Biochemical Pharmacology, 1964, Vol. 13, pp. 165–182. Pergamon Press Ltd., Printed in Great Britain.

BRAIN DAMAGE AND PARALYSIS IN ANIMALS EXPOSED TO HIGH PRESSURE OXYGEN—PHARMACOLOGICAL AND BIOCHEMICAL OBSERVATIONS

H. A. S. VAN DEN BRENK and D. JAMIESON

Radiobiological Research Unit, Cancer Institute Board, Melbourne, Australia

(*Received* 1 *July* 1963; *accepted* 19 *September* 1963)

Abstract—Single exposures of high pressure oxygen (OHP) at 30–66 ψ gauge pressure caused CNS damage and paralysis in rats and mice but guinea pigs, rabbits and man did not show such sequellae. The CNS damage in rats was greatly increased by CNS depressent drugs (pentobarbital sodium, paraldehyde, N_2O and sernyl) given before exposure to OHP. The CNS lesions were also potentiated by raised respired pCO_2, by acetazolamide and by NH_4Cl, whilst protection was afforded by methaemoglobinaemia (induced by PAPP) by the tris-buffer THAM, by 2–4 dinitrophenol and by 5HT against the barbiturate and CO_2 potentiation of OHP brain damage. OHP induced brain damage was *not modified* by (I) hypothermia (CP_z), (II) electroconvulsive shock treatment during OHP, (III) by cerebral X-irradiation, (IV) by adrenalectomy or cortisone, (V) by slow decompression rates, (VI) by spinal block with local anaesthetic, (VII) by "conditioning" of rats to OHP, (VIII) by hyper- and hypo-glycaemia, and (IX) by alterations in tissue histamine levels.

The results are discussed in relation to possible biochemical mechanisms and theories of oxygen poisoning.

IT WAS shown by Bean and Siegfried[1] that repeated exposure of unanaesthetised rats to high pressures of pure oxygen (OHP) at 65 lb/in^2 gauge pressure, often resulted in transient or permanent signs of damage to the CNS in the form of stuporous states and paralytic motor disturbance. In a preliminary study[2] it was reported that anaesthetic agents greatly potentiated this brain damage in rats which also resulted from a single exposure to high pressure oxygen. The present paper deals with further observations made of this phenomenon, with particular reference to the effectiveness of various pharmacological treatments in altering the severity of the paralytic effects of OHP in rats and other species.

MATERIALS AND METHODS

Canberra Black or Hooded Wistar strain rats weighing 150–200 g were used. Since preliminary observations failed to show that sex difference significantly affected the results, both male and female animals were used, but the sex proportions were kept constant for control and test groups respectively. Guinea pigs weighed 400–500 g and hybrid Walter and Eliza Hall mice weighed 30–40 g.

The animals were pressurized in the steel compression chamber and apparatus described previously.[3] After flushing of the chamber containing the animals with oxygen, the pressure was increased at a linear rate of 15 lb/in^2 per minute to the desired level, and this pressure was kept constant for the period defined as "duration of

OHP" under results. The animals were then decompressed at 15 lb/in^2 per min, removed from the chamber and housed for subsequent observation in an air conditioned room at 21°, and given a standard laboratory diet. In experiments where CO_2 mixtures were used under pressure, the CO_2 was added to the oxygen atmosphere and is expressed as partial pressure (mm Hg). In one experiment the decompression rate was lowered to 2·5 lb/in^2 per min.

Twenty four hours after pressurization, each animal was examined with care, and signs of brain damage recorded. These were expressed as a semiquantitative index as follows:

Neurological change	Score
No significant change	0
Hyperexcitability and slight incoordination of movements	1
Hyperexcitability and slight spasticity of limbs, normal righting reflexes.	2
Spasticity with splaying of hind limbs, righting reflexes present but impaired.	3
Severe spasticity with "Kangaroo" posture and loss of righting reflexes.	4
Severe spasticity and paralysis, lying on side and unable to move and eat or drink without assistance.	5
Dead at 24 hr preceded by signs of severe spastic paralysis.	6

Rats which were dead on decompression had severe lung damage and were excluded from the analysis.

Drugs and administration

The following compounds were injected in saline or small volumes of distilled water:

Pentobarbital sodium, paraldehyde, morphine sulphate, chloropromazine hydrochloride, acetazolamide sodium, histamine acid phosphate, lysergic acid diethylamide (LSD); methoxamine HCl; 1-(1-phenylcyclohexyl) piperidine HCl (sernyl), cystamine base; 2-aminoethyl isothiouronium bromide hydrobromide (AET); 5-hydroxytryptamine creatinine sulphate (5HT); cortisone acetate; tris (hydroxymethylaminomethane) (THAM). 2:4 dinitrophenol (DNP) was dissolved in NaOH solution; *p*-aminopropriophenone (PAPP) was injected in propylene glycol. Inorganic salts were dissolved in distilled water. All compounds were administered by intraperitoneal injection with the exception of the daily doses of cortisone, which were given subcutaneously, and paraldehyde which was given intramuscularly.

Pentobarbital sodium was administered 10 min before pressurization, while compounds given in conjunction with this anaesthetic were usually injected 5 min after pentobarbital sodium. AET and PAPP however were administered 10 min prior to pentobarbital. THAM and the inorganic salts used to produce alkalosis or acidosis were injected immediately prior to pressurization.

Subacute depletion of tissue histamine by compound 48/80 dissolved in distilled water was given in accordance with the schedule of Feldberg and Talesnik[4] in doses of 100 μg, 200 μg and 500 μg per rat administered intraperitoneally on successive days.

X-radiation of brain

The X-ray exposure factors were 250 kV X-rays, 15 mA, 30 cm FSD, HVL = 1 mm Cu, giving a dose rate 300 r/min in air. The whole of the rat body, with the exception of the head was heavily shielded with lead, the head packed in bolus to give full back scatter, and the tissue dose was calculated at 0·5 cm depth below the dorsal surface of the skull.

Determination of CO_2 content of blood

For comparisons of untreated rats and those injected with paraaminopropriophenone (PAPP) to produce methaemoglobinaemia blood samples were obtained by left ventricular puncture. One ml of blood was then transferred immediately to a Van Slyke apparatus and the CO_2 content determined.

In a further estimation of the effect of met Hb on CO_2 content of whole blood several millilitres of rat blood were withdrawn by left ventricular puncture. One half of the sample was equilibrated for 15 min with preheated humidified 4·8% CO_2/95·2% O_2 using a modification of an equilibration vessel described by Gray[5]. One ml of this equilibrated blood was then analysed for CO_2 content in the Van Slyke apparatus. The other half of the original blood sample was treated with 0·1 ml 20% K_3 Fe $(CN)_6$ solution per ml blood to convert the haemoglobin present to methaemoglobin. This blood was then equilibrated with 4·8% CO_2/95·2% O_2 as above, and analysed for CO_2 content.

