MONOGRAPH

ON

CARBON DIOXIDE

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CARBON DIOXIDE

Summary

Carbon dioxide, CO_2 - a colorless; odorless, noncombustible gas, (carbonic acid gas or carbonic anhydride) is widely distributed, not only on Earth - but throughout our solar system. It comprises about 0.03% of the normal ambient air on Earth and is an essential material for the respiration cycles of plants and animals. The photosynthetic process in plants utilizes CO_2 and water as the basic materials for the synthesis of carbohydrates; in animals, CO_2 and water are end-products of metabolism of carbohydrates, fats and to some extent - protein, and the CO_2 is excreted by lungs, kidneys, intestine and skin - either as CO_2 or as the HCO_3 ion (bicarbonate). The gas is soluble in water, volume for volume; the weak, dibasic acid formed, H_2CO_3 reacts with bases to form carbonates, and as such is found in many minerals. Limestone, calcium carbonate - when "burned" in the manufacture of lime, produces CO_2 as a by-product in commercial quantities. It is also obtained commercially as a by-product in industrial fermentations, as in converting glucose to ethyl alcohol and CO_2 by yeast.

Goddard (661) in discussing the biological role of CO_2 also describes the environmental content of CO_2 under the following headings: "The Atmosphere", usually considered to have 0.03% CO_2, but variable over range of 0.015 to 0.032%; "Fresh Water", a highly complex situation in which the CO_2 exists as dissolved CO_2, H_2CO_3, HCO_3 and CO_3; "Soil Carbon Dioxide", variable but ranging 10 to 100 times the CO_2 concentration of the atmosphere' and "The Seas", fairly constant at surface of the open ocean in temperate latitudes, with a total CO_2 (CO_2, HCO_3, CO_3) content of about 46 ml/l.

Some natural wells or hot springs process high concentrations of CO_2 and minerals, and have been known for centuries as spas or health "resorts". Clemons (332) in 1937 reviewed the history of the therapeutic aspects of CO_2:

The Ancient Record.

The application of carbon dioxide for therapeutic purposes has been traced to very early times. In fact, Dioscorides and Plinius spoke of the stone of Memphis in 45 B.C. They recorded that this stone was pulverized, dissolved in vinegar, and applied to the surface of the body in order to produce anesthesia.

The appearances of mineral springs attracted the attention of the early philosophers. They made various tales current,

relative to the marvels of certain springs, by relating supernatural properties of their effervescing waters.

Strabo related that the springs of Hierapolis imparted red colour to roots of trees and shrubs. He said that their juices, when they were mixed with water, produced the purple liquor which was used for dyeing wool. Philostratus, when writing about the sanguinary battle which the Greek army fought with Telephus, on the banks of the Caicus river, stated that the wounded soldiers, who resorted to Agamemnon's spring, were all restored. According to Herodotus, a spring in the country of the Ichthyophagi prolonged life beyond one hundred and twenty years. A certain spring in Chios caused insanity, according to tradition. Another one in Magnesia improved the voice of singers. Those waters at Lethe and Mnemosyne are often mentioned in classical literature. The former spring brought about oblivion. The latter one restored memory.

The Book of Genesis recorded the fact that Anah, the father of Esau's wife, discovered some thermal springs in the desert. In one book of Kings, mention was made of the spring near Jericho which made the ground barren. The prophet Elisha threw salt into it and said: "There shall not be from thence any more death or barren land."

From the New Testament we learn that the Jews used thermal waters before the time of Christ, as it is recorded that "There lay a great multitude of impotent folk, of blind, halt and withered, in the porches of lake Bethesda, by the sheep-market of Jerusalem, waiting for the moving of the waters; and, whosoever first after the troubling of the waters stepped in was made whole of whatsoever diseasehe had."

The Part Played by Springs in Ancient Religion.

Mineral springs played an important part in religion of the ancients, as the disciples of Esculapius erected temples to the God of Medicine in vicinities of mineral springs. These monuments were not only provided with theatres and places of amusements, as our fashionable watering-resorts, but also with hospitals and medical schools for instruction of students.

The Castalian spring had a temperature of two degrees Fahrenheit. In this spring Pythia had to bathe, before she ascended the tripod within the steaming cave of Apollo's oracle at Delphi, where copious eruptions of carbon dioxide occurred. Here, from the short and incoherent sentences, which the priestess uttered in paroxysms, the prophecies were drawn.

The Pioneers in Carbon Dioxide Therapy.

Hey, Withering, Percival, Dobson, Warren, McBride, Ingenhousz, Beddoes, Henry, Lee, Rotheran and White made systematic use of carbon dioxide for therapeutic purposes, before Faraday succeeded in liquefying the chemical in 1823. Twelve years later Thilorien solidified carbon dioxide. However, it was not therapeutically used, in this form, until 1899.

Percival reasoned that, since carbon dioxide has anti-putrid properties, it should be used for the treatment of malignant ulcerations. In fact, he confirmed his thought in the Infirmary of Manchester upon Dr. Withering's service where he demonstrated that suffusion of a cancerous wound with carbon dioxide gas caused a disappearance of purulent matter, relieved pain and gave a better aspect to a wound. He wrote that palliation in so frightful and hopeless a disease should be considered a very precious acquisition.

Dobson, who likewise used carbon dioxide in gaseous form for the treatment of cancer, did not report that he effected a cure, but that pain was relieved and the appearance of wounds improved.

The analgesic effect of carbon dioxide was soon established after Beddoes learned from John Ingenhousz of its remarkable property of almost instantaneously quieting very severe pain such as that produced by vesication, Ingenhousz having discovered that, during the time that a blistered finger, with its epidermis removed, was exposed to atmospheric air, the pain was severe. This pain became more intense, when the member was in an oxygen-bath. But, that pain immediately subsided after the finger was held within a container of carbon dioxide gas.

Beddoes applied the gas to malignant ulcerations, thus relieving pain and diminishing discharge. Subsequently, Ewart likewise reported that he treated a case of cancer of the breast. He stated that, upon the next day after the application of the gas, the ulcerated surface had a better appearance. The pain which had been very severe disappeared. The fetid odour became less noticeable, and the wound healed within two and a half months.

