Canad. Med. Ass. J.
Sept. 2, 1967, vol. 97

Hyperbaric Oxygen:

A Review of Treatment in Eighty-Three Patients

JOHN H. DUFF, M.D., F.R.C.S[C], H. R. SHIBATA, M.D., L. VANSCHAIK, M.D., R. USHER, M.D., R. A. WIGMORE and L. D. MacLEAN, M.D., F.R.C.S.[C], *Montreal*

FOR purposes of clinical and experimental research a hyperbaric chamber was installed at the Royal Victoria Hospital, Montreal, on October 22, 1963.[1] During a period of three and one-half years since that time, 83 patients with various ailments have been treated in the chamber. In all of these patients there was some physiologic rationale for therapy with hyperbaric oxygen, although review of the literature reveals that benefit from such therapy has been fully established in only a few types of human disorder.

PHYSIOLOGIC RATIONALE

Apart from the treatment of decompression sickness, the single potential benefit of hyperbaric oxygen is the increase in the amount of oxygen that can be dissolved in the plasma. In a normal subject breathing air at atmospheric pressure, the amount of oxygen dissolved in the blood is 0.3 ml. per 100 ml. of blood. If the subject breathes pure oxygen at three atmospheres pressure absolute (ATA), the dissolved oxygen can be increased to 5 to 6 ml. per 100 ml. of blood. Since the normal total body arteriovenous oxygen difference is 5 to 6 ml. oxygen per 100 ml. blood, it is evident that dissolved oxygen alone is theoretically capable of meeting a large part of the oxygen needs of the body.

Increased oxygen capacity of blood is of potential benefit to patients with anaerobic infections, ischemia and gangrene, and cancer. In patients with shock and low cardiac output, the achievement of high oxygen content of existing blood flow is desirable.

Table I indicates the variety of ailments treated and the number of patients receiving therapy.

GAS GANGRENE

Hyperbaric oxygen was first used for the treatment of gas gangrene by Boerema in 1960.[2] Working with Boerema, Brummelkamp[3] in 1965 reported a series of 40 patients with gas gangrene of whom 32 survived. In only two of the 40 patients did the authors consider the gangrene not cured. Clostridial antitoxin was not given to patients in this series, and in eight of the patients systemic antibiotics were not employed. Débridement was usually delayed until after treatment with hyperbaric oxygen.

TABLE I.

Diagnosis	*Number of patients treated*
Decompression sickness	17
Shock	8
Gas gangrene	6
Cancer	30
Chronic infection	6
Ischemia and gangrene	8
Respiratory distress syndrome	3
Ischemic skin grafts	2
Cardiac arrest	1
Carbon monoxide poisoning	2
Total	83
Total number of "patient dives"	347

In vitro experiments reported by Boerema and Van Unnik suggest that at a Po_2 of 240 mm. Hg production of alpha toxin by the clostridial organism ceases, although the organism is not killed.[3] Fredette[4] has shown that oxygen at 3 and 6½ atmospheres pressure has a bacteriostatic effect on *Clostridium perfringens in vitro.* These studies and the excellent clinical results of Boerema and Brummelkamp provide good reason for treating gas gangrene with hyperbaric oxygen.

In the present series, six patients with gas gangrene were treated with hyperbaric oxygen. In all but one of these patients, the treatment included surgical débridement. Table II summarizes the results. In four of the six patients treated, the rapidity with which the symptoms of toxicity decreased was impressive—the temperature and heart rate fell and delirium ceased shortly after hyperbaric oxygen therapy was started. Wound healing appeared to be influenced mainly by the surgical débridement.

From the Departments of Surgery and Obstetrics and Gynecology, Royal Victoria Hospital and McGill University, Montreal, Quebec.

Supported by Grant No. MA-2324 from the Medical Research Council of Canada and Grant No. 293-15 from the United States Public Health Service.

Reprint requests to: Dr. J. H. Duff, Department of Surgery, Royal Victoria Hospital, Montreal, Quebec.