To determine the CO_2 content of reduced and oxygenated blood, blood was withdrawn from the left ventricle and abdominal vena cava in each rat and immediately placed under paraffin. Not more than 1 hr later these venous and arterial samples were analysed for total CO_2.

RESULTS

Paralytic damage following pressurization without pretreatment of rats

In Table 1 results are shown for a total of seventy-two rats exposed to various pressures of oxygen (45–66 lb/in^2) for various times. At 45 lb/in^2, for exposure periods up to one hour, no brain damage was observed in survivors although this pressure gave rise to both convulsions and pulmonary damage. At higher pressures (48–66 lb/in^2) some residual brain damage occurred, but the overall effect was difficult to assess owing to the high incidence of pulmonary damage at these pressures. However, it seems clear that above 45 lb/in^2, residual brain damage increases with increasing pressures. The incidence of this damage is more directly related to the magnitude of the oxygen pressure, than to the duration of the pressure exposure or to the product of pressure and exposure time.

Progress of animals after brain damage

Nine rats with varying degrees of brain damage following pentobarbital sodium (see below) and exposure to OHP were kept for 6 months. One rat initially had very severe paralysis (score 5). In this animal righting reflexes improved to about 10 sec righting time after 7 days and continued to show a slow improvement over the next few months. After 6 months it was assessed as "moderate" paralysis (score 3). For two other rats with severe paralysis, the paralysis improved similarly in one, from score 5 to 3, but the other severely paralysed rat did not show objective improvement. Four rats initially showed slight to moderate brain damage (score 2–3). These rats

improved over 7–10 days and one continued to improve until it was classified at normal after 3 months, whilst very slight paralysis (score 1) persisted in the others. Two rats with slight paralysis (score 2) initially improved until they appeared almost normal (score 0–1) after 4–5 months. We were unable to decide in some rats whether

TABLE 1. EFFECT OF A SINGLE EXPOSURE TO OHP IN CAUSING CNS DAMAGE IN UNANAESTHETIZED RATS

Rat weight (g)	Number of rats	Oxygen pressure (lb/in²)	Duration of exposure to OHP (min)	Index of CNS damage ±S.E.
150–200	3	45	10	0·0
	6	45	15	0·0
	6	45	25	0·0
	9	45	30	0·0
	6	45	45	0·0
	6	45	60	(6 rats dead on decompression)*
	6	48	40	0·8 ± 0·4
	6	60	15	1·3 ± 0·4
	9	60	30	1·5 ± 0·5
	3	60	45	5·0 (2 rats dead on decompression)*
	6	66	15	2·3 ± 0·2
50	6	45	30	0·0

* Death due to lung damage

the improvement was due to a true reversal of the initial damage or to adaptation of the animal to its neurological lesion with compensation by the utilization of alternative neuromuscular pathways. However, in most rats recovery of the spastic paralysis was clearly due to true recovery of the neurological lesion. In a severely paralysed rat (score 5) recovery progressed to the stage that the animal was able to move freely and was almost normal except for some spasticity of the hind limbs, a slight delay in righting reflexes and hyperexcitability. In most of the affected rats, there was little objective improvement for 5–7 days after exposure to OHP, but considerable improvement was evident in the following 7 days after which further improvement was less rapid. In some rats kept for over 12 months this latter improvement seems to have ceased and with ageing the neurological manifestations have once more become more severe.

Pretreatment with CNS depressant drugs

(i) *Pentobarbital sodium.* The results for 145 rats pretreated with pentobarbital sodium (38 mg/kg body weight), and subjected to 30–66 lb/in² OHP for varying periods, are shown in Table 2. At 45 lb/in² brain damage increased as the exposure time was increased from 15–20 min. For 25–30 min exposures a maximum effect was obtained and further prolongation of the exposure time did not significantly increase the observed effect. At this level of pressure (45 lb/in²) it was also found that an additional pretreatment with pentobarbital sodium 24 hr before the second pentobarbital anaesthetic was followed by OHP exposure, did not affect the severity of residual paralysis. Less mature rats, weighing 50 g, exposed to 45 lb/in² OHP for 30 min after anaesthesia showed significantly less brain damage ($P < 0{\cdot}05$) than older

rats. When higher pressures of oxygen were used in conjunction with pentobarbital sodium, brain damage was markedly increased and the exposures required were shorter than at 45 lb/in², but again the maximum index of damage obtained was comparable to that at 45 lb/in² and lengthening the duration of exposure did not significantly increase the index of damage.

TABLE 2. EFFECT OF A SINGLE EXPOSURE TO OHP IN CAUSING CNS DAMAGE IN RATS (150–200 g) ANAESTHETIZED WITH PENTOBARBITAL SODIUM ADMINISTERED INTRAPERITONEALLY BEFORE COMPRESSION

Dose of pentobarbital sodium (mg/kg)	Number of rats	Oxygen pressure (lb/in²)	Duration of exposure to OHP (min)	Index of CNS damage ±S.E.
38	3	45	10	0·0
38	12	45	15	1·0 ± 0·2
38	22	45	25	3·8 ± 0·3
38	42	45	30	3·7 ± 0·2†
38‡	6	45	30	2·3 ± 0·3*
38	6	45	40	3·7 ± 0·7
38	3	45	45	3·7 ± 0·9
38	6	45	50	4·0 ± 0·6
38	3	45	60	4·0 ± 1·0
(170 g rats given two treatments with pentobarbital, 38 mg/kg 24 hr before exposure and repeated 10 min before compression)	6	45	30	4·5 ± 0·3†
38	7	30	30	1·7 ± 0·5
38	9	60	15	4·1 ± 0·3
38	3	60	30	3·0 ± 0·0
	3	60	45	4·0 ± 1·0
	5	66	15	5·0 ± 0·3
15	6	48	40	1·8 ± 0·4
38 mg/kg after mid dorsal spinal block§	7	45	30	3·0 ± 0·7
38	6	45	35	2·5 ± 0·7‖
38 mg/kg but slow decompression at 2·5 lb/in² per min	6	45	30	3·2 ± 0·8‖

* $P < 0{\cdot}05$.
† n.s.
‡ 50 g rats.
§ Temporary mid dorsal spinal block injecting 0·1 ml procain into cord immediately before pentobarbital anaesthesia and OHP.
‖ n.s.

It was also found that decreasing the dose of pentobarbital greatly decreased brain damage following OHP. Rats which were conscious and not fully relaxed during the OHP exposures suffered much less damage. However, no attempt was made to determine dose response curves for pentobarbital sodium in this respect, owing to the limited accuracy with which the brain damage could be quantitatively assessed, and since other complications such as pulmonary damage occurred at higher pressures.

