still capable of paralysing and killing up to 20% of monkeys inoculated intracerebrally, and of provoking central-nervous-system changes in the rest.

No doubt the d, t, and MS markers do represent useful investigational tools of polioviruses. But it is important to recognize our ignorance concerning the basic mechanisms involved in neurovirulence and the degree to which these various markers reflect this mechanism. The correlation of these markers with neuroactivity is imperfect, indicating that other and unknown factors also play a part in neurovirulence. This is in all probability to be expected, since the growth of a virus within a cell is dependent not only upon the environmental conditions outside the cell, as pH and temperature, but also upon the presence of proper receptors on the cell surface and perhaps an entire battery of as yet unknown enzymes necessary for initiating and carrying out the complex operation of virus synthesis and release. It seems, therefore, that at this stage of our knowledge one is hardly justified in considering as indicators of avirulence unstable secondary characteristics of as yet unproved significance, when the direct method of assay in monkeys is readily available and accords well with the field behaviour of the strains in the human host.

Summary

Baselines of normally expected neuroactivity for each type of Lederle attenuated polioviruses in consecutive lots of vaccine were established by grading on a scale of 1 to 4 the spread of lesions from the site of inoculation in 574 monkeys inoculated intracerebrally with consecutive lots of vaccine. Relative neuroactivity of a vaccine lot was expressed as the sum of the percentage of animals showing grades 3 and 4.

The average incidence of combined grades 3 and 4 was 21% for type I, 57% for type II, and 16% for type III virus. The greater intracerebral activity of type II virus, which expresses itself in histopathological lesions and not in paralysis, is in marked contrast to its clinical behaviour in man, where it shows the least "aggressiveness" of the three poliovirus strains, and to its marker pattern, which conforms most closely to the one proposed for avirulence.

All three Lederle poliovirus strains possess the t-character, which appears to have the best correlation with monkey avirulence, but differ in their d and MS markers; type I was found to be d+MS±; type II was d±MS-; and type III was d+MS+. Some of the discrepancies in the correlation between markers and monkey virulence are discussed.

Since the value of d, t, and MS markers as criteria of attenuation for polio vaccine viruses has not been definitely established, residual neurovirulence for monkeys remains the most acceptable measure of strain attenuation.

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VENTRICULAR FIBRILLATION DURING LEFT ATRIAL PUNCTURE RECOVERY AFTER EMERGENCY MITRAL VALVOTOMY

BY

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The technique of percutaneous left atrial puncture introduced by Björk *et al.* (1953) has been widely used in the investigation of mitral valve disease and is a routine method at this hospital (Hancock, 1959a). The case reported here developed ventricular fibrillation during the puncture. Immediate cardiac massage was followed by mitral valvotomy and the patient ultimately made a complete recovery.

Case Report

A married woman aged 37, who gave no rheumatic history, complained of increasing breathlessness on exertion since her first pregnancy five years ago, when mitral stenosis was diagnosed. She had had attacks of bronchitis during the previous three winters, with haemoptyses on the last occasion.

On examination the pulse was regular and there were the physical signs of mitral stenosis, with a soft early diastolic murmur at the lower left sternal border, attributed to slight aortic incompetence. The blood-pressure was 110/60. There was no evidence of systemic or pulmonary venous congestion.

A chest radiograph showed no cardiac enlargement, pulmonary venous congestion, or interlobular septal lines. Screening showed slight left atrial and right ventricular enlargement. An electrocardiogram showed P mitrale, slight right axis deviation, and right ventricular hypertrophy.

Left atrial puncture was undertaken to assess the severity of the mitral stenosis, as doubts existed whether the symptoms were due to the valve lesion or to pulmonary disease.