Soon after he published his report, Ewart wrote to Beddoes that, while the wound had healed, it reopened soon after the carbon dioxide suffusions had been discontinued. However, the ulcer had a less hideous appearance, and the gain had been controlled by the use of carbon dioxide gas.

Ten months later, Ewart again wrote to Beddoes and informed him that the woman was still alive, but that the recurrent ulcer had not cicatrized as the primary one had. However, the relief from pain had continued and the absence of odour was strikingly noticeable.

Such results which were obtained in other countries were too important not to be considered by the French practitioners of that time. In fact, everywhere in France experiments with carbon dioxide were made in order to verify that which had been learned.

The carbon dioxide gas treatment of cancer thus became popular throughout France, its popularity being foremost at the Academy of Science and Art of Dijon where great scientific activity developed, and where facts were established showing the beneficial effects of the gas upon all kinds of ulcerations.

J. L. Targioni of Florence reported to this academy the detail of a case of cancer of the breast which had been treated by carbon dioxide in gaseous form. The pain was relieved, the bad character of the suppuration subsided, and the general condition of the patient improved markedly. In fact, one-half of the cancerous ulcer had cicatrized.

In Paris case-reports were also published by the Royal Society of Medicine, proving the efficacy of the remedy. Then, the commission, whose chairman was Lalouette, confirmed that carbon dioxide gas, although it did not cure cancer, did relieve pain and acted upon ulcers in a beneficial way by modifying secretion and causing, to a certain extent, cicatrization.

At a later period, Demarquay, who wrote the history of the therapeutic use of carbon dioxide in gaseous form, reported analogous cases wherein long continued suffusions of malignant growths with carbon dioxide gas ameliorated the sufferings of cancerous individuals.

The Early Use of Carbon dioxide Inhalation.

Dating from the end of the eighteenth, and the first quarter of the nineteenth centuries, inhalation of carbon dioxide was employed for treatment of tuberculosis. Such treatment, in fact, became exceedingly popular, and it was widely accepted, as in most of the European countries numerous recorded case-records confirmed that inhalation of air containing a high percentage of carbon dioxide effected an improvement in, and sometimes a cure of, pulmonary tuberculosis.

Edmund Weill spoke in the Academy of Science at Paris.

He gave an account of experiences with carbon dioxide inhalations. The two to five minute sittings consumed from two to five liters of the gas once or twice a day. He reported that no unpleasant symptoms were experienced. The coughing spells were relieved and prevented. In fact, patients were soon rested and rejuvenated.

Owing to the significance of the transport of an excess of carbon dioxide in the blood-stream, it is not difficult for one to explain how such results were thus obtained. However, the disastrous consequences of intravenous injections of carbon dioxide, which were subsequently employed, might have placed the use of the gas in disrepute.

Clemons continues his paper with descriptions of the early use of CO_2 as an anesthetic, its use in treatment of coloric therapy and diagnostics, and in the solid, frozen form - therapeutic application to malignant growths.

More relevant to CO_2 as a food additive, Beattie (112) in 1970, describes the use of CO_2 in carbonated water or in soft drinks, particularly from the historical aspects of the British "lemonade", the invention of which probably goes back to the 17th century. Efforts to carbonate water were made as early as the 16th century, but commercial progress did not occur until the 19th century.

An excellent summary of CO_2 metabolism and toxicology has been presented by Vogelenzang (2023), 1962, preliminary to a description of a case involving mass CO_2 intoxication on shipboard with several fatalities (the case is reviewed in the section on Biology: Special Studies in this monograph). The following is taken directly from Vogelenzang:

Carbon dioxide gas (CO_2) is a colorless gas that is heavier than air. It has already caused many cases of intoxication in industry. These cases occurred predominantly in closed spaces (silos, vaults, ships' holds), in which CO_2 had accumulated as a result of fermentation processes. If the harmful effect of CO_2 on the human body is examined more closely, it is necessary to pay attention to the following factors:

1. The stimulating action of CO_2 on important brain centers and cerebral cortex;

2. The paralyzing effect of CO_2 on brain centers and cerebral cortex;

3. The irritating action of skin and mucous membranes.

The fact should not be omitted that many older investigators shared the belief that CO_2 is not toxic per se but causes dangers only in its role as an oxygen displacer.

CO_2 and Metabolism

CO_2 plays an important role in many vital functions. CO_2 and H_2O are produced in the cell by combustion. Respiration serves to remove the CO_2 and supply oxygen. The gas is transported via the blood. A small portion of the CO_2 is dissolved in the blood; the greatest part is chemically bound.

Sodium bicarbonate is produced with Na_2CO_3. Another fraction is bound to protein, chiefly to hemoglobin. The total carbon dioxide content at the alveolar carbon dioxide pressure of 40 mmHg in the arterial blood is approximately 50 vol.%, 60 vol.% in venous blood. The 10% difference is given off to the venous blood from the tissues. The H ion concentration of arterial and venous blood is practically the same so that the ratio between the free CO_2 and bicarbonate must be the same. The blood behaves as a buffer solution. The ratio of CO_2 to $NaHCO_3$ is 1:20; it is held as constant as possible. The CO_2 coming from the tissue is bound to alkali. The total quantity of substances present in the blood capable of binding CO_2 is called the alkali reserve.

As a result of the release of oxygen to the tissue, the 70-times stronger acid of the oxyhemoglobin passes over into the weaker acid of the hemoglobin. A greater fraction of the alkali bound to the oxyhemoglobin is released and stands in opposition to the CO_2. The opposite reaction takes place in the lung. Through the absorption of oxygen, oxyhemoglobin is produced which binds alkali again. The CO_2 is exhaled. This exhalation takes place in accordance with the laws of gas diffusion. The gas flows from places with higher to places with lower gas pressure.

The normal ambient air consists of 0.03% CO_2 and 21% O_2; the remainder is nitrogen. The exhaled air consists of 4-5% CO_2 and 16% O_2. The composition of the alveolar air is, besides nitrogen, 14.5 vol.% O_2 and 5.5 vol.% CO_2. The alveolar O_2 pressure is 120 mmHg, and the CO_2 pressure is 40 mmHg.