It was also found that if the distal half of the rat's body was paralysed by injecting the 0·1 ml of procaine HCl into the mid dorsal spinal cord before administering pentobarbital, the incidence of paralysis in such rats exposed to OHP was not significantly different from that in pentobarbital group not subjected to a spinal block.

In one group of pentobarbital treated animals the decompression rate after OHP was decreased to 2·5 lb/in² per min, but this slow decompression did not lessen the paralytic sequelae.

(ii) *Nitrous oxide.* When the partial pressure of N_2O in the respired gas was adjusted to 12 lb/in² and the oxygen pressure to 48 lb/in², brain damage was significantly increased compared to unanaesthetized controls (Table 3). Pretreatment with a low dose of pentobarbital sodium (15 mg/kg) before the N_2O/O_2 pressurization further increased the brain damage. This dose of pentobarbital sodium itself given before OHP caused little residual brain damage. The concentration of N_2O used in this experiment caused loss of consciousness and anaesthesia during the period of exposure but arousal during or within a few minutes after decompression.

(iii) *Paraldehyde.* In anaesthetic doses (1·2 ml/kg) this compound potentiated brain damage due to OHP, comparable in severity to potentiation by pentobarbital sodium (Table 3).

TABLE 3. EFFECT OF CNS DEPRESSANTS ON OHP INDUCED PARALYSIS IN RATS

Treatment	Number of rats	Oxygen pressure (lb/in²)	Duration of exposure to OHP (min)	Index of CNS damage ±S.E.
Unanaesthetized	6	48	40	0·8 ± 0·4
N_2O (12 lb/in²) + O_2 (48 lb/in²)	6	48 (total pressure 60)	40	2·7 ± 0·7
N_2O (12 lb/in²) + O_2 (48 lb/in²) preceded by pentobarbital sodium (15 mg/kg)	6	48 (total pressure 60)	40	3·7 ± 0·2*
pentobarbital sodium (15 mg/kg)	6	48 (total pressure 60)	40	1·8 ± 0·4*
Morphine sulphate (10 mg/kg)	6	45	40	0·2 ± 0·2†
Morphine sulphate (10 mg/kg) + pentobarbital sodium (15 mg/kg)	6	45	40	3·8 ± 0·4†
Unanaesthetized	6	45	40	0·0
Paraldehyde (1·2 ml/kg)	6	45	30	4·7 ± 0·2
Chlorpromazine (35 mg/kg)	3	45	30	0·0
Sernyl (50 mg/kg)	6	45	30	4·0 ± 0·8
Sernyl (25 mg/kg)	6	45	30	0·0

* $P < 0·01$
† $P < 0·01$.

(iv) *Sernyl.* A dose of 50 mg/kg, which produced catatonia but did not fully anaesthetize the rats, potentiated brain damage due to OHP. A dose of 25 mg/kg was ineffective in this respect.

(v) *Morphine.* In doses of 10 mg/kg morphine failed to significantly potentiate brain damage in unanaesthetized rats but combined with a low dose of pentobarbital sodium (15 mg/kg), a significant increase in brain damage resulted ($P < 0·01$ if compared with the effect of 15 mg/kg pentobarbital sodium alone).

(vi) *Chlorpromazine.* This compound administered in large doses (35 mg/kg) sufficient to induce drowsiness and hypothermia in the rats, failed to increase the paralytic effects of OHP.

Effect of respired carbon dioxide

Addition of CO_2 to the oxygen to give partial pressures of CO_2 ranging from 38 to 152 mm Hg caused marked enhancement of brain damage in the absence of anaesthesia (Table 4). Indeed this enhancement far exceeded that due to pentobarbital sodium and other CNS depressant drugs at comparable oxygen pressure exposures (compare results Tables 1, 2, 3 and 4). The highest partial pressure of CO_2 used (152 mm Hg) did not itself cause brain damage in the absence of raised pO_2.

TABLE 4. EFFECT OF RESPIRED CO_2 ON CNS DAMAGE RESULTING FROM OHP IN UNANAESTHETIZED RATS AND IN RATS ANAESTHETIZED WITH PENTOBARBITAL SODIUM (38 mg/kg)

Group	Number of rats	Oxygen pressure (lb/in²)	pCO_2 added to OHP (mm Hg)	Duration of exposure to OHP (min)	Index of CNS damage ±S.E.
Unanaesthetized	3	45	38	25	2·0 ± 0·0
	12	45	76	15	3·5 ± 0·5
	12	45	114	15	5·0 ± 0·4
	12	45	152	10	5·8 ± 0·1
	24	45	152	15	4·2 ± 0·3
	6	152 mm Hg.	152	25	0·0
Anaesthetized	5	45	76	15	4·0 ± 0·4
	6	45	114	15	2·4 ± 0·8
	9	45	152	15	4·3 ± 0·2

When pentobarbital sodium (38 mg/kg) was given before raised pCO_2/pO_2 exposures, the combined effect was much greater than for pentobarbital sodium alone, but not significantly different from that due to raised pCO_2/pO_2 treatments.

Drugs interfering with acid base balance (Table 5)

(i) *Acetazolamide.* In doses of 100 mg/kg, this compound greatly potentiated brain damage due to OHP, and furthermore increased the effect of pentobarbital sodium in this respect. However pretreatment with this compound before exposure to raised pCO_2/pO_2 atmospheres did not significantly increase or decrease the effect of carbon dioxide in potentiating OHP brain damage. Furthermore, given before exposures of 45 lb/in² oxygen for 15 min, pretreatment combinations of acetazolamide, carbon dioxide and pentobarbital sodium did not result in brain damage significantly greater than that due to CO_2 or acetazolamide given singly in similar dosages.

(ii) $NaHCO_3$ *and THAM.* The bicarbonate given intraperitoneally in doses of 0·3 g/kg body weight did not alter brain damage due to OHP in unanaesthetized rats. However it significantly reduced the potentiating effect of a barbiturate ($P < 0·05$). This effect was even greater for tris buffer THAM (tris [hydroxymethyl] aminomethane) which in doses 1·2 g/kg markedly decreased the effect of both pentobarbital sodium ($P < 0·001$) and raised pCO_2 ($P < 0·001$).

(iii) *NH_4Cl.* Pretreatment with this compound, in doses of 250 mg/kg, slightly increased brain damage due to OHP alone, and to pentobarbital sodium treatment combined with OHP, ($P < 0{\cdot}05$).

(iv) *Alloxan.* 180 mg/kg of this compound was given to rats 24 hr before pentobarbital sodium and exposure to OHP. The rats urine was tested for glycosuria and ketone bodies and showed 0·5–2% sugar content and ketone bodies in faint to moderate concentration. The CNS lesions in such diabetic rats were not significantly different from those in pentobarbital control animals (Table 5).