TABLE II.— GAS GANGRENE

Patient	*Age*	*Diagnosis*	*Treatment*	*Outcome*
O.B.	77	Gas gangrene of abdominal wall after aneurysm resection; shock and acidosis	Oxygen at 3 ATA for 1 hour	Died in chamber
A.D.	58	Gas gangrene of scrotum and perineum	Oxygen at 3 ATA for 1 hour; 4 exposures	Survived
G.R.	67	Recurrent carcinoma of cecum; gas gangrene of abdominal wall	Oxygen at 3 ATA for 1 hour; 5 exposures	Improved initially; died 4 days later
F.E.	44	Gas gangrene of abdominal wall after appendectomy	Oxygen at 3 ATA for 1 hour; 5 exposures	Survived
M.C.	55	Gas gangrene of scrotum and perineum	Oxygen at 3 ATA for 1 hour; 6 exposures	Survived
A.L.	42	Gas gangrene of leg following crushing injury	Oxygen at 3.5 ATA for 1 hour; 7 exposures	Survived

CHRONIC INFECTION

In 1963, McAllister *et al.*[5] reported that hyperbaric oxygen had an inhibitory effect on bacteria and fungi *in vitro*. Among the organisms inhibited by oxygen at 3 ATA were *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus viridans, Streptococcus hemolyticus, Escherichia coli,* Proteus and *Candida albicans.* Hopkinson and Towers[6] also demonstrated a bacteriostatic effect of hyperbaric oxygen *in vitro.* Ollodart *et al.*[7] noted a bacteriostatic effect on *Escherichia coli in vitro* of oxygen at 2 and 3 ATA. They also showed that in dogs with peritonitis caused by *Escherichia coli,* hyperbaric oxygen had an inhibitory effect on bacterial growth if the peritoneal cavity was opened and directly exposed to oxygen under pressure. On the other hand, *in vivo* studies by Grogan[8] revealed that oxygen at 3 ATA enhanced the growth of *Staphylococcus aureus* in the peritoneal cavity of mice.

Six patients with chronic infections resistant to conventional therapy were treated with five to 31 sessions of hyperbaric oxygen. These patients were placed in the chamber once per day where they breathed oxygen by mask. Table III summarizes the results in these patients. In two patients marked improvement and eventual wound healing occurred during hyperbaric therapy. Although wounds in two of the remaining patients eventually healed, no improvement or change could be seen during the period of hyperbaric therapy.

The results in this small series along with the *in vitro* studies described suggest that further study of a larger number of patients is indicated to assess the value of hyperbaric oxygen in chronic infection.

TABLE III.—CHRONIC INFECTION

Patient	*Age*	*Diagnosis*	*Organism*	*Treatment*	*Outcome*
W.P.	64	Post-traumatic osteomyelitis and leg ulcer—refractory to all previous treatment and facing amputation	*Staphylococcus pyogenes, Aerobacter aerogenes, Pseudomonas aeruginosa*	Oxygen at 3 ATA for 1 hour; 21 exposures	Improved and eventually healed
N.K.	54	Diabetic ulcer of the foot; osteomyelitis	*Staphylococcus pyogenes*	Oxygen at 3 ATA for 1 hour; 20 exposures	No improvement; amputation required
D.D.	25	Recurrent ulcerating infections of the forearm following trauma	Aerophilic streptococcus, Hemolytic staphylococcus	Oxygen at 3 ATA for 1 hour; 31 exposures	No immediate result from hyperbaric oxygen, although infection eventually cleared
A.K.	32	Infected pelvic cavity, following cesarean section and hysterectomy	*Escherichia coli, Streptococcus viridans*	Oxygen at 3 ATA for 1 hour; 8 exposures	Infection cleared; but there was no obvious change during hyperbaric oxygen therapy
C.P.	43	Post-traumatic osteomyelitis	*Staphylococcus pyogenes*	Oxygen at 3 ATA for 1 hour; 5 exposures	No obvious effect of hyperbaric oxygen
C.H.	58	Chronic leg ulcers	*Staphylococcus pyogenes, Pseudomonas aeruginosa,* Aerophilic streptococcus	Oxygen at 3 ATA for 1 hour; 21 exposures	Infection cleared; skin grafting successful