Carbon dioxide forms the adequate stimulus for the respiratory center by nervous regulation. Carbon dioxide is regarded as the respiratory hormone. (The stimulating effect of CO_2 on respiration has recently led to the addition of CO_2 to medical oxygen.) O_2 lacks a stimulating effect. Every shift in the CO_2 and $NaHCO_3$ ratio toward the acid side leads to increased respiration. Franke has demonstrated by numerous experiments the elevation of CO_2 in the blood as the cause of the stimulation

of the respiratory center. An elevated CO_2 pressure also has an effect on the blood circulation; it causes vasodilatation. According to Eppinger, carbon dioxide has a stimulating effect on the intestine.

The body responds to an increase in the carbon dioxide in the inhaled air with hyperventilation. If the carbon dioxide concentration of the ambient air approaches that of the alveolar carbon dioxide content of 5.5%, then the CO_2 can no longer be flushed out of the blood. The CO_2 pressure in the blood increases. This is first countered by hyperventilation; and finally it is no longer possible to maintain the respiration rate, and an increase in the CO_2 pressure of the blood is unavoidable. Paralysis of the respiratory center occurs, and death ensues with symptoms of cerebral stimulation and paralysis. The hyperventilation, in the extreme the respiration of Kuszmaul, can also arise at the other end, when other acids such as lactic acid and keto acids occur in the organism in large quantities. These bind too much alkali so that now sufficient alkali is no longer available for the CO_2 produced during the combustion.

As a result of the hyperventilation, the CO_2 pressure in the blood is reduced as far as possible, and in this way the state of acidosis can be compensated longer. During muscular work, lactic acid and phosphoric acid as well as a large amount of CO_2 are produced as a result of intensified oxidation processes.

The CO_2 stimulates the respiratory center as well as the vasomotor and heart-vagus center, and likewise the motor centers. It acts on the heart and vessel tonus and on muscle tonus. The effect is always biphasic. The stimulation phase is followed by the paralysis phase.

Prausznitz and Schultzik investigated the toxic effect of CO_2 in different concentrations in animals. Naturally, nothing was said of an O_2 deficit in these cases. They carried out the tests with dogs, cats, rabbits, and rats. Up to 10% CO_2, they saw nothing abnormal. With increasing concentrations, the respiratory frequency and depth increased. At 25-30% CO_2, the animals fell asleep, consciousness had apparently vanished, corneal and light reflexes were extinguished. At 50% CO_2, in most cases there was a brief excitation stage, the animals collapsing after a few seconds. At this time they displayed frequent tonic and clonic convulsions; there was very strong opisthotonus in cats and rabbits. In other animal tests, frequently briefly after the respiratory stoppage, there was a temporary relaxation of the extremities and tonic contractions of the stretching musculature in the manner of decerebration rigidity.

The Cheyne-Stokes respiratory type was observed very frequently; in dogs, a considerable saliva flow was registered. They often had a bloody foam on the mouth, which was regarded as a sign of damage to the respiratory tract, also loss of urine and defecation. The pulse was often accelerated and irregular. If the animals were restored to fresh air, then they often vomited but recovered soon. The rate of recovery depended less on the percentage CO_2 in the air than on being accustomed and on the length of the test. The high tolerance of the animals to the gas was striking when one considers that humans lose consciousness at 10% CO_2, while the animals still did not display any symptoms at this concentration. Even at a concentration of 60-70% CO_2, the rabbits remained alive for a few minutes. Only the behavior of cats and mice was noteworthy at 10% CO_2, they sat together in a quite friendly manner and entirely forgot their natural enmity.

According to Starling, increasing the CO_2 tension in the blood causes an enlargement of the heart volume during diastole and to a lesser degree during systole. The stroke volume increases with increasing CO_2 pressure from 2% to 8%. At higher CO_2 pressure of approximately 12-20% CO_2, the systole is influenced more strongly in the sense above, resulting in reduction of the stroke volume. Eppinger recorded in animal tests, in addition to blood pressure elevation, simultaneously a pulse retardation. In animals which at the beginning of the tests already had a low heartbeat frequency (vagus-tonus), a long-lasting extrasystole was frequently observed. It was known that the bradycardia produced by the inhalation of CO_2 was dependent on the action of the heart-vagus center. When the vagus was cut, no changes occurred.

Since the bradycardia always set in within a few seconds, it is assumed that the cause must be sought not only in the stimulation of the vagus nerve by the increase of the CO_2 content of the blood but also especially in a reflexive action (probable trigeminus vagus reflex of the nasal mucousa).

CO_2 intoxication, according to Eppinger, causes a strong expansion of the heart chambers, chiefly on the right, and the progressing intoxication causes disturbances of the stimulation conduction as well as a lengthening from auricle to ventricle. An anatomical examination revealed swollen heart valves and occasionally hemorrhages under the endocardium. The unopened heart was contracted (reduced heart tonus). American researchers found in animal tests that even small quantities of CO_2 caused dilation of the blood vessels.

The rhythmic activity of the vascular muscles was halted under the influence of CO_2 if the vasomotor centers were isolated. If the vessels were connected to the vasomotor center, then vasocontraction occurs. The result of the vascular contraction

was a blood pressure elevation. However, if the CO_2 concentration remained above 30%, then the stimulating effect was lost, and the blood pressure decreased below normal levels until total heart stoppage was reached. Elevated CO_2 pressure in the blood causes contriction of veins. The venous contriction was eliminated after the cutting of the nervous connections.

In every case of CO_2 intoxication, one must wonder whether a pure CO_2 intoxication is involved or not. One must distinguish between cases in which in addition to the elevated CO_2 content, the O_2 content of the air is normal, and those cases in which the O_2 content is strongly lowered. If the O_2 content of the air drops from a normal 21% to 14%, then the respiration is already considerably impaired. A content of 11% causes suffocation.