TABLE 5. EFFECT OF DRUGS AFFECTING TISSUE ACID-BASE BALANCE ON OHP INDUCED PARALYSIS IN RATS

Treatment	Number of rats	Oxygen pressure (lb/in²)	Duration of OHP exposure (min)	Index of CNS damage (±S.E.)
Acetazolamide (AZL) (100 mg/kg)	12	45	30	3·8 ± 0·6
AZL (100 mg/kg) and pentobarbital Na (38 mg/kg)	6	45	30	5·0 ± 0·0
AZL (100 mg/kg) and CO_2 (p_{CO_2} = 76 mm Hg)	12	45	15	3·0 ± 0·6
AZL (100 mg/kg) and CO_2 (p_{CO_2} = 114 mm Hg)	12	45	15	4·2 ± 0·6
AZL (100 mg/kg) and CO_2 (p_{CO_2} = 152 mm Hg)	12	45	15	3·7 ± 0·3
AZL (100 mg/kg) and pentobarbital Na (38 mg/kg) and CO_2 (p_{CO_2} = 76 mm Hg)	6	45	15	4·7 ± 0·4
AZL (100 mg/kg) and pentobarbital Na (38 mg/kg) and CO_2 (p_{CO_2} = 114 mm Hg)	6	45	15	3·3 ± 0·6
AZL (100 mg/kg) and pentobarbital Na (38 mg/kg) and CO_2 (p_{CO_2} = 152 mm Hg)	6	45	15	4·3 ± 0·5
Na HCO_3 (0·3 g/kg)	6	45	30	0·0
Na HCO_3 (0·3 g/kg) and pentobarbital Na (38 mg/kg)	12	45	30	2·5 ± 0·7*
NH_4 Cl (40 mg/kg)	6	45	30	0·7 ± 0·2
NH_4 (250 mg/kg)	6	45	40	1·0 ± 0·5
NH_4 Cl (250 mg/kg) and pentobarbital Na (38 mg/kg)	6	45	40	5·2 ± 0·3†
THAM (1·2 g/kg)	6	45	30	0·0
THAM (1·2 g/kg) and pentobarbital Na (38 mg/kg)	6	45	30	0·7 ± 0·2‡
THAM (1·2 g/kg) and CO_2 (p_{CO_2} = 38 mm Hg)	6	45	30	0·7 ± 0·2§
Alloxan (180 mg/kg) 24 hr before pentobarbital Na (38 mg/kg)	12	45	30	4·3 ± 0·4‖
Insulin (1·2 units/kg) 30 min before pentobarbital Na (38 mg/kg)	11	45	30	3·5 ± 0·6‖
Control group (pentobarbital Na 38 mg/kg only)	23	45	30	3·7 ± 0·4‖

Comparison with respective control groups * compared with pentobarbital only (3·7 ± 0·2 Table 2) $P < 0{\cdot}05$; † cf (3·7 ± 0·7 Table 2) $P < 0{\cdot}05$ ‡ cf (3·7 ± 0·2 Table 2) $P < 0{\cdot}001$ § cf (2·0 ± 0·0 Table 4) $P < 0{\cdot}001$. ‖ n.s.

(v) *Insulin.* In the highest tolerated subconvulsive dose (1·2 units/kg in these rats) given 30 min preceding pentobarbital and OHP, this substance failed to modify brain damage (Table 5).

Methaemoglobinaemia (PAPP)

In a series of experiments (Table 6) rats were injected with paraminopropriophenone (PAPP) in doses of 24 mg/kg to cause severe methaemoglobinaemia. These animals were subsequently exposed to (I) OHP alone, (II) pentobarbital sodium followed by OHP, or (III) OHP combined with raised pCO_2. In such rats the incidence of residual brain damage due to OHP was reduced to negligible values in all groups (orders of probability from $P < 0{\cdot}01$ to $P < 0{\cdot}001$). As PAPP was dissolved in propylene glycol, this latter compound alone was given to rats prior to injection with pentobarbital sodium and exposure to OHP; propylene glycol did not alter the potentiation of brain damage by pentobarbital sodium.

TABLE 6. EFFECT OF PARA-AMINOPROPRIOPHENONE (PAPP) ON OHP INDUCED PARALYSIS IN RATS

Treatment	Number of rats	Oxygen pressure (lb/in^2)	Duration of OHP exposure (min)	Index of CNS damage ($\pm$S.E.)
PAPP (24 mg/kg)	6	45	30	0·0
,,	6	45	40	0·0
,,	6	45	50	0·7 $\pm$ 0·4
,,	12	60	15	0·4 $\pm$ 0·3
Pentobarbital Na (38 mg/kg)	12	45	30	2·6 $\pm$ 0·4
,,	6	45	50	4·0 $\pm$ 0·6
,,	9	60	15	4·1 $\pm$ 0·3
PAPP and pentobarbital Na (24 mg/kg) (38 mg/kg)	6	45	30	0·0
,,	6	45	40	0·3 $\pm$ 0·3
,,	6	45	50	0·7 $\pm$ 0·4
,,	16	60	15	1·6 $\pm$ 0·9
PAPP and CO_2 (76 mm Hg)	6	45	15	0·0
,,	6	45	15	0·0
PAPP and pentobarbital and CO_2 (76 mm Hg)	6	45	15	0·0
,, (152 mm Hg)	6	45	15	0·8 $\pm$ 0·7*
Propylene glycol (5 ml/kg) and pentobarbital Na (38 mg/kg)	6	45	30	4·2 $\pm$ 0·5

* Two rats died during exposure to OHP/CO_2.

Effect of PAPP on blood CO_2 capacity

If the CO_2 capacity of blood in PAPP treated rats was greater than in untreated rats subjected to OHP, better removal of tissue CO_2 would probably result and by this mechanism possibly ameliorate the toxic effects of OHP. The total CO_2 content of arterial and venous blood samples of untreated and PAPP treated rats were determined (Table 7). It is seen that for arterial blood equilibrated with 4·8% CO_2/95% O_2 the CO_2 content was reduced if the methaemoglobinaemia was produced *in vitro* by potassium ferricyanide. For normal rats the mean arterio-venous CO_2 difference was

7·2 vols CO_2 per cent of blood and represents the amount of CO_2 released by a given volume of blood in its passage through the lungs. For PAPP treated rats this CO_2 release was 2·0 vols per cent and is less than in normal rats. Furthermore both the arterial and venous blood CO_2 content in normal rats were higher than the respective arterial and venous CO_2 levels in PAPP treated rats. These results suggest therefore,

TABLE 7. CARBON DIOXIDE CONTENT IN BLOOD OF NORMAL RATS AND RATS PRETREATED WITH PARA-AMINOPROPRIOPHENONE (PAPP) (25 mg/kg)

