strokes than does t-PA[61,62]. In contrast, an international study of its use in stroke treatment, published in *The Lancet*, December 1995[65], gave results which split the research teams involved[66]. As the pharmaceutical industry's magazine *Scrip* commented: "*The results are so uncertain that even the trial's steering committee could not agree on their interpretation*"[67].

Nevertheless, an accompanying editorial in *The Lancet* sums up the outcome of the trial: "*The report shows a clear excess of early deaths and cerebral haemorrhages, which was not fully offset by the possible long-term benefit. ... For many neurologists and stroke physicians these results will confirm their impression that thrombolytic therapy is at best not beneficial, and at worst probably harmful. ... Until the available trials have been systematically reviewed (and further trials completed), thrombolysis cannot be recommended as routine therapy for any category of patient with ischaemic stroke*"[68].

One such "*further trial*" (with the acronym NINDS) was published in America just one week later. Clinicians there had compared t-PA with placebo for the treatment of ischaemic stroke. They found that three months after t-PA treatment, survivors were at least 30% more likely than those treated with placebo to be almost or fully recovered[69].

The NINDS trial also found that patients treated with t-PA were still more likely to suffer secondary haemorrhagic strokes than were patients treated with placebo, but the incidence of such strokes was much lower in this trial than in all other published trials of either streptokinase or t-PA. The NINDS clinicians speculated that early treatment within three hours of the first stroke could be responsible for this, although the smaller doses of t-PA used may also have helped[69].

Their main conclusion is that t-PA treatment is more effective, and safer, if given within three hours of the stroke[69].

Yet t-PA significantly increases the risk of stroke even when it is used to treat heart attack patients[61,62], and not just stroke patients. Consequently, it is difficult to see how more rapid treatment of the initial stroke could be responsible for reduced inci-

dence of secondary stroke. Time may tell.

In the meantime, a cautious editorial accompanying the NINDS publication pointed out that the outcome measures used in NINDS to assess efficacy of treatment had not been validated. Further, in normal clinical practice it is unlikely that the stroke could be diagnosed and treated within such a short time span; it would involve neurological evaluation, computed tomography and obtaining informed consent for treatment; all of this takes time. The editorial concluded: "*The risk of cerebral haemorrhage should discourage the indiscriminate use of plasminogen activators when clearly defined criteria are not met. These criteria include a short time from the ischaemic event to treatment, and the absence of any sign of brain injury on computed tomography. ... In the clinical application of thrombolysis in acute stroke we should move forward cautiously with a clear awareness of the potential risks*"[54].

It is unfortunate that NINDS only compared t-PA with a placebo, and not with streptokinase as well. Given earlier treatment times and smaller doses, streptokinase might still be better than t-PA. Nevertheless, at present it seems that both t-PA and streptokinase cause secondary (and more dangerous) strokes when used as treatment for stroke in patients. Although clearing the blocked blood vessel in the brain is beneficial in animal models of stroke, most clinical trials have shown it to be useless or even dangerous in human stroke victims; only the NINDS trial has found a benefit. Since NINDS was only published in late December 1995, at the time of writing this NAVS report other stroke clinicians had not had time to express their opinions on the NINDS criteria, techniques, results and conclusions; NINDS may yet be dismissed by other stroke clinicians.

If t-PA does ultimately prove useful in the treatment of stroke, it will be through carefully controlled clinical trials like NINDS showing the correct way to use it. This information obviously has not been made available by years of animal experiments, or it would already be in clinical use. Whatever the final outcome, and it is still unclear, it will be a direct result of clinical study of stroke patients and not of research on gerbils and baboons, wherever the latter is carried out. As we have seen, the whole concept of treating stroke with clot-busting drugs came about through clinical study.

an alternative

In vitro studies with cultured nerve cells have contributed greatly to our knowledge of the cellular and ionic mechanisms involved in stroke. In vitro, nerve cell damage can be assessed by light microscopy, using phase contrast or vital staining. In living cell cultures, the concentration of the enzyme lactic dehydrogenase in the medium can be used as a measure of cell damage, and is known to correlate in a linear fashion with neuronal damage[42].

The toxicity of chemicals and potential value of drugs, such as receptor agonists, can be readily compared in vitro. The damage caused by ischaemia can be studied by using metabolic poisons or by removing oxygen from the incubation medium. In vitro studies of the protective action of potential drugs have contributed greatly to our knowledge of the factors which cause cell death in ischaemia. Nevertheless, in vitro tests can not reproduce the entire range of other factors which come into play in life[42].

Equally, experiments on animals cannot reproduce the range of factors involved in clinical stroke in humans; it is clear that the best way forward is the use of clinical study and in vitro study of human brain tissue.

What researchers would regard as "*clinical material*" is actually far more easily available than a lay person might believe. In addition to patients suffering from naturally occurring strokes, hospitals carrying out heart surgery generate an endless stream of patients suffering minor brain damage.

It is estimated that despite improved surgical techniques, about 25-35% of survivors of coronary artery bypass or heart valve-replacement operations suffer from some brain disorder as a result of their operation. Many of these disorders resolve naturally over time, but some are irreversible[70].