Left Atrial Puncture

Premedication: papaveretum $\frac{1}{2}$ gr. (20 mg.), hyoscine 1/150 gr. (0.43 mg.). Local analgesia with 2% lignocaine (without adrenaline). A No. 16 thin-walled cannula with

inner needle was introduced 4 cm. to the right of the midline in the ninth intercostal space, with the patient prone, and advanced without difficulty into the left atrium (Hancock, 1959a). After left atrial pressure had been recorded, a fine "polythene" catheter was passed through the cannula into the left atrium and ventricle; two records during withdrawal through the mitral valve were obtained (Fig. 1). At the time, the gradient (observed on the oscilloscope monitor) was noted to be small and of doubtful significance. The pressures were L.A. c 34, x 29, v 39, y 25 (mean 30) and L.V. 110/29; the mean diastolic gradients on the two withdrawals were 0 and 6 mm. Hg.

After these pressure records had been made, the patient complained of pain across the lower chest, felt ill, and vomited; the E.C.G. monitor showed sinus arrest and an idioventricular rhythm (rate 45-60) with a broad Q.R.S. (0.18 sec.) (Fig. 2 A and B). Ordinarily, a dye-dilution curve for estimation of cardiac output and assessment of mitral incompetence (Hancock, 1959b) would have been performed at this stage. On this occasion the needle was promptly removed and the patient turned into the supine position. She vomited again, and the E.C.G. now showed coarse ventricular fibrillation (Fig. 2 C).

An immediate left thoracotomy was performed and cardiac massage begun through the intact pericardium; oxygen was administered by facepiece, using a Boyle anaesthetic apparatus. A thoracic surgeon and an anaesthetist arrived within five minutes of the onset of cardiac arrest. The surgeon opened the pericardium and began direct cardiac massage; there was no evidence of intrapericardial bleeding. An endotracheal tube was introduced and manual inflation of the lungs with oxygen continued.

A good carotid pulse was palpable during massage, and the myocardial tone was good. In spite of numerous (approximately 12) attempts at electrical defibrillation (150-200 v.) and intra-atrial injections of 1:10,000 adrenaline and 1% calcium chloride, ventricular fibrillation continued for 30 minutes, after which time co-ordinate ventricular contraction was restored. At this stage it was necessary to adminis-

ter 15 mg. of D-tubocurarine intravenously to control voluntary respiratory efforts; anaesthesia was continued with 50% nitrous oxide and oxygen.

It was then felt that the patient stood little chance of survival if severe mitral stenosis were left unrelieved. In spite of the evidence already obtained of little or no significant stenosis, the mitral valve was then explored digitally through the left atrial appendage. Tight mitral stenosis (0.75-1 by 0.5 cm.) was found, without calcification or regurgitation. The medial commissure was split to the ring, and the lateral commissure also split well but not completely. Partially fused chordae and papillary muscles beneath the valve cusps formed a funnel-like subvalvar stenosis; some separation of the chordae was achieved and a final functional orifice of 3.5 cm. obtained. Ventricular fibrillation recurred, but this responded to a single shock and sinus rhythm returned (P-R interval 0.2 sec., QRS duration 0.16 sec.) (Fig. 2 D and E).

After restoration of sinus rhythm the systemic blood-pressure was unrecordable, but by aortic palpation was within the range of 40 to 50 mm. Hg. The circulation was accordingly assisted by intermittent cardiac massage for approximately 10 minutes, and over this period the systolic pressure gradually rose to 80 mm. Hg. The numerous bleeding-points which had now become apparent were ligated and the chest wall was closed with underwater-seal pleural drainage. Though the patient was breathing spontaneously 1/75 gr. (0.87 mg.) of atropine sulphate followed by 2.5 mg. of neostigmine was given in order to abolish any residual curarization. The pulse was very rapid (about 140 beats a minute) and digitalization was started by an intravenous injection of 1 mg. of digoxin. The patient was then transferred to the recovery ward and placed in an oxygen tent.

One hour later it was noted that respiration was fast and somewhat gasping. She was unconscious, with generalized flaccidity and pupils which did not react to light. The systolic blood-pressure was 70 mm. Hg, and the rectal temperature 37° C.