Sample (Number of animals)	Vol CO_2 per cent ±S.E.	Mean difference between samples (significance P)
1. No treatment—fresh left ventricular blood (6)	52·2 ± 3·2	8·9
2. PAPP treated—fresh left ventricular blood (6)	43·3 ± 1·9	($P < 0·05$)
3. No treatment—venous blood (6)	59·4 ± 1·7	14·0
4. PAPP treated—venous blood (6)	45·4 ± 2·1	($P < 0·05$)
5. No treatment—fresh left ventricular blood equilibrated *in vitro* with 4·8% CO_2/95% O_2 (6)	58·0 ± 1·9	10·5 ($P < 0·05$)
6. No treatment—fresh left ventricular blood treated *in vitro* with K_3 Fe $(CN)_6$ and then equilibrated with 4·8% CO_2/95% O_2 (6)	47·5 ± 3·1	

that PAPP treatment, which produces methaemoglobinaemia, reduces CO_2 carrying capacity of the blood and would tend to enhance CO_2 retention by the tissues rather than the reverse.

Other chemical pretreatments

(i) *Treatments specifically affecting tissue 5HT.* Pretreatment with 5-hydroxytryptamine (16·5 mg/kg) significantly reduced brain damage due to barbiturate and OHP ($P < 0·001$) and this effect was reversed by the 5HT antagonist LSD administered in doses of 0·5 mg/kg before the 5HT. LSD alone did not significantly alter brain damage due to barbiturate and OHP (Table 8). Attempts made to assess the effect of depletion of tissue 5HT by means of large doses of reserpine were unsuccessful owing to the marked physiological disturbance caused by this pretreatment *per se*. Another biogenic amine examined (methoxamine) failed to reduce the CNS damage due to barbiturate and OHP.

(ii) *Treatments specifically affecting tissue histamine.* Pretreatment with histamine (100 mg/kg) failed to significantly influence the effect of either OHP alone or barbiturate plus OHP in respect to paralytic damage. Similarly rats depleted of tissue histamine by means of compound 48/80 behaved no differently from non depleted animals in this respect (Table 8).

(iii) *Other compounds.* The radioprotective compounds cystamine and AET, previously found to protect against OHP induced convulsions, lung damage and death in rats and mice[6, 7] did not significantly alter the barbiturate potentiation of residual brain damage after OHP (Table 8). Similarly another compound tested, NaCN, failed to influence this effect. However 2–4 dinitrophenol (DNP) in doses of 15 mg/kg significantly reduced the paralytic effects of the barbiturate plus OHP treatment ($P < 0·001$)—an unexpected result in that this drug was found to decrease

TABLE 8. EFFECT OF MISCELLANEOUS CHEMICAL PRETREATMENTS ON OHP INDUCED CNS DAMAGE IN RATS

Group	Treatment	No. of rats	Oxygen pressure (lb/in²)	Duration of OHP exposure (min)	Index of CNS damage (±S.E.) (significance P)
5HT and 5HT antagonists	pentobarbital Na (38 mg/kg)	42	45	30	3·7 ± 0·2
	pentobarbital Na (38 mg/kg) + 5HT (16·5 mg/kg)	6	45	30	2·3 ± 0·3 ($P < 0·001$)
	pentobarbital Na (38 mg/kg) + LSD (0·5 mg/kg)	6	45	30	4·2 ± 0·6
	pentobarbital Na (38 mg/kg) + 5HT + LSD	6	45	30	3·7 ± 0·7
Methoxamine	pentobarbital Na (38 mg/kg) + methoxamine (10 mg/kg)	6	45	30	3·7 ± 0·7
Sulphydryl compounds	pentobarbital Na (38 mg/kg) + cystamine (−SS) (75 mg/kg)	9	45	30	3·7 ± 0·7
	pentobarbital Na (38 mg/kg) + AET (300 mg/kg)	3	45	30	5·7 ± 0·6
Metabolic inhibitors	pentobarbital Na (38 mg/kg) + NaCN (1 mg/kg)	6	45	30	4·3 ± 0·2
	pentobarbital Na (38 mg/kg) + 2–4 DNP (15 mg/kg) (dissolved in NaOH)	12	45	30	0·6 ± 0·4 ($P < 0·001$)
	pentobarbital Na (38 mg/kg) + 5% NaOH (1 ml/kg)	4	45	30	4·0 ± 1·0
Histamine and histamine depletion	Histamine (75 mg/kg)	3	45	30	0·0
	Histamine (75 mg/kg) + pentobarbital Na (38 mg/kg)	3	45	30	5·0 ± 0·6
	Compound 48/80 ("subacute depletion")*	3	45	30	0·0
	48/80 + pentobarbital Na (38 mg/kg)	3	45	30	4·7 ± 0·4
	48/80 + histamine (75 mg/kg)	3	45	30	0·0
	48/80 + histamine + pentobarbital Na (38 mg/kg)	3	45	30	4·3 ± 0·4

* See methods.

survival in mice exposed to OHP[6]. As pretreatment with compounds causing alkalosis were found to have some effect on OHP induced paralysis, and since DNP was prepared in 0·6% NaOH solution for injection, a control experiment was carried out; the vehicle, 0·2 ml of 0·6% NaOH, used for DNP injections, was given to a group of rats prior to treatment with pentobarbital sodium and OHP. The quantity of NaOH did not affect residual paralysis (Table 8).

Other pretreatments

(i) *Adrenalectomy and cortisone.* (Table 9). The paralytic sequelae of the barbiturate and OHP treatment, were not significantly affected by previous adrenalectomy nor by large repeated doses of cortisone acetate.

TABLE 9. EFFECT OF VARIOUS TREATMENTS ON OHP INDUCED CNS DAMAGE IN RATS

Treatment	Number of rats	Oxygen pressure (lb/in²)	Duration of OHP exposure	Index of CNS damage ± S.E.
Adrenalectomized 7 days before pentobarbital Na (38 mg/kg) and OHP	6	45	30	4·7 ± 0·3
Cortisone acetate 1 mg per rat twice daily for 3 days before pentobarbital Na (38 mg/kg) + OHP	6	45	30	4·2 ± 0·6
Cortisone acetate 5 mg per rat twice daily 3 days before pentobarbital Na (38 mg/kg) + OHP	6	45	30	4·2 ± 0·6
Pentobarbital Na (38 mg/kg)	3	45	10	0·0
"	3	45	10	0·0
+ 100 V shocks for 2 sec at 0, 5 and 10 min during OHP exposure				
Pentobarbital Na (38 mg/kg)	5	45	25	3·2 ± 0·7
"	6	45	25	2·8 ± 0·4
+ 100 V shocks for 2 sec every 4 min throughout exposure to OHP				
I. Rats "*conditioned*" *to OHP* exposure to OHP every day for 14 days (exposure times increasing from 5 to 20 min at 45 lb/in²) before *final* exposure after pentobarbital Na (38 mg/kg) anaesthesia.	7	45	40	4·0 ± 0·8
II. As in I but each second conditioning exposure to OHP preceded by pentobarbital Na (38 mg/kg).	7	45	40	3·2 ± 0·5
III. "*Unconditioned*" controls—single OHP exposure after pentobarbital Na (38 mg/kg).	7	45	40	3·6 ± 0·7

(ii) *Electro-convulsive shock treatment during OHP.* (Table 9) Electro-convulsive seizures were induced in barbiturate anesthetized rats during the OHP exposure. In the group receiving 100 V shocks of 2 sec duration every 4 min during the 25 min OHP exposure the residual brain damage was reduced from 3·2 ± 0·7 to 2·8 ± 0·4 but this reduction is not significant.