Many cases are due to damage caused by ischaemia, as a result of inadequate brain oxygenation, or formation of blood clots[70]. In other words, they are due to accidental surgically induced strokes, of varying duration and degree, and ranging from global to focal.

Severe brain damage in this group of patients contributes substantially to the mortality figure of up to 15% for heart surgery. In non-comatose patients, the neurological signs range from global cerebral dysfunction of variable degree, to focal defects including memory disorders, spatial disturbance, impairment of language function, inability to recognise objects visually[70].

Then there are patients undergoing brain surgery. Recall the Institute of Neurology team's own comment in 1993 that: "*In cerebral vascular surgery, either global or focal changes of cerebral perfusion commonly occur due to deliberate arterial hypotension or temporary clipping of the parent vessels of lesions*"[33].

We are not recommending wholesale experimentation on these unfortunate victims of medical damage. But it must surely be possible for experienced clinicians to derive some useful knowledge from such patients, given the advanced state of non-invasive imaging. After all, these patients are in the right place, at the right time, and their doctor-induced accidents need to be diagnosed anyway.

Some specialists recommend routine monitoring for such accidents whilst surgery is in progress. In 1984 it was stated that: "*The purpose of intraoperative neurophysiological monitoring of cortical function is to allow recognition of these insults at the moment of occurrence thus permitting immediate therapeutic intervention where possible. In addition, such information is important in planning future improvements in surgical, perfusion and anaesthetic techniques*". The same researchers bemoaned the fact that most surgical teams do not even bother to monitor the brain's electrical activity during surgery, probably because the equipment involved is inconvenient to use[70].

3.2.3 Depts of Neurological Surgery and Neurochemistry

J. Creamer/National Anti-Vivisection Society

Two researchers (P.J.Goadsby and N.M.Branston) in these separate departments of the Institute of Neurology have recently applied for, and received, Home Office project licences to carry out different animal experiments into migraine.

background: migraine

Migraine is more common in women than men, and it affects up to 5% of the population. It is a severe headache which usually lasts from 6 to 24 hours, and is associated with transient visual and / or gastrointestinal disturbances. The headache is often preceded by a visual aura of flashing and moving dots or zig-zag lines which partly prevent vision; there may also be loss of vision in discrete areas, and blurring of vision or loss of visual field. The majority of patients also suffer from nausea and vomiting in at least some attacks[71].

Migraine can be provoked by many factors, including fatigue, alcohol, menstruation, hunger, and chemical constituents of dietary items such as chocolate, cheese, shellfish and red wine[71]. These chemical factors were all discovered through clinical study.

For example, the role of tyramine in migraine was suggested through clinical study of patients who took monoamine oxidase inhibitor drugs to treat depression; the role of phenylethylamine was discovered through patients who developed migraine after eating chocolate; the role of phenols and the enzyme phenolsulfotransferase was discovered through clinical study of patients who developed migraine after taking cocoa, alcohol (especially red wine), dairy products, citrus fruits and coffee; the possible role of prostaglandins and histamine were discovered when these substances were used clinically for other purposes; all of these, and many other factors, have all been identified through clinical study and a vast body of clinical evidence has subsequently built up[72].

Again, it was clinical studies which suggested that brain serotonin might play some part in migraine, and a vast amount of clinical and in vitro evidence has accumulated on this subject. An American clinical neurologist even cites one of Goadbsy's earlier experiments in monkeys, but only as a source of "*supportive*" evidence to back up existing clinical theories[72].

cats destined for migraine research

Goadsby is Wellcome Senior Research Fellow in the department of Clinical Neurology[73]. We have an incomplete sequence of photos of his project licence application and its Home Office approval, and Home Office personal licence approvals for Goadsby and two colleagues.

Goadsby says he has been experimenting on rats, cats, guinea-pigs and macaques since 1981. He has worked in Australia, New York, Paris and at the National Hospital for Neurology in Britain[73]. He has been supported by Wellcome for several years, even whilst working in Australia. Publications dated 1992 from The Prince Henry Hospital, Little Bay, Australia, list him as a Wellcome Senior Research Fellow[74,75].

Cats at the Institute of Neurology.