The individually different sensitivity plays a great role in CO_2 intoxiation. It is higher in cases of physical stress, heart and lung diseases, and pregnancy. A certain adaptation to CO_2 can take place. Of decisive importance for the symptoms occurring in a case of CO_2 intoxication is the concentration of the CO_2 and the duration of its inhalation. Opinions are divided concerning the harmful concentration. Paul found a content of 1% CO_2 to be harmless. Ordinarily, a concentration of 2% is regarded as innocuous.

Tests on American submarines revealed respiratory disturbances already at 2% CO_2, and most authors observe a distinct effect on the average only above 3%. The increase in respiratory efficiency is essentially based more on the deepening of the individual breaths rather than on an increase in respiratory rate. At 5-6% CO_2, the respiratory value is doubled. Prausnitz found a frequency increase from a normal 18 to 28.3 at this concentration. With increasing CO_2 concentration in the air, the dyspnea increases rapidly. Strong persons can tolerate a concentration of 8% CO_2 for a long time, although they are quickly fatigued by the stepped-up respiration. . Weak persons collapse already at a concentration of 6% CO_2. The duration of inhalation is decisive.

Schultzik recorded manifest dyspnea after remaining 15 minutes in a room containing 5% CO_2. At 7%, CO_2 dyspnea already occurred after 12 minutes, and at 8%, it occured immediately. Lower concentrations evoke the same effects after longer inhalation at higher concentrations in a shorter time. The dyspnea cannot be tolerated longer than 15 to 20 minutes at 8% CO_2. Remarkable is the fact that the stimulating effect on the respiratory center still persists in spite of the loss of consciousness. Only at 30% CO_2 does the critical point occur between the stimulating and paralyzing action on the respiratory center. At this concentration, paralysis of the respiratory center occurs with great probability. Accord-

ing to some, the latter occurs already at 20% CO_2 in a few seconds, according to others only after some time.

It is not simple to establish precisely at which concentration of CO_2 death finally occurs. From the foregoing, however, it appears that animals possess a much greater tolerance to CO_2 than humans.

Symptomatology

The symptomatology of CO_2 intoxication is as follows:

Initial Symptoms:

0.1% is regarded as the outer limit of harmlessness of the CO_2 content of the air. Above 2% CO_2, the first symptoms of headache. Within increasing concentration, there is a stronger brief feeling of pressure in the head, usually coupled with a buzzing in the ears and visions of stars. At 3%, intensified respiration occurs. Many persons, chiefly mine workers, who have had experience with the effect of CO_2-rich air note as the first symptom at approximately 2% CO_2 a stimulation of the skin and mucous membranes, conjunctivitis, an irritating taste on the tongue or a mild stinging in the nose. Generally symptoms appear from the direction of the skin and mucous membranes only after 4-5% CO_2.

The exposed persons note on tender parts of the skin such as the scrotum and the inside of the thigh a stimulation, and in the mouth and cheek a dry feeling and an irritation of the conjunctiva. Frequently there are disturbances of sensation as well as a reduction of pain and the sense of touch.

Upon inhalation of CO_2 concentrations above 10%, a brief excitation stage appears immediately (paralysis of the cerebral hemispheres, unrest, narrowing of consciousness, contraction of the pupils, sleep, and possibly vomiting), anesthesia and narcosis. During the tolerance stage (spinal cord paralysis) we see the disappearance of the reflexes and of the slow regular pulse. During the collapse stage (medulla paralysis) we see dilated pupils, irregular respiration, weak pulse, cyanosis and subsequently death through paralysis of the respiratory and vasomotor center.

The complete intoxication picture is as follows: from 5% CO_2 on, we see increasing depth of respiration and to a lesser degree increased respiratory frequency.

At 8% CO_2, distinct dyspnea arises, which is tolerated for about 15-20 minutes; 9.5% CO_2 is the upper limit that is tolerated. There is an increase in the blood pressure, a further elevation of the pulse rate, reduced stroke volume, an increase of the flowing blood volume, activation of resting

blood depots, and an elevation of venous tone.

After 10% CO_2, anesthesia and ataxia occur, which pass over into narcosis.

At 20% CO_2, narcosis occurs directly. In the outer air, the CO_2 disappears rapidly from the blood. Before awakening, frequently states of recovery appear, motor unrest, tongue biting, incontinence, and vomiting are observed. The patients often behave as if they were in an alcoholic fit or suffered from epilepsy. A strong euphoria also appears and an absolute lack of an subjective feelings of illness. When fresh air is suddenly admitted, occasionally seizures of epileptiform character are seen.

The paralysis of the respiratory and vasomotor center begins with approximately 30% CO_2. The reflexes vanish, a collapse of the blood circulation occurs with reduction of heart and vessel tone, and a drop of the blood pressure to shock values.

The after-effects consists of headache, dullness, vertigo, convulsions in the limbs, thirst, and a slight temperature elevation. Frequently an albuminuria and a glucosuria persist for several days. Lung changes can also appear, which form the early stage of lung edema.

Frequent rhythm disturbances and dilatation are found in the heart. In heart patients, we also occasionally see auricular fibrillation. The therapy consists of placing the patient in the fresh air and administering pure O_2. In cases of lost consciousness, artificial respiration must be applied as quickly as possible, since here only a few minutes decide between life and death. Protection against chilling is also important. Intracardiac injection of epinephrine 1 on 1000 and cardiac massage may be applied. Artificial respiration must be continued on the patient until recovery or until rigor mortis sets in. During states of recovery, the patients must be sedated so that the O_2 consumption is not increased by motor unrest, which could counteract the recovery. Transportation must always be accomplished with the patient prone and never without accompaniment.

A most comprehensive, detailed review of CO_2 metabolism may be found in a Russian report of 1973 by Gulyi and Mel'nichuk (716), which includes over two hundred references. The conclusions only are presented here; a complete translation is included in the documents section of this monograph:

1. Carbon dioxide is contained in animal tissues in the form of soluble carbon dioxide gas, carbonic acid, bicarbonate, carbonates, and carbamates. About 94-95% of the total amount of carbon dioxide in the blood of animals occurs as bicarbonates. The carbon dioxide content in other biological liquids and tissues is in direct proportion to its content in the blood.