(iii) "*Conditioned*" *rats.* Since there is some evidence that rats can be rendered less sensitive to OHP toxicity by repeated exposures to gradually increasing amounts of oxygen in terms of pressure and duration of increased pressure, such "conditioned" animals were exposed to a final test exposure of OHP (45 lb/in² for 40 min) preceded by pentobarbital anaesthesia. The details of the "conditioning" treatment are shown in Table 9 together with the final results. No significant alteration in barbiturate potentiation of the OHP toxicity in respect to persistent brain damage was found for the method of "conditioning" adopted.

TABLE 10. EFFECT OF LOCAL X-IRRADIATION OF BRAIN OF RATS ON SUBSEQUENT OHP INDUCED BRAIN DAMAGE

Treatment	No. of rats	Oxygen pressure (lb/in²)	Duration of OHP exposure (min)	Convulsions during OHP	Index of CNS damage (±S.E.)
I. 5000 rads to head after pentobarbital Na (38 mg/kg)	3	(not exposed)	—	0/3	0·0
II. As in I but X-ray dose 8000 rads	3	(not exposed)	—	0/3	0·0
III. As in I but followed immediately by OHP	4	45	30	2/4	4·0±0·0
IV. As in II but followed 24 hr later by further pentobarbital Na (38 mg/kg) and OHP.	4	45	30	1/4	4·0±0·0
V. As in I but followed 24 hr later by OHP alone.	3	45	30	3/3	0·0
VI. As in V but X-ray dose 8000 rads	3	45	30	2/3	0·0

(iv) *Effect of cerebral X-irradiation.* The results are shown in Table 10. All irradiations were given to anaimals anaesthetized with pentobarbital sodium, 24 hr preceding OHP exposures. The mean tissue doses to the brain were either 5000 or 8000 rads, administered as a single radiation exposure. Such animals, subsequently, exposed to OHP without barbiturate pretreatment showed an increased tendency to convulsions during OHP exposures. However the residual brain damage caused by OHP combined with barbiturate pretreatment was not significantly increased by whole brain X-irradiation given immediately beforehand or 24 hr previously.

CNS damage in other animal species and humans

(i) *Mice.* As shown in Table 11, a 25 min exposure at 45 lb/in² oxygen resulted in no paralytic after effects in unanaesthetized mice despite the marked incidence of convulsions in this species during exposure to OHP. However pretreatment with a barbiturate resulted in residual brain damage as in rats.

(ii) *Guinea pigs.* In this species, no brain damage was observed after 45 lb/in² oxygen exposures for over an hour, and barbiturate anaesthesia for OHP exposures for as long as 75 min failed to induce brain damage. In three such anaesthetized animals exposed to 105 min OHP (45 lb/in²) oxygen, death occurred either before or during decompression.

(iii) *Rabbits.* Eight rabbits were deeply anaesthetized with intravenous pentobarbital sodum and exposed to 45 lb/in^2 O_2 for 60 min. No residual brain damage or other deleterious effects were observed after decompression.

TABLE 11. EFFECT OF OHP IN CAUSING CNS DAMAGE IN MICE AND GUINEA PIGS

Group	Treatment	No. of animals	Oxygen pressure (lb/in^2)	Duration of OHP exposure (min)	Index of CNS damage (±S.E.)
Mice	Unanaesthetized	10	45	25	0·0
	Pentobarbital Na (38 mg/kg)	20	45	25	5·5 + 0·3
Guinea pigs	Unanaesthetized	3	45	25	0·0
		3	45	45	0·0
		3	45	75	0·0
		3	45	105	(all died)
	Pentobarbital Na (38 mg/kg)	3	45	25	0·0
		3	45	45	0·0
		3	45	75	0·0

(iv) *Humans.* A series of over 200 humans with advanced neoplastic disease have been treated by one of us (van den Brenk) in conjunction with other clinical staff of the Cancer Institute Board, Melbourne, by means of megavoltage X-irradiation delivered under conditions of high pressure oxygen (45 lb/in^2 gauge pressure) with the patients deeply anaesthetized with pentobarbital sodium[8]. Each of these patients received not less than two, and mostly three treatments spread over a period of 1–4 weeks. The duration of the pressurization exposure necessary for each treatment averaged 45 min (not less than 30 min, and as long as 90 min). In this group of patients, no deaths attributable to either pressurization or anaesthesia occurred, there were five convulsions during exposure to the pressure or on decompression. No cases showed subsequent clinical signs of brain damage, paresis, or significant pyschological disturbance.

DISCUSSION

The observations made clearly show that the phenomenon described by Bean and Siegfried[1] can be consistently produced in rats surviving a single acute exposure of OHP. The clinical lesion varies from mild hyperexcitability to severe spastic paralysis and death. The animals tend to recover, the maximum rate of recovery occurring during the second week but in more severely paralysed rats, such recovery is incomplete and residual paralysis and motor disturbance persists and somewhat worsens at a later stage. The incidence of the lesions varies greatly in the different species examined, being high in rats and mice, but not demonstrated in guinea pigs, rabbits and man. Severe pharmacological depression of central nervous system function greatly enhanced OHP induced brain damage in rats and mice, but failed to do so in guinea pigs, rabbits and man.

For the central nervous system depressant drugs examined it has been shown that the production of loss of consciousness and muscular relaxation are prerequisite for inducing or potentiating brain damage after OHP. The anaesthetic treatment is ineffective if it does not coincide with the period of OHP exposure. Also if anaesthetic

and depressant agents are used in combination in reduced dosages, their effect in this respect is additive and possibly synergistic (e.g. in the case of morphine) although this latter action has not been confirmed by strict experimental methods. The effect of the anaesthetic agent cannot be reasonably attributed to hypothermia since chlorpromazine failed to potentiate OHP paralysis, and there was no correlation between animal temperature and this potentiation for various agents.