Cris Iles/National Anti-Vivisection Society

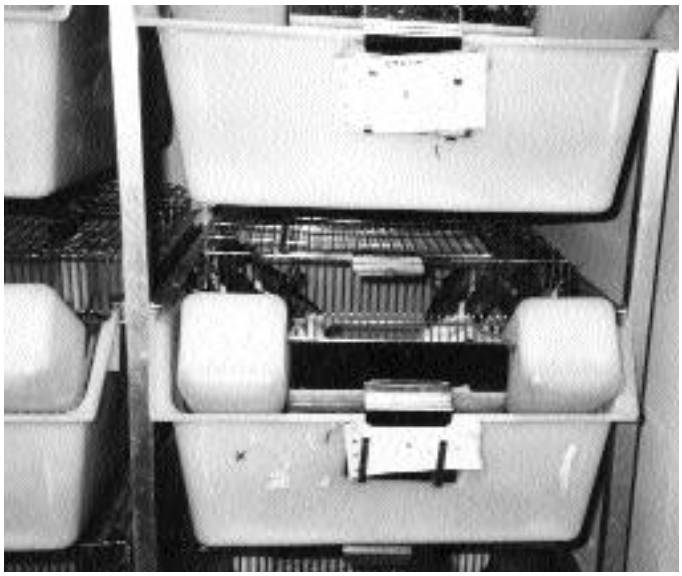
Goadsby signed his project licence application on 9th May 1995, and it was countersigned by the Institute Secretary on 11th May[73]. The five-year project licence (PPL 70/3725) was issued on 25th July 1995, at the same time as a personal licence for Goadsby (PIL 70/12483). Two other personal licences in respect of the same project were issued on the same date; one for Ms.Yolande Edna Knight (PIL 70/12562) and one for Ms.Karen Lisa Hoskin (PIL 70/12563)[76].

All three licensees are permitted to starve cats overnight. All other approved procedures relate to both cats and rats, and includeadministration of drugs by any route; anaesthesia; withdrawal of blood from superficial vessels; catheterisation of blood vessels; measurement of brain blood flow by Doppler flow probe; opening of the skull; injection of substances into the brain; insertion of recording electrodes into the brain; removal of the brain; exposing the spinal cord by removing bone from the spinal column; use of neuromuscular blocking agents in conjunction with terminal anaesthesia; isolation of blood vessels in the head for electrical, mechanical or chemical stimulation; killing the animals by perfusion of the heart[76].

It is not possible to describe the proposed project, since most of the experimental protocol details are missing from our sequence. However, it seems that all procedures will be carried out on cats under terminal anaesthesia. The total number of

cats required is impossible to determine from available documentation. However, the experiments involve at least 4 separate protocols, and protocol 3 alone is stated to require 25 adult cats per year[73].

The potential benefits of this work are said to be: A better understanding of the way in which sensory neurones influence blood circulation in the brain; definition of the neurotransmitters involved in craniovascular pain, which will assist the treatment of headache; an understanding of the neural control of the cerebral circulation, which will ultimately affect management strategies for problems such as stroke and sub-arachnoid haemorrhage[73].



Cats Illus/National Anti-Vivisection Society

Rats at the Institute of Neurology.

rats destined for migraine research

N.M.Branston is a Senior Lecturer in the Department of NeurologicalSurgery[77]. We have a complete sequence of photographs of his project licence application and Home Office approval.

Branston is using rats to study migraine. All of his experiments will be under terminal anaesthesia, and he expects to use 420 rats over a five year period. The project is being funded by a three-year grant from the Migraine Trust[77].

However, note that Branston was and still may be involved with ALL of the baboon research into stroke, discussed in detail earlier. It is not clear whether he was the project license holder for the baboon studies.

critique of migraine research in animals

Current knowledge of the biological aspects of migraine has been obtained by rigorous application of scientific method, and has led to much debate. It has also all been obtained through clinical study, for there is no animal model of migraine[72].

Animal experiments have also proved useless in finding new cures for migraine. This is not surprising, for the animal models used do not measure headache.

Animal models of pain necessarily omit the motivational and affective aspects that differentiate pain (a sensory and emotional experience) from a physiological response - a crucial distinction in human beings[78]. And, although animals may get headache, they cannot tell us about it[79]. Consequently, according to researchers at the Massachusetts General Hospital: "*Very few animal models have been developed to evaluate compounds of potential importance for the treatment of migraine and related headaches. Moreover, traditional models do not assess the effects of drugs on nociception* [ability to feel pain], *but utilize measures of blood flow, blood velocity or assess the abili - ty of compounds to constrict or dilate vascular smooth muscle*"[80].

Theories as to the precise cause of migraine have also been confused by animal

experiments. It is likely that in the future, as in so many other areas of medicine, we will be able to recount how the reliance of so many researchers on inaccurate animal models has delayed progress in migraine research by many years.

spreading oligemia

A landmark series of clinical studies reported in 1981 gave rise to the theory of "*spreading oligemia*" [oligemia means reduced blood flow]. These experiments studied blood flow in the human brain, by measuring the washout of the radiolabelled inert gas ^{133}Xe, a quantity of which had been injected into the internal carotid artery[72,81].

By simultaneously examining the regional blood flow in 254 areas of the human cerebral cortex on one side of the brain, during onset of a migraine attack in seven patients, clinicians showed that migraine followed a slowly spreading reduction in the cerebral blood flow of up to 30%. This clinical study produced the most precise data to date on this phenomenon[72,81].

Subsequent clinical studies have confirmed the finding, and have also suggested that the slowly spreading reduction in local blood supply in the cortex may explain the localised neurological symptoms, but does not seem to be necessary for them to occur. Hence it is now accepted that classic migraine is accompanied by a slowly spreading reduction in cortical blood supply [oligemia] that persists for several hours, but it seems unlikely that these disturbances are caused by a primary circulatory disturbance. Hence, the precise cause of migraine is still obscure[72].