2. In tissues of animals and man, the contents of, and proportions between, different forms of carbon dioxide and hydrogen ions may vary, and thus they determine 9 states of the organism: normal, metabolic acidosis and alkalosis, respiratory acidosis and alkalosis, respiratory acidosis and alkalosis with partial compensation, and compensated acidosis and alkalosis.

3. More than 10 carbon dioxide fixation reactions occur in animal tissues, in which the carbon of carbon dioxide is transformed into the carbon of organic compounds. These reactions are divided into three groups: those processing due to ATP energy, those due to the energy of reducing forms of pyrimidine nucleotides, and those not requiring outside energy.

4. The intensity of processes of carbon dioxide fixation in tissues is determined by the following factors: 1) the concentration of substrates underlying carboxylation; 2) the concentrations and proportions of different forms of carbon dioxide in tissues; 3) the degree of energy and corresponding coenzymes of carboxylase provided (biotin, nicotinamide, vitamin B6, tetrahydrofoliate); 4) the presence in definite concentrations of activators (ions of magnesium, manganese, and zinc) and inhibitors of carboxylase (ions of heavy metals, etc.), as well as a number of cofactors (acetyl glutamate, glutamate, citrate, carnitine, acyl-CoA, etc.), which are allosteric regulators of carboxylase activity; 5) corresponding hormonal status in the organism.

5. Carbon dioxide, being utilized in carboxylation reactions in animal tissues, takes part in the biosynthesis of fatty acids, lipids, many replaceable amino acids, and therefore proteins, in the synthesis de novo of carbon, in the formation of pyrine and pyrimidine bases, and thus nucleotides and nucleic acids, as well as in the reactions of the tricarbon cycle.

6. There is a dependence of the intensity of biosynthesis of the organic compounds listed above on the processes of carbon dioxide fixation, which is expressed in the fact that stimulation or depression of the latter increases or decreases respectively the process of the former.

7. Increasing, within definite limits, the content of carbon dioxide and ions of magnesium, manganese, and zinc in tissues may noticeably increase the intensity of biosynthetic processes and stimulate tricarbon cycle reactions in the organism. This confirms that carbon dioxide in the animal organism is not only a regulator of processes of respiration and the major, central buffer system in

animals and man, but it is also a regulator of basic, biosynthetic processes in the organism.

8. The indicated regulatory role of carbon dioxide in the biosynthetic processes of the organism may be effectively utilized for practical purposes. Increasing the biosynthetic processes by stimulating processes of carbon dioxide fixation, one can noticeably increase the productivity of farm animals and birds, as well as significantly accelerate the process of tissue regeneration, in particular, in hepatectomy, burns, and post-hemorrhagic anemias.

9. Besides the participation in the processes of carboxylation and regulation of reactions of the medium, carbon dioxide has the property of causing specific changes in the activity of several enzymes (ATP-ase, aconitate-hydrolase, and glucose-6-phosphate-phosphohydrolase), which have no direct relation to carbon dioxide fixation processes.

Various representative CO_2 inhalation studies have been included in this monograph and are abstracted in the following biological and biochemical sections; brief notes only are presented here.

Anselmo et al. (49) described a case of acute CO_2 poisoning nearly a quarter of a century ago in which a family of five persons where overcome by CO_2 in a well; two members were fatally affected.

Rat studies of acute and chronic CO_2 toxicity were reported by Barbour and Seevers (87). Atmospheres of increasing CO_2 concentrations showed that 10-20% CO_2 was generally tolerated at the lowest level, but some succumbed at 15%, and 80% of the animals died within 4 days of 20% CO_2. At 50% CO_2, all rats died within 6 hours. Thirty days of 10% CO_2 produced only loss of weight; other studies of similar duration at 20-25% CO_2 gradually built up produced considerable weight loss of up to 50% of the original body weight.

Severe damage to brain and spinal cord was observed in rats kept at levels of CO_2 increased from 20-43% over a period of about 20 hours by Stephens (1868). Similar but less pronounced damage was produced in rats kept at 6-10% CO_2 for 107 days.

Friedlander and Hill (590) recorded EEG patterns in 37 young human adults given 30% CO_2 for 50-52 seconds. The alpha generally disappeared at about 10-15 seconds in cases where no high voltage pulses and slowed activity were seen; in the latter instances, 128-131 seconds elapsed before return of the alpha, but when no high voltage pulses were seen, the alpha returned in about 100 seconds. An unconscious period of about 24-28 seconds was observed in most patients.

Shaefer (1691) has reported extensively from submarine studies of the toxicity of CO_2 in that closed environment. An inverse time - concentration relationship has been demonstrated under conditions of

prolonged exposure to CO_2 in which time can replace concentration. Tolerance limits for CO_2 exposure are proposed: at levels of 3% or greater CO_2, performance deteriorates and basic physiological functions become altered; at 1.5% CO_2, basic performance and physiological functions are unaffected, but adaptive process are retarded and could reach pathophysiological states after long exposure; and at levels 0.5-0.8% CO_2, probably no significant effects occur.

Carbon dioxide accidentally discharged into a ship's engine room resulted in the mass poisoning of 30 persons with 7 fatalities according to a report in 1962 by Vogelenzang (2023). Some survivors suffered retrograde amnesia, headache, vomiting, auricular fibrillation, or various other symptoms. Serious EKG disturbances in several young men indicated myocardial damage.

The EEG and reaction times in human subjects exposed for 5 minutes to 0-7.9% CO_2 showed that both the alpha frequency and reaction time were significantly influenced by the CO_2 concentration in studies by Harter (759).

Therman (1934) in a review of the neurophysiological effects of CO_2, notes that basically, CO_2 increases the slow electrotonic component of the total membrane potential of nerves thus increasing the threshold of stimulation, increasing duration and height of the action potential, and decreasing the speed of nerve impulse conduction.

Woodbury and Karler (2121) have also reviewed the neurophysiological and neurochemical responses of the central nervous system to CO_2. The effects of exogenous CO_2 on brain function are described as neurophysiological or metabolic. Drugs which influence endogenous CO_2 metabolism in brain function, such as salicylates, DNP, acetazolamide, etc., are discussed. The role of carbonic anhydrase in the central nervous system is described.