The failure to recover consciousness, coupled with the neurological signs present three hours after closure of the thoracotomy, unquestionably pointed to anoxic cerebral damage of a severe degree. Treatment was based on two principles—namely, dehydration and hypothermia. Initially 100 ml. of triple-strength plasma was given rapidly and was followed 15 minutes later by a further 200 ml. Within minutes the limbs were moved spasmodically and she protested incoherently. Coincident with this was a faint pupillary response to light and a definite improvement in muscle tone. The improvement in the level of consciousness was maintained, and over a period of three hours a total of 500 ml. of the concentrated plasma was given. It is emphasized that during this period the systolic blood-pressure remained within the range of 80 to 90 mm. Hg. During this phase the body temperature was reduced rapidly by surface cooling to 33.5° C. and maintained at approximately that level for 30 hours. Shivering was controlled by intramuscular chlorpromazine, and analgesics were given as required.

Some 12 hours after starting this regime the patient was conscious, though disorientated in respect of time. There was a gradual improvement in the mental state, and before hypothermia was discontinued she was quite rational but emotionally labile and intensely depressed. This alteration in personality was noted by relatives. Some three weeks after opera-

FIG. 1.—Left ventricular and left atrial pressure record during withdrawal across mitral valve. Above, first record showing no gradient; below, second record showing small diastolic gradient (mean 6 mm.).

tion she was aware of a sudden mental change, which is best related in her words as "an awakening as if from a bad dream" to revert to what she considered her normal bright and cheerful self. She complained of moving patterns in the lower half of the visual fields, but there was no actual loss of visual field. There was also some ataxia, giddiness, and weakness of the right arm. Apart from extensor plantar responses, little objective evidence of central nervous abnormality was discovered, but the neurologist (Dr. Ian Mackenzie) thought that there had been some anoxic brain damage, principally of basilar distribution.

On discharge from hospital seven weeks after emergency thoracotomy, her physical and mental capacity appeared to be completely normal.

FIG. 2.—E.C.G. standard lead II. A, Immediately before withdrawal of needle. B, After withdrawal of needle. C, Onset of ventricular fibrillation. D, After mitral valvotomy. E, After closure of chest.

Discussion

Management of the Emergency

Experience with this patient emphasizes once again the crucial importance of opening the chest and starting cardiac massage immediately cardiac arrest has been diagnosed (Milstein, 1956). In this case the monitoring electrocardiogram left no doubt that ventricular fibrillation had occurred, but no time was wasted stimulating the heart mechanically, administering drugs, or awaiting assistance before embarking on thoracotomy.

It is also well established that a valve stenosis should be relieved before cardiac arrest can be overcome. That this is so when cardiac arrest occurs during the course of an operation on the heart, particularly for the relief of aortic stenosis, is perhaps more familiar than the sort of situation described in this paper. It was very striking that co-ordinate ventricular contraction was achieved with the utmost difficulty before the mitral valvotomy and with relative ease afterwards. There can be no doubt that her survival depended on the performance of mitral valvotomy as well as on the immediate institution of cardiac resuscitation when the emergency arose.

Management of the Post-operative Period

The principal effects of anoxic damage to the central nervous system are nerve-cell injury and the appearance of cerebral swelling. The mechanism, extent, and clinical significance of this latter process are not clear

(White *et al.*, 1942; Edstrom and Essex, 1956), but the increase in brain volume is of serious significance (Atkinson, 1952). A rational if empirical method of treatment is therefore a reduction of brain volume. Hypothermia is of great value in that not only does it reduce cerebral metabolism and confer a degree of protection under anoxic conditions (Lougheed and Kahn, 1955; Brock and Ross, 1955), but it is also believed to produce a reduction in brain volume where there is established brain injury as reported by Lundberg *et al.* (1956) and Sedzimir and Dundee (1958). There is ample experimental and clinical evidence that hypothermia will result in a reduction of normal brain volume (Rosomoff and Holaday, 1954).

Rapid reduction of brain volume is achieved by intravenous hypertonic solutions. Concentrated solutions of dextrose, plasma, and more recently urea (Javid and Settlege, 1956) have been used for this purpose. We decided to use triple-strength plasma because it is locally non-irritant and at the same time undoubtedly efficient, as shown by the work of Maciver *et al.* (1958). Clinical experience with intravenous urea is not as yet extensive, and the concentrated sugar solutions, apart from the intense local irritation, may cause renal tubular damage (Lindberg *et al.*, 1939).