Further clinical studies should be able to elucidate this, with the advanced technology now available. Changes in cerebral blood flow are now quite easy to measure in humans, and such techniques are becoming commonplace.

At the time of the clinical research in 1981, Positron Emmission Tomography (PET) had not been used in migraine research; the clinicians involved with ^{133}Xe research said PET would give more accurate results, but that it was more expensive and difficult to apply[81].

PET is now being used in many areas of clinical research, including the measurement of blood flow in the brain. For example, clinical researchers at the MRC Clinical Sciences Centre and Royal Postgraduate Medical School recently wrote: "Positron Emission Tomography (PET) permits the non-invasive measurement of changes in regional cerebral blood flow as an index of regional neuronal activation. We defined central nervous pathways activated by angina by dynamic PET with ^{15}O-labelled water to measure regional cerebral blood flow changes during drug-induced angina"[82]. Since migraine too can be induced at will by many factors, PET is potentially a very valuable tool for clinical migraine research.

spreading depression

On the other hand, some possible causes of migraine have also been suggested from experiments on rats and monkeys. For example the theory of "*spreading depression*" as proposed by Leao in 1944[72,79].

Spreading depression is a wave of electrical changes moving slowly over the cerebral cortex of the brain. Spreading depression can be produced easily in small mammals

J.Creamer/National Anti-Vivisection Society

This page and opposite, cats waiting to die at the Institute of Neurology.

with smooth brains (lissencephalic animals), like the rat[72], by injecting depolarising chemicals such as potassium chloride, by electrical stimulation, or by mechanical deformation of the cortex[83]. Spreading depression has even been produced experimentally in the monkey, an animal with a convoluted cerebral cortex (gyrencephalic animal, like humans), albeit with great difficulty[72].

The spreading depression theory has serious flaws; spontaneous, unprovoked spreading depression has never been seen in ANY animal[72], and despite one claim to the contrary[84] it has never been convincingly shown in humans either[72].

As neurologist J.N.Blau at the National Hospital for Neurology and Neurosurgery in London, wrote in 1992: "*Leao stimulated the smooth non-gyrate cortex of rabbits, pigeons, and cats by applying high concentrations of potassium to the cortical surface or piercing it with a needle. These non-physiological stimuli provoked a band of electrical silence accompanied by pallor which migrated across the cortex at 3-5mm per minute. The band of reduced blood flow was succeeded by hyperaemia* [increased blood flow] *so that blood in adjacent cerebral veins became arterialised. Does this phenomenon occur in the human brain? It has not been reported by the numerous neurosurgeons who have needled the exposed human cortex in search of epileptic foci and it was specifically denied from Penfield and Jasper's laboratory after examination of nearly a thousand electrocorticographic recordings*"[79].

This clinical finding cannot easily be dismissed.

By "*Penfield and Jasper's laboratory*", this neurologist was referring to the world-famous Montreal Neurological Institute at McGill University in Montreal, Canada. Wilder Penfield [1891-1976] became Director of the Neurological Institute in 1934. Penfield and his colleagues have done more than any other team in the world to map the human brain in conscious patients[85]. They brought the use of electrical stimulation in human brain mapping to a high level of development[86].

As of 1986 a neurologist called Pierre Gloor was Professor of Clinical Electroencephalography and Experimental Neurophysiology, in the Dept. of Neurology and Neurosurgery at McGill University and the Montreal Neurological Institute. It was this clinical laboratory, in which Gloor has apparently worked since the mid 1950's, which failed to find any sign of spreading depression in human brains[87].

Gloor's thoughts on spreading depression are so important that they require quoting

in full. In 1986 he wrote: "*In the exposed cortex of animals, spreading depression can be elicited by a variety of stimuli applied to the cerebral cortex, among them mechanical and electrical. Over a 30-year period I have personally recorded the electrical activity of the exposed human cerebral cortex in the operating theatre in nearly 1,000 conscious, locally anaesthetised epileptic patients whose cortex was widely exposed prior to surgical excision of their epileptogenic focus. During these procedures, the cortex was repeatedly subjected to both mechanical deformation (e.g. by insertion of depth electrodes) or electrical stimulation, both methods that are used experimentally to induce spreading depression. Yet I have never observed anything that remotely resembles spreading depression during these surgical procedures. Some of these patients had migraine in addition to epilepsy, although I do not know how many suffered from classical migraine. Observations on animals have shown that the more highly evolved (the more convoluted ?) the cortex becomes, the more difficult it is to elicit spreading depression. It is exceedingly easy to provoke it in rabbits, more difficult in cats and quite difficult in monkeys. This evidence and my lack of ever observing the phenomenon in the exposed human cortex make me doubt that spreading depression plays any role in human cerebral pathophysiology, including that of migraine*"[87].

Blau, at the National Hospital for Neurology and Neurosurgery, gave further clinical evidence against the spreading depression theory. He wrote: "*There are reasons for doubting its relevance (even if it does exist in man) to migraine; hypoperfusion traverses the cortex like a carpet being unrolled, not a band; hypoperfusion persists in the occipital region for 4-6 hours, whereas migraine aura last 5 to 60 min; reduced blood flow takes 5 hours to reach the hand area, whereas upper-limb numbness occurs immediately after the visual aura or precedes it; and hypoperfusion affects the leg area, which is seldom involved in migraine. To account for migraine we do need a slow neural or neurochemical process, and Leao's spreading depression could provide a model; but if a model fails to correspond with reality, it is the model that has to be discarded*"[79]. [Our emphasis].