Since the completion of this work, an internal report by Pfeiffer and Gersh[9] has been discovered which also draws attention to brain damage resulting from OHP in cats. They report that "when daily oxygen convulsions were prevented with pentobarbital Na, temporary functional impairment of the CNS occurred". These workers used a protocol for assessing the effect of the test pressures in which the same animals were exposed on repeated occasions, with or without drug pretreatments. Effect of OHP (105 lb/in^2 gauge pressure) was assessed in terms of "preconvulsive time" (min) the animals being decompressed rapidly in 1 min at the first sign of generalized convulsions. They also recorded "late CNS damage" for pentobarbital sodium (15 mg/kg), for neosynephrin HCl (2·0 mg/kg), and for ammonium chloride (250 mg/kg); magnesium chloride (200 mg/kg) caused "slight depression". They confirmed the report by Behnke *et al.*[10] that carbon dioxide shortened this preconvulsive time for OHP seizures but apparently observed no late CNS damage following this treatment, whilst "complete methaemoglobinaemia" induced by PAPP (15 mg/kg) had no effect on preconvulsive time but caused death on decompression. Both lactic acid and Na lactate were considered to increase the delaying effect of pentobarbital sodium on preconvulsive times, but ammonium salts did not possess this property.

In the experiments with rats reported in this paper marked brain damage was consistently produced if the partial pressure of respired CO_2 was high during OHP. The severity of this effect was at least comparable to that obtained with CNS depressant drugs such as pentobarbital sodium, N_2O, paraldehyde and sernyl. Furthermore additive effects between subthreshold doses of individual CNS depressant drugs were obtained and also between raised pCO_2 and depressant compounds. The marked effect of CO_2 could be simulated by the carbonic anhydrase inhibitor acetazolamide Na and similarly this drug was additive with both CO_2 and pentobarbital sodium in potentiating OHP brain damage. The added finding that sodium bicarbonate and tris-buffer (THAM) reduced the brain damage resulting from pentobarbital sodium and raised CO_2 during OHP exposure, suggests that a lowering of pH in the vulnerable CNS tissue loci may be an important mechanism in this type of oxygen toxicity. CO_2 caused similar potentiations of OHP damage to rat lungs[7, 11] but in this tissue pentobarbital sodium greatly diminished OHP damage and antagonised the action of CO_2. For the effects of various compounds on preconvulsive times in mice and rats exposed to OHP, great discrepancies exist if comparison is made with post-OHP induced CNS damage. Thus for CO_2 preconclusive times in cats were not altered[8] whilst in other species, such times are shortened[12–14]—findings which agree with the effects of CO_2 on lung damage, paralysis and survival time in OHP. However central nervous system depressants, which "mask" convulsions in animals naturally appear to ameliorate OHP damage in terms of incidence of convulsions and the preconvulsion times. Therefore one must beware of accepting the incidence of convulsions due to OHP and modification of this phenomenon by pharmacological agents as an expression of oxygen damage to CNS. It is considered that the incidence of post OHP

paralytic changes are a much more reliable index of oxygen toxicity in the CNS. Whilst CO_2 accentuates this damage, the mechanism of oxygen poisoning remains obscure. Several hypotheses have been advanced which attribute a primary role to CO_2 in oxygen poisoning. Thompson[15] originally proposed that CO_2 retention by tissues was the cause of oxygen poisoning—a view not supported by the finding of Draper *et al.*[16] that concentrations of CO_2 as high as 55 per cent respired by dogs for 45 min did not cause similar toxic manifestations. The autointoxication theory was also proposed by Gesell,[17] and Lambertson *et al.*[18] explained the action of CO_2 as due to causing cerebral vasodilation resulting in higher CNS oxygen tensions. Recent measurements[19] of pO_2 in cerebral tissues of rats exposed to high oxygen pressures support Lambertson's proposals in that cerebral pO_2 was higher if the pCO_2 respired was increased during OHP. However the results obtained with central depressants such as pentobarbital sodium and conversely with PAPP are difficult to attribute to this effect, as we have shown by measuring cerebral pO_2 during these treatments combined with OHP[19]—neither compound significantly altered the cerebral pO_2 values attained during OHP in contrast to CO_2. Several workers[14, 20, 21] have found that tissue depot pCO_2 rises in the preconvulsive stage of OHP exposure—an effect found by Bahnson and Mathews[22] to occur only when animals were in a terminal state. More recently Walker[23] also using preconvulsive time as an index of oxygen toxicity to CNS, has implicated impairment of CO_2 transport caused by OHP. His views are based on his observation that mice previously "conditioned" to CO_2 breathing subsequently convulsed later during OHP exposure. In our studies we have found that OHP paralysis was not affected by "conditioning" of rats to either pentobarbital sodium or previous OHP or combinations of both.

Some of the results obtained (e.g. the ameliorating effect of THAM and bicarbonate and the potentiating effect of ammonium chloride) do suggest that tissue pH and endogenous buffering mechanisms play an important role in OHP induced CNS paralysis—a finding supported by Bean[24] in that THAM delays the onset and reduces the intensity of OHP siezures in rats and also the mortality and lung damage in this species. However we were unable to demonstrate significant alterations in toxicity in alloxan diabetic animals, nor by lowering of blood sugar by means of subconvulsive doses of insulin. Furthermore the relative resistance of animal species such as guinea-pigs, rabbits and man to OHP induced paralytic sequelae further complicates the picture and makes any simple biochemical exposition of oxygen toxicity unconvincing. Indeed we have previously shown[7] that severe treatments with OHP failed to influence dehydrogenase activity in the rat brain in contrast to the reduction of these enzymes in OHP damaged lung tissues.

In other studies recorded in this paper, it is seen that discrepancies exist between various compounds tested for modification of OHP brain damage, if incidence of convulsions is compared with their effects on residual paralytic damage. Thus, whilst 5HT protects against both convulsions[6] and residual paralysis, AET protected against convulsions but not paralysis. Whilst the metabolic inhibitor NaCN had no effect on residual paralysis but greatly enhanced OHP convulsions and decreased survival,[6] the compound 2–4 dinitrophenol decreased survival, had no significant effect on convulsions[6] but reduced residual brain damage. Histamine protected against convulsions and death[6] but had no effect on residual brain damage. The lack of parallelism between convulsions and residual brain damage is further demonstrated by the results

obtained with adrenal cortical hormones and adrenalectomy (see also refs. 25–29), and with the effects of electro-shock convulsion induced during OHP and the effects of cerebral X-irradiation.