Interpretation of the Haemodynamic Findings

When the withdrawal records were obtained it was observed on the oscilloscope monitor that there appeared to be little or no diastolic gradient between left atrium and ventricle. It was concluded at the time that the mitral stenosis was mild, and this conclusion to some extent influenced the course of action later. Fortunately, however, the decision to explore was finally taken, since it was thought that the patient's chances of survival were slight if unrelieved severe mitral stenosis was present. It had been noticed during massage that filling of the left ventricle was impaired.

Subsequent study of the photographic pressure records (Fig. 1) showed that the mean diastolic gradient on the two withdrawals was 0 and 6 mm. Hg. In view of the surgical demonstration of severe stenosis, this requires explanation. If the 6-mm. gradient is regarded as a valid measurement, and the mitral valve area taken as 0.7 sq. cm. (surgical assessment), then the cardiac output at the time, calculated with a modified Gorlin formula,* was approximately 2.5 l./min. Though the patient's condition had not then given cause for worry, the electrocardiogram (Fig. 1) did show lengthening of the P-R interval (0.2–0.22 sec.) and QRS duration (0.1 sec.), and it may well be that the output of the heart had already fallen to this extent. It is more difficult to explain why the first withdrawal had shown no gradient. Scrutiny of the records shows that the difference between the two withdrawals was that the left ventricular diastolic pressure was higher in the former. A possible explanation is provided by the surgical observation of a funnel-like subvalvar stenosis produced by fused and shortened chordae. It is conceivable that the tip of the catheter lay below the mitral valve ring in the subvalvar zone; in systole the ventricular pressure was recorded, but in diastole the pressure was intermediate between atrial and ventricular. This explanation is not entirely satisfactory, but no better one can be put forward.

*M.V.A. (sq. cm.) = $\frac{C.O. (l./min.) \times 1,000}{45.5 \times D.F.P. (sec./min.) \times \sqrt{\text{mitral diastolic mean gradient}}}$
D.F.P. = diastolic filling period; C.O. = cardiac output; M.V.A. = mitral valve area. (D.F.P. and mitral gradient directly measured.)

It is important, however, to recognize that in severe mitral stenosis the gradient measured at left-heart catheterization may occasionally, under circumstances such as have been described here, be misleadingly low or even absent.

Risks of Left Atrial Puncture

Right posterior transthoracic left atrial puncture is now a well-established diagnostic procedure. It is recognized to have an appreciable morbidity and mortality, though the incidence of serious and fatal sequelae is small. Bougas (1957) collected 1,770 cases from the literature with a mortality of 0.5%, and Crymes *et al.* (1959) collected 2,178 cases with a mortality of 0.69%.

Some of the deaths reported have been in patients already seriously ill at the time of the procedure (Bougas *et al.*, 1956;† Bougas, 1957; Gadboys *et al.*, 1957; Fisher, 1959), and in these the procedure may have been merely a minor contributory factor. Other deaths have been due to pericardial tamponade or to surgical intervention for actual or supposed tamponade (Björk, 1954;‡ Blakemore *et al.*, 1957; Musser and Goldberg, 1957). Non-fatal haemopericardium, with or without tamponade, requiring aspiration has been reported in several series (Fisher, 1955, 1959; Lawrence, 1956; Blakemore *et al.*, 1957; Bagger *et al.*, 1957; Kavanagh-Gray and Drake, 1958); haemothorax has also been reported (Björk, 1954; Fisher, 1955; Bagger *et al.*, 1957; Samet *et al.*, 1959). In these cases a long and difficult puncture, the use of a heparinized drip for an associated right-heart catheterization, and the accidental puncture of a high-pressure chamber (usually aorta) have been recognized as important factors predisposing to haemorrhagic complications; Björk's (1954) case had an aneurysm of the ascending aorta which was punctured.