J.Creamer/National Anti-Vivisection Society

comment on goadsby's project licence application

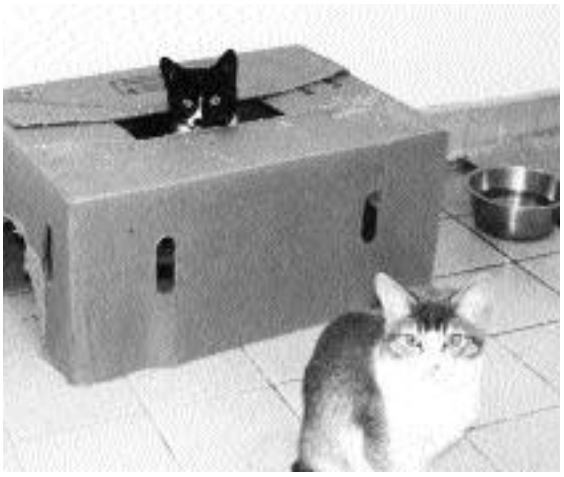
Even though spreading depression has never been seen in humans, or even in animals unless it has been experimentally provoked, some researchers still believe that the spreading reduction in blood supply (spreading oligemia) seen in migraine patients may be a result of spreading depression[72].

Goadsby apparently thinks there may be some connection between the two; in his Project Licence application he writes that spreading depression is "*not dissimilar to the changes in regional blood flow*" reported by clinicians during the early 1980's. He

also says that during the 1990's he has been using anaesthetised cats to "*examine various aspects of cortical spreading depression*"[73].

Several of his recent publications from Australia and Paris confirm this; they make plain that he has been studying spreading depression for some years. For example, in his summary of an Australian cat experiment reported in 1992, he wrote: "*Cortical spreading depression is characterized by a wave of depolarization that moves across the cortex leaving in its wake a state of hyperpolarization. Characteristic changes in cerebral blood flow are also seen and these consist of a wave of hyperemia followed by an oligemia, the latter lasting some hours in some experimental animals including the cat*"[74].

Later the same year, in his description of another Australian cat experiment, he wrote: "*To initiate an increase in metabolic activity and, pari passu, blood flow spreading depression was elicited by needle stick injury. Spreading depression when initiated causes a wave of depolarization, measured as an increased firing rate and associated marked increase in local cerebral blood flow*"[75].



In 1992 he wrote from Paris: "*The cerebrovascular and metabolic changes associated with spreading depression may have an important clinicalimplication since similar mechanisms may be involved in the pathology of migraine*"[88].Of the results of that particular experiment he stated: "*These data generalize the consideration of spreading depression away from the aura and provide a plausible link for involvement of spreading depression in other aspects of the migraine syndrome perhaps linking the phenomenon into migraine without aura in a way not adequately done in the past*"[88].

In 1994, back in Australia, he reported studies in cats of several anti-migraine drugs which are effective and in regular clinical use; he found that the drugs had no affect at all on spreading depression in cats, but even so decided that his findings "*do not provide evidence against the view that spreading depression is important in the aura phase of migraine*"[89].

So, during the 1990's, Goadsby has been using cats to study the very theory already discredited by a 30 year clinical study in 1,000 patients. In our opinion, one of the main principles underlying Goadsby's research on cats is seriously flawed. The decreased blood flow seen in cats' brains may or may not be caused by spreading depression, but the decreased blood flow seen in human migraine patients can't be; humans don't develop spreading depression, ever, even under experimental conditions.

Goadsby's current project is entitled "*The neural innervation of the cerebral circulation and migraine*". However, since our copy of his Project Licence application is incomplete, we can't tell whether spreading depression forms part of his proposal; bearing in mind his prolonged interest in the subject over recent years, and the fact that he mentions it in his justification for the project, spreading depression may play at least some part in his current work.

Thus, the Home Office should say whether, at the time they approved Goadsby's project licence application, they knew about the clinical studies which undermine so much of his past (and possibly present) animal work. We believe we have a complete sequence of the scientific references used by Goadsby in support of his application; there does not appear to be a reference to the research which clinically discredits spreading depression.

comment on branston's project licence application

Branston's Project Licence application makes no mention of spreading depression. Instead, he proposes to study the possible role of the trigeminal nervous system in causing migraine, a possibility once again suggested by clinical studies[77].

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Rat at the Institute of Neurology.

Branston's choice of the rat as an experimental model is particularly unfortunate; it has a smooth (lissencephalic) brain, unlike humans with their convoluted (gyrencephalic) brain and, as already pointed out, this has previously led to dubious and now discredited theories in migraine research.

J. Creamer/National Anti-Vivisection Society

Elisa a metal plate, electrodes and cannulae, permanently botled into her head at the Institute of Neurology.