Canad. Med. Ass. J.
Sept. 2, 1967, vol. 97

TABLE IV.—SHOCK

Patient	Age	Diagnosis	Treatment	Partial pressure of oxygen achieved mm. Hg	Arterial lactate* mg. % Before	During	After	Outcome
T.K.	55	Myocardial infarction	Oxygen at 3 ATA for 4 hours	1390	108	91	118	Died
C.H.	—	Septic shock	Oxygen at 3 ATA for 3½ hours	1450	26	25	32	Died
O.B.	77	Septic shock; gas gangrene	Oxygen at 3 ATA for 1 hour	1650	64	80		Died in chamber
G.S.	65	Septic shock; pneumonia	Oxygen at 3 ATA for 1 hour	340				Died
J.G.	62	Aspiration pneumonia	Oxygen at 3 ATA for 1 hour	570				Died
			Oxygen at 2 ATA for 2 hours	132				
I.A.	55	Cardiogenic shock; cirrhosis; aspiration pneumonia	Oxygen at 3 ATA for 1 hour; 2 exposures	1260	143	151	194	Died
C.S.	16	Bilateral lung contusions; aspiration pneumonia	Oxygen at 3 ATA for 20 minutes then at 2 ATA for 40 min.	1000	44	48		Died
J.C.	75	Septic shock; gas gangrene	Oxygen at 3 ATA for 1 hour; 4 exposures	—				Died

*Normal value—less than 13 mg.%

SHOCK

Current investigations suggest that shock is a failure of adequate perfusion of vital body tissues. This being so, one would expect tissue hypoxia to be a major effect of shock. Peretz, McGregor and Dossetor[9] have confirmed this effect in patients by showing a correlation between the severity of shock and the level of arterial blood lactate. Accumulation of lactic acid results from anaerobic glycolysis in hypoxic tissue. Being freely diffusible, the increased lactic acid can be followed by measurement of arterial blood lactate. Fig. 1 correlates survival with the level of arterial blood lactate in 63 patients in shock. On the basis of this and other studies, hyperbaric oxygen was administered to eight patients in severe shock refractory to other forms of therapy. In three of the patients, hypoxia resulting from pneumonia or lung trauma was a major component of the patient's illness. Arterial blood lactates were measured before, during and following therapy, where possible. Table IV summarizes the course of these patients.

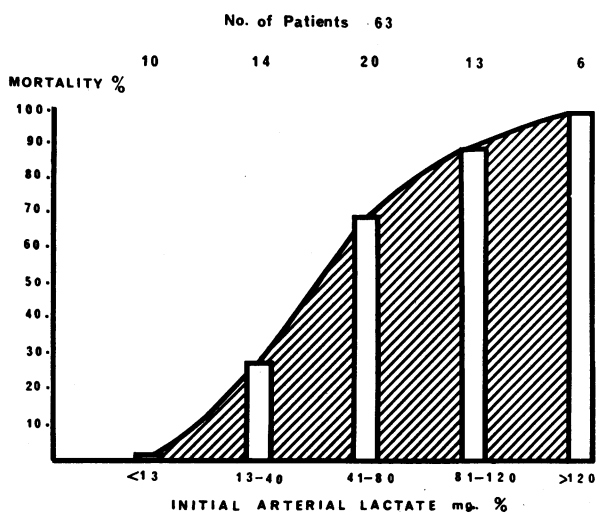

Fig. 1.—Initial arterial blood lactate level of patient in shock plotted against per cent mortality.