Proved or probable arterial emboli (Musser and Goldberg, 1957; Boshier, 1958; Samet *et al.*, 1959) have been responsible for deaths, and non-fatal emboli have also been reported (Bagger *et al.*, 1957; Kavanagh-Gray and Drake, 1958). Other important complications include pneumothorax, syncope, hypotension, and knotting, or "shearing off," of the plastic catheter used for entering the left ventricle.

It is generally believed that bronchoscopic puncture of the left atrium is a safer procedure, but deaths have been reported due to ventricular fibrillation associated with a reaction to local anaesthetic (Rubin and Shah, 1958), coma and asystole possibly due to a cerebral embolus (Crymes *et al.*, 1959), and convulsions on withdrawal of bronchoscope and needle (Davila *et al.*, 1958). Emboli may occur with this technique (Rubin and Shah, 1958), as also may knotting, or "shearing off," of the catheter or tamponade (Crymes *et al.*, 1959), though this appears to be very rare.

The occurrence of ventricular fibrillation during the left atrial puncture itself has been reported by Samet *et al.* (1959) and also by Björk in two cases in association with tamponade (Bagger *et al.*, 1957). One of Björk's cases had transient ventricular fibrillation and another had transient cardiac asystole immediately after angiocardiology with injection through the left atrial needle (Bagger *et al.*, 1957). Our patient developed ventricular fibrillation within a very short time of the

removal of the needle and in the absence of significant bleeding into the pericardial sac.

At this hospital, early experience with left atrial puncture was unfortunate (Brock *et al.*, 1956), but this was due to the use of the technique in the assessment of advanced aortic stenosis, many of the patients being in left ventricular failure. The technique was therefore abandoned in favour of left ventricular puncture in aortic stenosis, but was adopted again in 1957 for the investigation of mitral valve disease and has been used routinely since then (Hancock, 1959a). Fifty patients have been investigated. Thirty-one are included in Hancock's series; there were two important complications in these cases—a mesenteric embolus and atrial fibrillation (Hancock, 1959a); 19 additional patients with mitral or mitral and aortic valve disease (mild lone aortic stenosis in one) have had left atrial punctures, with significant complications in four. One is the subject of this report, the other three are briefly reported.

A man aged 47, with mitral stenosis and atrial fibrillation, remained hypotensive for several hours after an uneventful puncture; the next day he awoke with aphasia and a right hemiparesis probably due to cerebral embolism, from which he has made an almost complete recovery. He also had haematuria, attributed to a renal embolus.

A woman aged 51, with mitral and aortic stenosis, had a difficult left atrial puncture, at which the right atrium was entered first, and moderate hypotension developed. She was subsequently found to have a fairly extensive area of consolidation in the right lower lobe with a small pleural effusion.

A man aged 31, with mitral stenosis, had an uneventful left atrial puncture. He discharged himself from hospital against advice, and four days after the puncture was readmitted with pleural pain and haemoptysis, and with severe dyspnoea due to early pulmonary oedema. A chest radiograph showed an area of consolidation in the axillary subsegments of the right upper lobe, which resolved quickly.

This last patient probably had a pulmonary infarct, but in the other patient with a pulmonary opacity this could have been related to the passage of the needle and may have represented an area of haemorrhagic consolidation. In both these patients heparinized saline had been infused, and in one the heparin was thought to have contributed to the development of this complication. Since that time heparin has not been used during left atrial puncture, even when right-heart catheterization has been undertaken simultaneously.

Thus there have been six significant complications in 50 cases (12%), but no deaths.

Summary

The case of a patient with mitral stenosis who developed ventricular fibrillation at left atrial puncture is reported. Immediate thoracotomy and cardiac resuscitation were followed by mitral valvotomy; the patient has recovered completely. The crucial importance of relieving a stenotic lesion when cardiac arrest occurs is stressed. The post-operative care is described. Difficulties in the interpretation of withdrawal records obtained in this case are discussed.

The risks of left atrial puncture are reviewed, and complications observed in three other patients (cerebral and renal embolism in one and pulmonary consolidation in two) briefly described.

We thank Dr. R. Kauntze and Mr. D. N. Ross for permission to publish this case. We are particularly indebted to Mr. D. N. Ross for invaluable advice, and acknowledge the vital contribution made by the recovery ward staff.