3.2.4 Sobell Department of Neurophysiology

In this department, Roger Nicholas Lemon is using macaques for a study of the nervous connections between the brain and muscles of the hand. The project is not listed in Current Research In Britain[90], but according to our investigator the 2nd phase of the experiment began in August 1995. So far there have been no publications on this project, but we have photographs of Lemon's application for a project licence from the Home Office.

Lemon has been working with conscious monkeys, using chronic recording and stimulation tech-

Elisa.

niques, for the last 20 years; this work has been carried out in Australia, the Netherlands, and the UK[91].

background to motor areas of the brain

There are several levels of control of movement (i.e., motor control) in the central nervous system. In addition to the primary motor area of the cerebral cortex in the brain, movement is also controlled by several other sub-cortical areas of the brain. This was first suggested through clinical studies of epileptic patients by John Hughlings Jackson in the mid 19th century[92].

Connections from the motor cortex are made through nerve cells running through the "*corticospinal tract*" (also known as the "*pyramidal tract*"), to the spinal cord; there they join or "*synapse*" with motor nerves running to the muscle cell. The way in which these connections are made differs according to the evolutionary development of the species[92].

In the so-called "*lower*" mammals like the rabbit, the nerve fibres of the corticospinal tract barely reach the spinal cord. The final connection to motor nerves within the cord is made through a second cell or "*interneuron*". In more developed animals such as cats, the fibres of the corticospinal tract reach most of the spinal cord, but still only make contact with the motor nerves through interneurons[92].

In contrast, in primates the nerve cells running through the corticospinal tract synapse directly with motor nerves in the spine; an interneuron still exists, but the corticospinal tract synapses with both the interneuron and the motor nerve[93].

This direct connection with the motor nerve increases the speed of transmission of the nerve impulse between the motor cortex and the muscle[92], so the corticospinal tract is responsible for precise and skilled movements such as threading a needle or

writing[93]. This too was first suggested by clinical researcher Hughlings Jackson, more than 100 years ago. He noticed that loss of the corticospinal connections does not in itself paralyse muscles; rather, it prevents the use of the muscles in connection with certain movements[94].

Many nerve fibres also arise from the secondary sub-cortical motor areas of the brain. Some of these synapse with, and influence, the primary motor cortex itself. Others pass along the corticospinal tract to synapse with motor nerves within the spinal cord; and some pass to the spinal cord in tracts other than the corticospinal tract, for example within the "*reticulospina*l" and "*vestibulospinal*" tracts; the latter are called "*indirect pathways*"[92,93].

comment on lemon's project licence application

Lemon says that loss of movement of the fingers, and therefore skilled use of the hand, is one of the most debilitating consequences of damage to the motor areas of the brain. He clearly has stroke in mind; he has already found variations in response to non-invasive electromagnetic stimulation of the motor cortex in healthy humans and stroke patients[91].

Using conscious monkeys, during the 1980's he located specific neurones running from the motor-cortex of the brain and synapsing directly to motor nerves which control specific hand muscles. During the early 1990's he also found that monkeys lack direct synapses to the motor-neurones in the first few months of life; he suggests that is why they lack manual dexterity until the direct connections develop later in life[91].

He notes that following stroke in humans there is a substantial reorganization of the motor pathways, but it is still not known how this is achieved; nor is it known how the mechanisms involved could assist recovery after stroke. To

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The small restraining cage in which Elisa was held during experiments.

clarify this situation, he needs to know two things[91].

Firstly, what part is played in controlling the different muscle groups by corticospinal nerve fibres from parts of the brain other than the primary-motor cortex[91].

Secondly, what is the role of the nerve fibres which run along the indirect pathways. Lemon says the presence of these indirect pathways is "*well estab - lished*" in non-primates such as cats, which have no direct nervous connections between the motor cortex and muscles; but he also says: "*their existence in primates is not yet established*"[91].

The purpose of the present project is to understand the roles of the various direct and indirect nerves controlling hand movements in macaques, and to compare these with experimental results from cats. Experiments will also be carried out on New World squirrel monkeys for comparative purposes; like cats, squirrel monkeys have no direct connections between the motor cortex and the hand muscles[91].

Lemon says that his earlier work on laboratory primates has already enabled better interpretation of the results of electromagnetic stimulation of the brain in humans, and enabled investigation of the reorganization of the motor system in stroke victims and amputees. For the current project, invasive work in monkeys is being carried out inclose parallel with non-invasive studies of stroke patients[91].

macaca nemestrina - the experiment in the video

In total 5 types of experimental procedure will be carried out. Since we have video of the Pig-tailed macaque, Elisa (Macaca nemestrina) procedure (i.e., procedure number 1), we describe here what is being done for that part of theproject.

Procedure number 1 is a long-term experiment, involving training to perform lever-pulling etc. Lemon writes that Macaca nemestrina has been selected for this, because they are intelligent, generally docile and friendly, and can tolerate procedures over a 12 month period. They also have large hands, which will make it easier to take electrical recordings from individual muscles[91].