Bain and Klein (75) used cats in experiments to determine the influence of CO_2 in the brain, assaying tissues for glucose, lactate, phosphates and pyruvate. Blood samples, EEG and EKG data together with measurements of blood gases and pH were taken. Inhalation of 10-30% CO_2 lowered lactate and pyruvate levels inversely to blood CO_2 levels, pH, O_2 and cerebral blood flow. Glucose and phosphate levels remained relatively unchanged.

The introduction of air or O_2 into veins or arteries for diagnostic roentgenology in pneumoperitoneum, angiocardiography, etc. risks gas embolism. Stauffer et al. (1860) studied the circulatory disturbances created in dogs by intravenous air or CO_2 injection. CO_2 was much better tolerated than either air or O_2. It was suggested that selective CO_2 angiocardiography might well be developed for human subjects. A report four years later by Winters (2105) describes the safe use of CO_2 in the study of pericardial disease, in which 50-100 ml CO_2 is injected into a large vein of the patient's left arm for x-ray diagnosis. The CO_2 bubble

which forms in the right heart disappears rapidly by solution in the pulmonary blood and by expiration.

Specific phobias, such as a spider phobia or severe obsessional compulsive nerosis regarding fecal substances (cases described) have been removed by augmented respiration of mixtures of CO_2 and O_2 (30-70% CO_2) according to Orwin (1429) in a recent report (1973). It was speculated that the subcortical excitation occasioned by CO_2 facilitates association of visceral relief with the phobic stimulus.

An early paper (1938) by Waters (2055) describes some of the hazards involved in the use of CO_2 in therapy, particularly in individuals who may be hyper susceptible to CO_2. Cautious use of CO_2 by trained personnel who are aware of the dangers is urged.

Pregnant Sprague-Dawley rats exposed for 24 hours to an atmosphere of 6% CO_2, 20% O_2 and 74% N_2 during various stages of pregnancy, showed an increased mortality rate among male fetuses; stillborn offspring numbered more than twice those in control animals, and skeletal malformations numered 58 in the test group versus 1 in controls. Cardiovascular defects were seen in about one-fourth of the offspring of mothers exposed to 6% CO_2, and 86% had lung abnormalities, according to a report by Haring (756).

Loomis (1165) has proposed a theory to explain cytostosis and malignancy in animal tissues by self-limiting growth in the production of inhibitory levels of free CO_2 by respiration. Embryonic and malignant cells use adenosine triphosphate glycolytic systems which do not generate CO_2. Kieler and Bicz (964) have discussed the theory, and present observations from studies of CO_2 effects an aerobic glycosis and respiration in tumor cells which tend to support the Loomis theory.

It has been suggested that CO_2 is stored within body tissues as either bicarbonate or dissolved CO_2 according to levels of perfusion, metabolic rates and dissociation curves, in a multicompartment theory. Muscle, because of its low rate of perfusion, could serve as a repository for CO_2 produced by other tissues. The idea was tested in dogs by Heymann and Cherniack (809); the assumption was that cessation of ventilation should decrease the CO_2 content of venous blood below that found in arterial blood. In the dog, at one minute of apnea, the venous and arterial CO_2 concentrations were reversed from normal, and at 6 minutes the average venous CO_2 tension was 13 mm Hg less than that of arterial tension, or 3.3 vol.% less. For each minute of apnea, muscle stores about 3 ml CO_2 produced elsewhere, or about 4% of the total CO_2 produced in the body per minute.

Shephard (1768) found in human volunteers that 30 minutes of CO_2 inhalation under conditions of experimental hypercapnia led to a total CO_2 retention of 1000-2000 ml. in both normal and in congenital heart disease cases. Only small amounts of retained CO_2 were found in pulmonary gas volume, lung tissues and blood; most CO_2 was retained in other body tissues.

The transport of CO_2 in the body is chiefly as the bicarbonate ion according to a report by Enns (495); about five-sixths of the CO_2 in equilibrium solution in the blood is bicarbonate ion. the exchange between dissolved CO_2 and the bicarbonate ion is very slow; red cell carbonic anhydrase greatly accelerates the production of bicarbonate ions for CO_2 transport.

Goddard (661) in 1960 presented an excellent review of the biological role of CO_2. During the first three or four decades of this century, the roles of CO_2 in plant photosynthesis and in animal oxidative processes were assumed to be the sole functions. CO_2 is involved in a variety of reactions, however; with ATP and a hydrogen donor; in carbohydrate metabolism and several decarboxylation reactions; or in reactions involving malic acid, isocitrate and alpha-ketoglutaric acids.

Drury et al. (457) injected ^{14}C-labeled bicarbonate intravenously or intraventricularly into cats to determine the rate of CO_2 elimination. It was found that the mixing of labeled CO_2 with the body CO_2 pool was slow; about 65-75% of the body pool is in tissues with a low blood flow rate compared to CO_2 content, such as muscle, skin and blood - lung air. The rates of exchange between various components of the body's CO_2-HCO_3 pools in normal male subjects were studied by Winchell et al. (2101) more recently, also using ^{14}C-labeled bicarbonate injected intravenously. A compartmental model was used for mathematical treatment of labeled $^{14}CO_2$ excretion data. Several body pools are postulated: one with a high ratio of blood flow rate to tissue CO_2-HCO_3 content such as found in abdominal and visceral tissues; and a second pool in tissues with a small ratio of blood flow rate to tissue CO_2-HCO_3 content, such as muscle. Some CO_2 goes from first to second pool, with some feedback; other fractions from the first pool are expired in the breath or "fixed" in the body, later excreted in urine, feces and sweat.

The effects of breathing 5-7% CO_2 on urine flow and mineral excretion in human volunteers were reported by Barbour et al. (86). Urine flow was considerably increased by CO_2 when patient is recumbent, but not when patient is in sitting posture. No changes were seen in the excretion rate of creatinine, sodium, potassium or chloride. Pitressin negates the diuretic effect of CO_2.