A significant fall in arterial blood lactate was not seen during treatment with hyperbaric oxygen, in spite of increased oxygen delivery to hypoxic tissues. A possible explanation is that in advanced shock, hypoxic tissues are unable to utilize oxygen even when available at high partial pressure. In those patients with severe hypoxia of pulmonary origin, colour improved and an increase in Po_2 to above normal levels was noted consistently. Other evidence of clinical improvement, however, was not apparent and none of these patients survived.

Similar results were obtained in animal studies. Dogs with endotoxin shock were treated with blood transfusion and isoproterenol to restore cardiac output to normal or above normal levels. The animals were then treated with hyperbaric oxygen. The survival rate was unchanged and arterial blood lactate levels were the same as those in untreated animals.[10] Cowley *et al.*[11] reported similar results with regard to endotoxin shock in dogs but were able to show significant improvement in survival of dogs with hemorrhagic shock when treated with hyperbaric oxygen. It remains to be shown whether or not hyperbaric oxygen will have a role in the treatment of human patients in shock.

TABLE V.—ISCHEMIA AND GANGRENE

Patient	*Age*	*Diagnosis*	*Treatment*	*Outcome*
J.C.	73	Gangrene of hand; vasospastic disease	Oxygen at 3.5 ATA for 50 minutes; 5 exposures	Transient improvement in colour; amputation required
R.P.	67	Gangrene of foot; occlusive vascular disease	Oxygen at 4 ATA for 30 minutes; 3 exposures	Transient improvement in colour; amputation required
A.P.	65	Gangrene of toes	Oxygen at 3 ATA for 1 hour; 8 exposures	Transient improvement in colour; amputation required
J.C.	75	Gangrene of feet, hand and tip of nose; septic shock	Oxygen at 3 ATA for 1 hour; 4 exposures	Transient improvement in colour; died
M.R.	46	Vascular insufficiency of leg; pain at rest	Oxygen at 3 ATA for 1 hour; 5 exposures	Pain relief in chamber; no permanent improvement
G.L.	40	Optic nerve ischemia; constricted visual fields	Oxygen at 3 ATA for 1 hour; 10 exposures	No change in visual fields
W.C.	37	Frostbite of hands, feet and knees with dark cyanotic patches of skin at these sites	Oxygen at 3 and 4 ATA; 11 exposures	Complete disappearance of cyanosis during therapy; cyanotic area returned 2 hours after therapy; eventual healing without skin grafting
A.D.	13	Frostbite of great toe	Oxygen at 3 ATA for 1 hour; 1 exposure	Disappearance of cyanotic area after one treatment

ISCHEMIA AND GANGRENE

Treatment of ischemia and gangrene by hyperbaric oxygen is based on the premise that reversible hypoxia is present in ischemic tissue or tissue bordering gangrenous areas. Improved oxygenation of such tissue is theoretically possible under hyperbaric conditions as the amount of oxygen delivered can be increased by 5 volumes per cent or more. Unfortunately, treatment of patients with ischemic disease is limited by oxygen toxicity which occurs after prolonged exposure. Intermittent exposure to hyperbaric oxygen reduces the risk of toxicity but is capable of achieving only transient improvement in ischemic tissue (Table V, W.C.). Such treatment may benefit patients by maintaining viability of tissue with impaired circulation during the period of recovery.

Experimentally, Wang and Jacobson[12] showed that hyperbaric oxygen provided protection against hypoxia in dog hind limbs made ischemic by aortic occlusion. Similar observations were made in human patients undergoing resection of abdominal aortic aneurysms.[12]

Illingworth[13] reported improvements in patients with ischemic leg pain. These patients presumably had incomplete vascular occlusions. Decrease in pain was transient in many of the patients and the ultimate results of treatment were not reported.