In total, procedure number 1 will use 8 Macaca nemestrina and they are expected to experience "*moderate*" pain[91].

Briefly, these monkeys will be trained to accept restraint, then to perform simple hand tasks such as gripping and lever pulling for food reward; these training sessions will be preceded by a 24 hour starvation period. Lemon writes: "*The tasks are in no way painful or stressful to perform, and all of our monkeys demonstrate enthusiasm when offered the opportunity to work on the experimental task. I do not think the monkeys would be able to perform these tasks if they were in any way uncomfortable or stressed by*

the restraint"[91]. This justification to the Home Office seems disingenuous and misleading, in view of what is to happen next.

The monkeys will then be anaesthetised and given MRI scans of the brain, to identify particular structures for later electrode implantation. Later, they will be anaesthetised again so that an impression of the skull can be taken for precise manufacture of thestereotaxic device[91].

One to two months after the skull impression is taken, these monkeys will be anaesthetised once again, and fitted with the stereotaxic device and stimulating/recording electrodes in various parts of the brain. At the same time, the headpiece will also be connected directly to wires which run subcutaneously to recording electrodes in selected muscles of the forelimbs[91]; these subcutaneous wires are not visible in the video, but the right arm does appear to have been shaved at some time.

J. Creamer/National Anti-Vivisection Society

Above Macaca fascicularis at the Institute of Neurology.

Subsequently, the effects of brain stimulation on the learned tasks will be studied, as will the effects of blocking with drugs the nerve pathways in the brain involved in controlling the task[91].

Finally, under anaesthesia, small quantities of tracer chemicals which stain nervous tissue will be injected into those parts of the brain which have been studied. The animals will be allowed to recover, but three days later they will be killed. The brain and spinal cord will be removed for histological study, to trace the nerve connections that have been stained[91].

Iemon's other macaques

For short-term procedures of up to 3 months, Macaca fascicularis is to be used. Some of the experimental techniques, including training, will be similar to those already described[91].

critique of brain experiments in monkeys

Until more is known about the structure of the human brain no progress will be made by using laboratory animals, even those species like macaques which are supposed to closely resemble humans.

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J.Greaves/National Anti-Vivisection Society

As researchers at two prestigious institutions, the Salk Institute and the University of California, wrote recently: "*What is known about the neuroanatomy of the human brain? Do we have a human cortical map, corresponding to that for the macaque? And what does the human equivalent of the connectional map look like? The shameful answer is that we do not have such detailed maps because, for obvious reasons, most of the experimental methods used on the macaque brain cannot be used on humans. ... We can provisionally make the assumption that the connectional map for the visual areas of the human cortex will be similar to that for the macaque, but this assumption will have to be checked. For other cortical regions, such as the language areas, we cannot use the macaque brain even as a rough guide as it probably lacks comparable regions. We wish we had more concrete suggestions for new techniques. Although we have not, we feel we should make a wide audience aware of this pressing need, especially as most neuroanatomists seem scarcely to have noticed it*"[96].

They reiterated: "To interpret the activity of living human brains, their neuroanatomy must be known in detail. New techniques to do this are urgently needed, since most of the methods now used on monkeys cannot be used on humans"[96].

Whilst the technique of electromagnetic stimulation of the cortex can be used in both humans and macaques, it is clear that Lemon would be better occupied concentrating on human volunteers and patients (which he already uses anyway), and post-mortem human brain specimens. It might take several years longer, but at least he will be learning about humans, not monkeys.

As a neurologist at the University of Newcastle upon Tyne wrote 25 years ago: "*It is often maintained that a scientific attitude involves the mastery of instrumentation that can be acquired only from years of work in a laboratory. But today the neurosurgical theatre or the clinical investigation unit is in fact a superbly equipped laboratory, with the important advantage that its experiments are carried out on man rather than the guinea-pig*"[97]. Or, as in this case, on the monkey.

3.2.5 Astra Neuroscience Research Unit

This research is being done under Project Licence number 70/03313[98], and is not listed in Current Research In Britain[90]. At least two personal licensees are involved: 90/01986 - unidentified person, and 70/01737[98], the latter being a Mrs.J.A.Jones[99].

Telephone extensions on cage cards[98] can be traced to a J.Williams and a Dr.M.Colado; all are working in the Astra Neuroscience Unit[99]. Published research shows that Colado is the main person involved.

Colado's publications from the Institute of Neurology show that her permanent address is the Faculty 0of Medicine at Complutense University in Madrid[100]. A search shows her carrying out similar research at both places.

background - ecstasy

Ecstasy (methylenedioxymethamphetamine) is a derivative of amphetamine which has received a great deal of attention recently, because of its use as an illicit recreational drug. As it is so well known through media stories and articles, there is little point in going into further detail.

studies of ecstasy in rats

In 1993 Colado's team published two studies of ecstasy, in Lister Hooded rats supplied by Harlan Olac, Bicester.

In the first, Colado had studied the loss of brain serotonin (5-HT) caused by ecstasy and another amphetamine-derived drug, fenfluramine. In particular, she wanted to know if two other drugs (chlormethiazole and dizocilpine) could prevent the loss of brain serotonin. The rats were dosed with the amphetamine derivatives shortly before or after treatment with the protective drugs, then four days later were killed for examination of the brain. Colado was funded by a fellowship from the European Science Foundation[101].