CARBON DIOXIDE

Chemical Information

I. Nomenclature

A. Common Names

1. Carbon dioxide
2. Carbonic acid gas
3. Carbonic anhydride

B. Chemical Names

1. Carbon dioxide
2. Carbonic anhydride

C. Trade Names

None found

D. Chemical Abstracts Registry Number

000124-38-9

II. Empirical Formula

CO_2

III. Structural Formula

Not Applicable

IV. Molecular Weight

44.01

V. Specifications

From NAS/NRC sources:

Limiting Characteristics for Carbon Dioxide

Minimum CO_2	99.9%
Water	60 PPM
Dew Point	-52°F.
Hydrocarbons (Non-condensible)	35 PPM (as methane)
Hydrocarbons (Condensible)	5 PPM (weight)

Oxygen	30 PPM
Nitrogen	120 PPM
Carbon Monoxide	10 PPM
Oxides of Nitrogen (NO, NO_2)	5 PPM
Sulphur Dioxide	5 PPM
Phosphine (PH_3)	0.3 PPM
Hydrogen Sulphide	0.1 PPM
Organic Reducing Compounds	None
Carbonyl Sulfide (COS)	0.5 PPM
Odor	None
USP-18	Yes
BB-C-101b	Yes

Note: Analysis based on sampling liquid CO_2 and vaporizing. All results on a volume basis except as noted.

VI. Description

A. General Characteristics

Colorless, odorless, noncombustible gas with a faint acid taste.

B. Physical Properties

From The Merck Index (1259).

Usually a nonsupporter of combustion, although burning magnesium continues to burn when transferred into a CO_2 atm. *Caution: When shipped in steel cylinders, CO_2 is in the form of gas over liquid and at 20° exerts a pressure of 830 psi. Humans cannot breathe air contg more than 10% CO_2 without losing consciousness. Use gloves when handling dry ice, as its temp is at least −78.5°; momentary skin contact with dry ice has caused serious frostbites and blisters.* At atmospheric pressures the solid form changes into the gaseous phase without liquefaction. d_{gas} 1.527 (air = 1); d_{gas} 1.557 (N_2 = 1); abs d 0.1146 lb/cu ft at 25°; vol at 25° = 8.76 cu ft/lb. d^{0}_{gas} 1.976 g/l at 760 mm; d^{0}_{liq} 0.914 at 34.3 atm; $d^{-56.6}_{solid}$ 1.512. $Sublimes_{760}$ −78.48°. $mp_{5.2atm}$ −56.6°. The gas is not affected by heat until temp reaches about 2000°. Crit temp 31.3°; crit press 72.9 atm; crit density 0.464. Triple point −56.6° at 5.11 atm. Vapor press at −120° = 10.5 mm; at −100° = 104.2 mm; at −82° = 569.1 mm. Heat of formation 94.05 kcal/mol. Latent heat of vaporization 83.12 g cal/g. Specific heat 0.19 to 0.21 Btu/lb. Solubility in water (ml CO_2/100 ml H_2O at 760 mm): 0° = 171; 20° = 88; 60° = 36. More sol at higher pressures. Less sol in alcohol, other neutral organic solvents. Absorbed by alkaline solns with the formation in carbonates.

C. Stability

Is usually shipped, as noted above - in liquid form under considerable pressure. At atmospheric pressure, CO_2 is very stable as gas.

VII. Analytical Methods

From AOAC (2171): CO_2 in beer, Sec. 10.033-10.034 (manometric method); CO_2 in wines, Sec. 11.048 through 11.052 (manometric method). Numerous techniques have been reported for measuring CO_2 in blood: Reyes and Neville (1582) use less than 1 ml of blood, the bound gas is converted into physically dissolved form and the resultant CO_2 tension is measured electrochemically; gas chromatography is used by Lenfant and Aucutt (1114) by first extracting the gas from the blood; the determination of ^{14}C-labelled CO_2 in blood following studies of the oxidation of ^{14}C-labelled substances in vivo is described by Hagenfeldt (729) and similarly by Nemec et al. (1372) by scintillation spectrometry; infrared analysis of CO_2 in blood is described by Ortega et al. (1428), Ammann and Galvin (36) have pointed out some of the problems in the determination of CO_2 by infra-red absorption.

VIII. Occurrence

Occurs in the atmosphere of many planets. In our solar system, e.g., on Venus, the optical layer thickness due to CO_2 is 100,000 cm/atm, but only 220 cm/atm on Earth. Analyses of the air in the temperate zones of the Earth show 0.027 to 0.036% (v/v) of CO_2. Constituent of carbonate type of minerals and product of animal metabolism. Necessary for the respiration cycle of plants and animals. Obtained industrially as a by-product in the manufacture of lime during the "burning" of limestone; $CaCO_3$ + heat $\longrightarrow$ CaO + CO_2. Also produced by burning coke or other carbonaceous material: $C + O_2 \longrightarrow CO_2$ + 94.4 kcal. In the U.S.A. large amounts are produced by fermentation (Backus process and Reich process). When glucose is fermented by yeast, the chief products are ethyl alcohol and CO_2. Prepared in the laboratory by dropping acid on a carbonate: $CaCO_3 + H_2SO_4 \longrightarrow CaSO_4 + H_2O + CO_2$ (1259).

Biological Data

I. Acute Toxicity

Carbon dioxide toxicity studies have been largely confined to inhalation of the gas, or to injection for therapeutic or diagnostic purposes, and are described in the section, Special Studies.

II. Short-Term Studies

See above.

III. Long-Term Studies

See above.

IV. Special Studies

Inhalation Toxicity

An interesting case of acute poisoning from CO_2 of five members of a family was reported from the Philippines in 1951 by Anselmo et al. (49). A young boy (11 years) descended into a well to recover a pail, was overcome by what later was shown to be a high CO_2 concentration (about 12.5%) resulting from contamination from a nearby distillery. Successive efforts by members of the boy's family to save the boy resulted in 5 persons being overcome by CO_2 inhalation; two of the victims failed to recover.