Table V summarizes the diagnosis, treatment and results in eight patients with ischemic disease of various tissues. Although improvement in colour was noted in many of the patients during treatment, it can be seen that the outcome of the ischemic lesion was seldom influenced by therapy with hyperbaric oxygen. This evidence suggests that when complete vascular occlusion is present, the resulting ischemic tissue is not helped by hyperbaric oxygen. On the other hand, hyperbaric oxygen may be of value in situations where blood flow is temporarily decreased, as for example from arterial spasm or partial vascular occlusion.

DECOMPRESSION SICKNESS

Decompression sickness or "bends" was encountered in 17 patients during this period of review. A continuing industrial hazard, decompression sickness, is increasing in incidence owing to the growing population of SCUBA (self-contained underwater-breathing apparatus) divers. Twelve patients were caisson workers who developed "bends" as a result of inadequate decompression at industrial sites. Four of the patients were divers who for various reasons had to surface without adequate decompression stops. One physician developed decompression sickness after spending 6¼ hours in the chamber while treating an infant with respiratory distress syndrome.

Pain in one or both knees was by far the most common presenting symptom. Shoulder pain was also a common complaint. Pain was always severe enough to prevent the patient from sleeping. Serious manifestations of decompression

Canad. Med. Ass. J.
Sept. 2, 1967, vol. 97

sickness, such as visual disturbances and neurological deficits, were not encountered in this series. All patients were recompressed as soon as possible in accordance with Recompression Tables I and II of the United States Navy Diving Manual.[14] Two patients required recompression to 6 atmospheres absolute before satisfactory relief of pain occurred. All patients, except one man with associated injury, had practically complete relief of pain and were able to leave the hospital the day of or day following recompression.

CARBON MONOXIDE POISONING

Carbon monoxide combines with hemoglobin at one-tenth the rate of oxygen but dissociates from hemoglobin 250 times as slowly as oxygen[15, 16] Such prolonged binding of hemoglobin sharply impairs the oxygen transport system of the blood so that varying degrees of hypoxia occur. Hyperbaric oxygen is capable of increasing the oxygen dissolved in plasma by 6 volumes per cent (the normal total body arteriovenous oxygen difference). This dissolved oxygen is able to supply the tissue needs of most organs until hemoglobin dissociates from carbon monoxide and again assumes its full oxygen-carrying capacity.

In 1960 Smith and Sharp[17] reported the successful treatment of carbon monoxide poisoning with hyperbaric oxygen at 2 ATA. Later Smith[18] reported a series in which 68 of 70 patients survived carbon monoxide poisoning after treatment with hyperbaric oxygen.

In the present series two patients were treated for carbon monoxide poisoning. Improvement in both patients during treatment was remarkable. Both were found unconscious in a parked car in which the motor was running. They were taken to a local hospital where, still unconscious, they were referred to the Royal Victoria Hospital for hyperbaric oxygen therapy. The patients were placed in the chamber 1½ hours after being found. At this time one patient (W.D.) had improved and responded to spoken stimuli; he was completely disoriented and restless. The other patient (L.V.) remained unconscious. After breathing oxygen at 3 ATA for one hour, W.D. became fully conscious and rational. L.V. recovered dramatically. After 1½ hours of treatment, the patient became fully conscious and though lethargic could speak rationally. This lethargy continued for three days following treatment. Both patients were normal at the time of discharge. Electroencephalographic examinations before discharge were normal.

CANCER CHEMOTHERAPY AND HYPERBARIC OXYGEN

In 1954, Churchill-Davidson, Sanger and Thomlinson[19] first used radiotherapy and concomitant exposure to hyperbaric oxygen to treat patients with inoperable cancers. Based on the observation that cells with increased oxygen supply are more susceptible to radiation than poorly oxygenated cells, the results of treatment in 80 patients were encouraging. Krementz and Knudson[20] showed that treatment of Ehrlich ascites tumour in mice with nitrogen mustard and hyperbaric oxygen improved survival when compared to controls.