Perhaps the most interesting compound tested in respect to modifying residual brain damage was PAPP which exerted marked protection against the effects of both CO_2 and pentobarbital sodium, and yet did not modify convulsions due to OHP in cats,[9] although we did find it reduced preconvulsive times in mice.[19] This compound, despite causing severe methaemoglobinaemia did not reduce cerebral pO_2 at high pressures in rats[19]. Furthermore the suspicion that it enhanced the transport of CO_2 as a carbamino-compound of methaemoglobin which was not affected by the Bohr shift found for the natural haemoglobin, was not supported by biochemical analysis of CO_2 uptake by blood *in vivo* or *in vitro*. Indeed methaemoglobinaemia reduced CO_2 content of both arterial and venous blood and in this respect would tend to favour CO_2 auto-intoxication.

Other compounds of particular interest in regard to reducing oxygen toxicity are the so-called "antioxidant" group of drugs which have been found to afford very marked protection against residual paralysis and will be reported in a separate study.

CONCLUSION

It is concluded that residual paralysis induced in rats and mice by OHP must be regarded as a definite entity, with a pharmacological basis which appears to resemble other forms of oxygen toxicity (viz convulsions, lung damage and death) but which also differs from these in many respects. The nature of the cerebral lesion and its location is not established and serial sections of brains removed from such affected animals have revealed no obvious vascular or cellular lesion although these studies are incomplete and will be reported when more detailed results are available. Such brains showed no increase in weight or oedema and vascular permeability to Evans blue was unimpaired.[30] However the lesion results in essentially permanent effects, although there is evidence that some recovery takes place with time. There are very marked species differences in susceptibility to OHP paralysis, with guinea pigs, rabbits and man being very tolerant in this respect. No gross biochemical changes in brain dehydrogenase enzymes have been revealed.[7] The lesion is greatly potentiated by severe central nervous depression (anaesthesia) caused by drugs such as pentobarbital sodium—an effect inhibited by a suitable antagonist such as $\beta\beta$-methylethyl glutaramide.[2] It is also greatly potentiated by raised tissue CO_2 induced by CO_2 breathing or by administration of a carbonic anhydrase inhibitor and conversely is inhibited by an amine buffer (THAM) and bicarbonate. Whilst high brain oxygen tension is necessary to induce the paralytic sequelae, the modifying actions of raised respired pCO_2, central nervous system depressants and methaemoglobinaemia cannot be attributed to reducing or increasing the levels of brain pO_2.[19] It is considered that the most fruitful avenue for further investigation lies with an examination of redox potentials in brain tissue under OHP, combined with various treatments. Localization of the lesion by histochemical methods and electron microscopy are a further field for enquiry. It is emphasized that scoring of residual brain damage takes precedence over the scoring of convulsions and preconvulsion times as an index of the damage caused by high concentrations of oxygen to CNS structures.

Acknowledgements—We wish to thank Mrs. K. Elliott, Misses H. Hutchings, K. Ladner and V. Ferguson for their excellent technical assistance. We also acknowledge the generous gifts of compound 48/80 (Wellcome Foundation London) LSD (Sandoz Australia Ltd.) and serny! (Parke Davis & Co.).

REFERENCES

1. J. W. BEAN and E. C. SIEGFRIED, *Amer. J. Physiol.* **143**, 656 (1945).
2. H. A. S. VAN DEN BRENK and D. JAMIESON, *Nature, Lond.* **194**, 777 (1962).
3. H. A. S. VAN DEN BRENK, *Brit. J. Cancer* **15**, 61 (1961).
4. W. FELDBERG and J. TALESNIK, *J. Physiol.* **120**, 550 (1953).
5. L. H. GRAY, *Radiation Biology*. Ed. J. H. MARTIN, Butterworths Scientific Publications, p. 76, (1959).
6. H. A. S. VAN DEN BRENK and D. JAMIESON, *Int. J. rad. Biol.* **4**, 379 (1962).
7. D. JAMIESON and H. A. S. VAN DEN BRENK, *Aust. J. exp. Biol. med. Sci.* **40**, 51 (1962).
8. H. A. S. VAN DEN BRENK, *J. Coll. Radiol. Aust.* **6**, 94 (1962).
9. C. C. PFEIFFER and I. GERSH, Naval Medical Research Institute U.S. Report X-192, No. 2, (1944).
10. A. R. BEHNKE, L. A. SHAW, C. W. SCHILLING, R. M. THOMSON and A. C. MESSER, *Amer. J. Physiol.* **107**, 13 (1934).
11. H. A. S. VAN DEN BRENK and D. JAMIESON, *Aust. J. exp. Biol. med. Sci.* **40**, 37 (1962).
12. L. HILL, *Quart. J. exp. Physiol.* **23**, 49 (1933).
13. L. A. SHAW, A. R. BEHNKE, and A. C. MESSER, *Amer. J. Physiol.* **108**, 652 (1934).
14. H. J. TAYLOR, *J. Physiol.* **109**, 272 (1949).
15. W. G. THOMPSON, *Med. Rec.* **36**, 1 (1889).
16. W. B. DRAPER, R. W. WHITEHEAD and J. N. SPENCER, *Fed. Proc.* **6**, 323 (1947).
17. R. GESELL, *Amer. J. Physiol.* **66**, 5 (1923).
18. C. J. LAMBERTSEN, J. H. EWING, R. H. KOUGH, R. GOULD, and M. W. STROUD, *J. appl. Physiol.* **8**, 255 (1955).
19. D. JAMIESON and H. A. S. VAN DEN BRENK, *J. appl. Physiol.* **18**, 869 (1963).
20. J. A. CAMPBELL, *J. Physiol.* **68**, vii p. (1929).
21. K. SEELKOPF and R. VON WERTZ, *Arch. exptl. Pathol. Pharmakol.* **205**, 351, (1947).
22. H. T. BAHNSON and C. M. MATHEWS, *Amer. J. Physiol.* **175**, 87 (1953).
23. I. G. WALKER, *Canad. J. Biochem. Physiol.* **39**, 1803 (1961).
24. J. W. BEAN, *Amer. J. Physiol.* **201**, 737 (1961).
25. R. GERSCHMAN and P. NADIG, *Fed. Proc.* **12**, 50 (1953).
26. J. W. BEAN, P. JOHNSON and C. W. SMITH, *Abst. of Comm. XIX Intern'l. Physiol. Congress Montreal*, p. 945 (1954).
27. D. W. TAYLOR, *Abst. of Comm. XIX Intern'l. Physiol. Congress Montreal*, p. 821, (1953).
28. R. GERSCHMAN and W. O. FENN, *Amer. J. Physiol.* **176**, 6 (1954).
29. J. W. BEAN and P. C. JOHNSON, *Amer. J. Physiol.* **180**, 438 (1955).
30. D. JAMIESON and H. A. S. VAN DEN BRENK, *Aust. J. exp. Biol. med. Sci.* **40**, 309 (1962).