C. Iles/National Anti-Vivisection Society

Rat at the Institute of Neurology that has recently had head surgery.

In the second 1993 publication, the team described a study of ecstasy in living Lister Hooded rats. This time the rats were given intraperitoneal chlormethiazole and dizocilpine shortly before neurotoxicity was induced by four injections of ecstasy. Three days later some of the rats were killed for examination of the brain, but others had been fitted with microdialysis probes[102].

In "*freely moving*" rats these probes were perfused with artificial cerebrospinal fluid, so that at 20 minute intervals small samples of the neurotransmitter dopamine could be washed out of the brain and its quantity measured. The effects on brain dopamine levels, of ecstasy and the potential therapeutic drugs, were then measured in conscious animals. Again Colado was funded by the European Science Foundation[102].

Colado's most recent publication

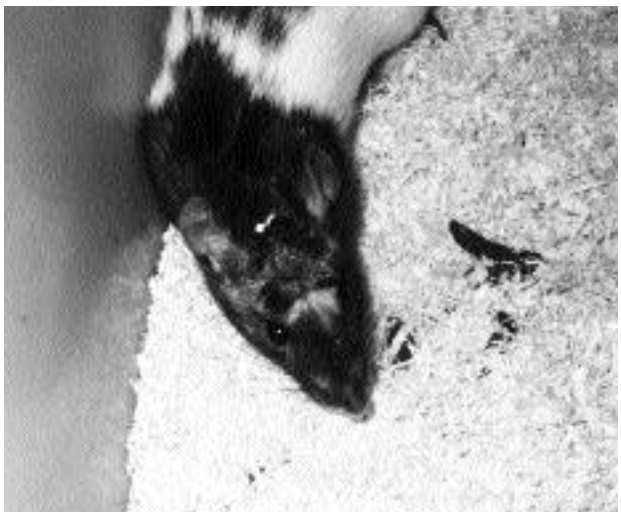
from the Institute of Neurology was in January 1994. It was a study in Lister Hooded rats (again supplied by Harlan Olac, Bicester) of potential drugs for treating, or preventing, the brain damage caused by ecstasy. Rats were dosed with ecstasy ataround the same time as the hopefully protective drugs under investigation. Some of the animals were killed 4 hours later, others 4 days later, in both cases by breaking their necks. The brains were removed for study[100].

critique of ecstasy research in rats

Despite the restrictions imposed by various governments on the use of ecstasy, its popularity as a recreational drug has increased in the USA and Europe[103].

The neurotoxicity of ecstasy appears to be related to its metabolism, so extensive studies have been done on this. They have all been done on animals; virtually nothing is known about the human pharmacokinetics of ecstasy since, because it is a controlled drug with no clinical application, it has not been investigated clinically[104].

Most of the neurochemical and behavioural research into the effects of ecstasy in rodents has focused on the neurotransmitters serotonin and dopamine, with some small glimpses from drug abusers of its effects in humans[105,106]. Since no controlled studies have been done on the adverse consequences of ecstasy, the only clinical information available comes from individual case reports[103].



In animals, ecstasy damages those brain cells which depend on serotonin, but there is still no evidence that it damages such cells in humans. Some of the disturbances seen in drug abusers could be a result of serotonin deficit, but on available evidence it is too early to jump to this conclusion[107].

Serotonin function can only be assessed in humans by indirect methods, and none of them are conclusive. However, the results of three clinical studies of serotonin neurotoxicity have been conflicting; of two using the same technique, one reported loss of serotonin, the other did not. A third experiment using a different technique was inconclusive[103].

So whether humans develop neurotoxic changes like animals, and if so whether they are of functional significance, remains to be seen. Controlled studies are needed to find out whether humans exposed to neurotoxic amphetamines develop lasting changes in their brains. Analysis of already-available human clinical data from drug abusers could help to identify changes in subtle behavioural functions, such as those in which serotonin might be involved[107].

Such clinical research would not only help to define the public health consequences of exposure to these drugs, but it could also enhance our understanding of the roles of dopamine and serotonin in human brain function[107], both in normal and in diseased states such as major depression, anxiety disorders and Alzheimer's disease[103].

Meanwhile, at Britain's flagship Institute of Neurology, researchers are generating yet more information about rats; whilst we still know virtually nothing about the clinical effects on humans of a drug that a significant proportion of British children apparently take on a regular basis. If this is a result of government restrictions on the use of ecstasy, then the British government should take a lead and make exceptions in the case of well-planned clinical research.

Recall too that in our discussion of clot-busting drugs at CXWMS, we related how species differences in serotonin receptors had caused great confusion in serotonin research; that confusion applies equally to studies of the brain, making the results from rats even less applicable to humans.

NOTE: We have done a full critique (see earlier) of fenfluramine research carried out by a quite separate group at the Institute of Neurology; the fenfluramine aspects of Colado's research will be subject to the same limitations outlined in that report.

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