In the present series 30 patients with inoperable tumours were treated with a combination of hyperbaric oxygen and radiomimetic drugs. Eight of 30 had inadequate therapy for various reasons and have not been included in the series. Twenty-two patients breathed oxygen at 3 ATA for one hour daily for 20 to 27 days while taking methotrexate or methotrexate-methylhydrazine in combination. Objective improvement was noted in five and subjective improvement by six. It was observed initially that when the recommended doses of anticancer agents were combined with hyperbaric oxygen signs of toxicity such as leukopenia, oral ulceration and gastrointestinal upset appeared earlier. Lower doses of drugs were then given to permit the combination of hyperbaric oxygen and chemotherapy to be continued over a longer period of time. Similar observations were made by Hitchcock.[21] These findings may indicate enhancement of the effect of anticancer agents by hyperbaric oxygen. This trial is continuing.

RESPIRATORY DISTRESS SYNDROME

Three premature infants with hypoxia from respiratory distress syndrome were treated with hyperbaric oxygen. Although there was initial improvement, the outcome was fatal in all three infants. Two showed transient improvement in arterial blood Po_2 and lactate levels. The third infant is representative of the course of all three.

The birth weight of this male infant was 1620 g. and the gestational age was 32 weeks. He was severely ill from birth with respiratory distress syndrome and had repeated apneic spells during the first 24 hours. The pH of capillary blood at 41 hours was 7.20 despite bicarbonate administration. The Pco_2 rose from 50 mm. Hg at three hours to 90 mm. Hg at 72 hours. At this time the arterial blood Po_2 was 22 mm. Hg with an ambient oxygen concentration of 85%. Blood lactate was 97 mg. per 100 ml. and the infant was unresponsive. At 77 hours he was placed in the chamber at 2 ATA and given 85%

oxygen to breathe. After 20 minutes at this pressure, there were signs of improvement including respiratory effort, increased activity and improved skin colour. Arterial Po_2 increased to 60 mm. Hg and arterial lactate dropped to 50 mg./ml. However, arterial Pco_2 rose to over 100 mm. Hg. At the age of 80 hours, the infant began to regress. Arterial Po_2 dropped to 35 mm. Hg and the lactate level rose to 60 mg./100 ml. He died at 83 hours of age. Postmortem examination showed moderate hyaline membrane formations in the lungs with minimal pneumonia. There was a small subdural hematoma and the liver showed centrilobular necrosis with thrombi in the umbilical veins.

The clinical improvement noted in all three infants with improved oxygenation suggests that hypoxia is a major part of the terminal state of infants with respiratory distress syndrome.

CONCLUSIONS

A broad experience in therapy with hyperbaric oxygen has been reviewed. For patients with gas gangrene, the results in this and other series suggest that hyperbaric oxygen is a worthwhile addition to current therapy. Although carbon monoxide poisoning is becoming rare in Canada, two patients so affected improved dramatically when given oxygen at 3 atmospheres pressure. Both made a complete recovery. The necessity of a hyperbaric chamber for the treatment of decompression sickness is undisputed. This condition is an increasing industrial and recreational hazard. In the treatment of patients with chronic infections such as leg ulcers, the use of hyperbaric oxygen is less impressive, although further trials in this area are indicated. In the treatment of carcinoma, the results of treatment with a combination of hyperbaric oxygen and radiomimetic drugs are also equivocal and trials are still in progress. Hyperbaric oxygen was not found to be of benefit in the treatment of ischemia and gangrene or in patients in shock. In respiratory distress syndrome transient improvement was achieved but all three infants died.

Summary Eighty-three patients were treated in a hyperbaric chamber. Aside from those with decompression sickness, the therapy in all these patients was directed towards increasing the amount of oxygen dissolved in the blood. Four of six patients with gas gangrene survived and in each there was a rapid decrease in the toxicity due to this severe anaerobic infection. Marked improvement occurred in three of six patients with chronic infections resistant to conventional therapy and eventually their wounds healed. Eight patients in shock refractory to other forms of therapy were not improved by hyperbaric oxygen. Ischemic lesions of various organs in eight patients were not influenced by hyperbaric oxygen therapy, although transient improvement was noted. Transient improvement occurred in three infants with respiratory distress syndrome but all died. Results with combined therapy (hyperbaric oxygen and radiomimetic durgs) in patients with inoperable tumours are equivocal, and this study is continuing.