†Case also referred to by Bailey (1955) and Musser and Goldberg (1957).

‡Case also referred to by Crafoord (1955) and Bagger *et al.* (1957).

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- Murphy *et al.*, 1957). It has been demonstrated (Dietrich, Friedland, and Kaplan, 1958) that the inhibitory action of 6-aminonicotinamide on certain diphosphopyridine nucleotide (D.P.N.)-dependent reactions results from its rapid substitution for nicotinamide in the D.P.N. molecule to form an inactive D.P.N. analogue.

These results show that 6-aminonicotinamide causes malformations by interfering with some function of nicotinamide, probably with its role in D.P.N. synthesis. The rapidity with which 6-aminonicotinamide acts, and the ability of nicotinamide to protect against its effects, provided an opportunity to study the effects on embryonic development of precisely timed short exposures of pregnant mice to this nicotinamide antagonist.

Materials and Methods

A/Jax male mice, 3 to 4 months old, were placed overnight with C57BL/6 females which were observed for vaginal plugs in the morning. Similarly C57BL/6 males were mated with A/Jax females. Animals were maintained on Purina Lab Chow, supplemented with milk and lettuce once a week, and water *ad lib*.

At approximately 9½, 10½, or 11½ days after a vaginal plug was observed pregnant females were given a single intramuscular injection of 6-aminonicotinamide, the standard dose being either 0.4 or 0.5 mg., depending on whether the animal weighed less than or more than 23 g. on the morning a vaginal plug was observed. Females receiving 0.4 mg. of 6-aminonicotinamide were treated, two hours later, with 0.15 mg. of nicotinamide (the "single protective dose") or 0.3 mg. (the "double protective dose"). Females given 0.5 mg. of 6-aminonicotinamide received proportionately more nicotinamide (0.19 or 0.38 mg. respectively). Control females received the 6-aminonicotinamide and a single protective dose of nicotinamide simultaneously.

At 18½ days after insemination the animals were killed and the offspring removed. After gross examination most embryos were prepared for skeletal staining with alizarin, and some were fixed for histological study.

Results

The types of skeletal malformation produced by treating pregnant female mice of heterogeneous genetic background with 6-aminonicotinamide have been described previously (Pinsky and Fraser, 1959). Similar types of malformation were produced by the present method, except that the frequency of cleft-lip, with cleft-palate, reported as showing an equivocal increase over the control figure in the previous experiment, showed a marked increase in C57BL × A/Jax embryos treated on day 9½ in the present experiment.

Protective Action of Nicotinamide

In evaluating our results four criteria of teratogenic activity were used: (1) frequency of *resorption sites*, as identified by the presence of a recognizable placenta with no embryo, or a resorbing embryo; (2) frequency of *cleft-palate*, without cleft-lip; (3) frequency of *cleft-lip*, with cleft-palate; (4) frequency of major *hind-limb defects*, defined as absence or extreme hypoplasia of the tibia and/or severe digital anomalies.

To find out whether a "single protective dose" of nicotinamide is, in fact, fully protective two tests were used. Firstly, the "single protective dose" was given simultaneously with the 6-aminonicotinamide. This

CONGENITAL MALFORMATIONS AFTER A TWO-HOUR INACTIVATION OF NICOTINAMIDE IN PREGNANT MICE

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This paper describes a method by which the action of a specific vitamin (nicotinamide) can be inhibited for a very brief period of embryonic development by an analogue (6-aminonicotinamide), and presents preliminary observations on the types and frequencies of malformations so produced in the mouse.

Major skeletal malformations have been observed in the offspring of pregnant rats (Murphy, Dagg, and Karnofsky, 1957) and mice (Pinsky and Fraser, 1959), and in newborn chicks from eggs (Landauer, 1957) treated with 6-aminonicotinamide. Simultaneous treatment with nicotinamide gave protection against the inhibitory effects of 6-aminonicotinamide as measured by the LD₅₀ in mice (Johnson and McColl, 1955) and by teratogenicity in chicks and rats (Landauer, 1957;