Résumé Le traitement en chambre hyperbarique a été appliqué à 83 malades. En dehors de ceux qui souffraient du mal des caissons, le traitement avait pour objet, chez tous ces malades, d'augmenter la quantité d'oxygène dissoute dans le sang. Sur six malades souffrant de gangrène gazeuse, quatre ont survécu. On a noté chez ceux-ci une diminution rapide de la toxicité causée par cette infection anaérobique grave. Chez trois des six malades souffrant d'infections chroniques graves qui résistaient aux traitements classiques, on a constaté une nette amélioration et finalement la cicatrisation de leurs plaies. L'oxygène hyperbarique n'a pas agi chez huit malades en état de choc. Chez huit autres malades souffrant de lésions ischémiques de divers organes, l'oxygénothérapie hyperbarique n'a pas influencé les lésions ischémiées, malgré une amélioration temporaire. Chez trois nourrissons souffrant du syndrome de la souffrance respiratoire, survint une amélioration transitoire, mais tous finirent par mourir. Chez des patients porteurs de tumeurs inopérables, le traitement associé (oxygène hyperbarique et médicaments radiomimétiques) n'a pas été très supérieur à la seule chimiothérapie.

REFERENCES

1. DUFF, J. H. *et al.*: *Canad. Med. Ass. J.*, **91**: 1051, 1964.
2. BRUMMELKAMP, W. H., HOGENDIJK, J. L. AND BOEREMA, I.: *Surgery*, **49**: 299, 1961.
3. BRUMMELKAMP, W. H.: *Ann. N.Y. Acad. Sci.*, **117**: 688, 1965.
4. FREDETTE, V.: *Ibid.*, **117**: 700, 1965.
5. MCALLISTER, T. A. *et al.*: *Lancet*, **2**: 1040, 1963.
6. HOPKINSON, W. I. AND TOWERS, A. G.: *Ibid.*, **2**: 1361, 1963.
7. OLLODART, R. M. *et al.*: *Clin. Res.*, **12**: 37, 1964 (abstract).
8. GROGAN, J. B.: *Surg. Forum*, **16**: 81, 1965.
9. PERETZ, D. I., MCGREGOR, M. AND DOSSETOR, J. B.: *Canad. Med. Ass. J.*, **90**: 673, 1964.
10. DUFF, J. H. *et al.*: *Surgery*, **58**: 174, 1965.
11. COWLEY, R. A. *et al.*: *Ann. N.Y. Acad. Sci.*, **117**: 673, 1965.
12. WANG, M. C. H. *et al.*: *Surgery*, **59**: 94, 1966.
13. ILLINGWORTH, C.: *Brit. Med. J.*, **2**: 1271, 1962.
14. United States Navy Department: U.S. Navy diving manual. Navships 250-538, Superintendent of Documents, Government Printing Office, Washington, D.C., 1959, p. 131.
15. HARTRIDGE, H. AND ROUGHTON, F. J. W.: *Proc. Roy. Soc. (Biol.)*, **94**: 336, 1927.
16. *Idem*: *Physiol. Rev.*, **20**: 313, 1941.
17. SMITH, G. AND SHARP, G. R.: *Lancet*, **2**: 905, 1960.
18. SMITH, G.: *Ann. N.Y. Acad. Sci.*, **117**: 684, 1965.
19. CHURCHILL-DAVIDSON, I., SANGER, C. AND THOMLINSON, R. H.: *Lancet*, **1**: 1091, 1955.
20. KREMENTZ, E. T. AND KNUDSON, L.: *Surgery*, **50**: 266, 1961.
21. HITCHCOCK, C.: Personal communication.