Studies of acute and chronic toxicity of CO_2 with particular reference to its narcotic action were reported by Barbour and Seevers (87) in 1943. Albino rats were placed in a closed circuit system with an atmosphere of constant 21% O_2 and various concentrations of CO_2. The acute maximum concentration of CO_2 tolerated generally by the animals was between 10 and 20%; all rats survived 10% indefinitely, some few succumbed at 15%, and 80% of the animals died within 4 days of 20% CO_2. There were no survivors past 36 hours of 25% CO_2 and 50% CO_2 was uniformly lethal within 6 hours. Death from CO_2 was always associated with evidence of pulmonary injury; lungs were edematous and filled with sanguineous exhudate when seen at autopsy.

Ten rats were kept in 10% CO_2 for 30 days without showing untoward effects except for loss of weight, ranging from 14 to 27%, probably due to reduced food intake. Another group of rats was maintained for 5 days at a gradual increase in CO_2 concentration up to 20%; after 6 days at this level, the concentration of CO_2 was increased to 25% during the next 3 days. A single animal died after 4 days; the CO_2 was reduced to 20%.

In a similar experiment, the animals were kept for 29 days in CO_2 concentrations varying between 20 and 25%. The weight loss was considerable - 50% of the original body weight. When returned to room atmosphere, the animals recovered completely, gaining weight, within a few days.

At concentration of CO_2 of 11.%, O_2 consumption fell immediately, reaching a minimum at 2.5 to 5 hours. Within 24 hours, normal O_2 consumption was regained. In prolonged exposure to gradually increasing CO_2 concentration, no change in O_2 consumption was observed until a 10% CO_2 level was reached. Chronic exposures to 11% CO_2 for 30 days resulted in the plasma pH falling as low as 7.0 within 30 minutes and remaining there during remainder of the experiment. A marked reticulocytosis was seen, but leukocytes and erythrocytes remained unchanged in number.

A synergistic reaction to pentobarbital in atmospheres of 10-20% CO_2 was observed; sleeping time was prolonged beyond that with pentobarbital alone.

The morphological effects of CO_2 on the central nervous system which may result from the use of CO_2 in psychiatric therapy was subject to studies reported by Stephens (1868). Albino rats were placed in chambers with a controlled atmosphere in which the CO_2 concentrations could be varied; the O_2 concentration was kept fairly constant (18-21%) during the experiments. Twelve adult rats were subjected to CO_2 concentrations of 20% during the first 2 hours, after which it was raised to 38%, and finally, 43% at the close of the experiment. Survival times varied from 2.5 to 19.3 hours. After a 15% level of CO_2, the animals became quiet, eyes widely open and necks and bodies extended.

Examination of the central nervous system showed the greatest changes in the optic thalamus, brain stem and spinal cord. Nissl preparations demonstrated nerve cell changes including swelling, vacuolation and shrinkage of nuclei. Chromatolysis was marked, oligodendroglia were swollen and neuroglia nuclei stained deeply. Throughout both brain and spinal cord, severe damage was seen, especially in those animals that survived longest in the toxic atmosphere. Significant changes in the viscera were also observed.

A longer experiment, using 26 adult albino rats, was performed, similar to the foregoing, but with CO_2 concentrations maintained at 6-10%. Some of the animals became sluggish, ate poorly, lost weight and died in from 10 to 107 days; those animals which became acclimatized, remained active and ate well. Microscopic observations revealed effects similar to those of the "acute" studies, but generally less severe. The changes were minimal in the animals that survived the longer, chronic exposures.

Friedlander and Hill (590) administered a mixture of 30% CO_2 and 70% O_2 to 37 young adults and recorded EEG patterns under controlled conditions. The average time of inhalation of the mixture was 50-52 seconds, or in-

halations of 29-30 times. The term "build-up" was used to describe the appearance of high voltage pulses and slow activity, well defined as regular sinusoidal waves on EEG records. The following figure shows typical EEG records resulting from CO2 inhalation:

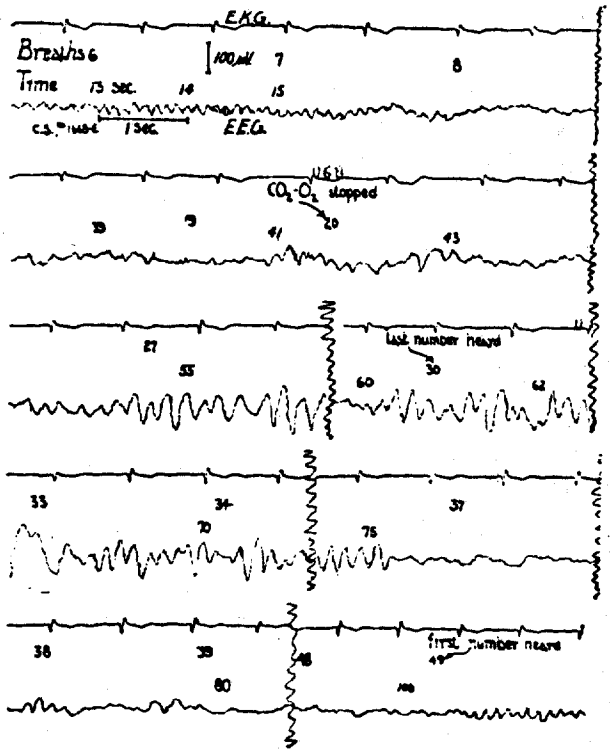


Figure 1. Portions from a record showing the entire sequence of changes: alpha, loss of alpha, high voltage 5/second slow waves ("build-up"), low voltage, irregular slow waves, and reappearance of the alpha. This particular tracing was chosen because of the minimal amount of artifact; however, it is atypical in that the "build-up" begins after the gas was stopped and there is not the usually greater amount of slowing after the "build-up" than before the "build-up."

Generally, the alpha disappeared from the EEG tracing after about 10-15 seconds, or 5-7 breaths of CO_2; it returned after about 100 seconds if no "build-up" occurred, and after 128-131 seconds if there was a "build-up". Changes in consciousness were tested by having the person count aloud the patients' respirations and asking the patient what the last number he heard was and when he again became aware of the counting. The method is very unreliable, but on the average, unconsciousness lasted 24-28 seconds, being fully regained within 110. seconds after treatment was begun. The "build up", arbitrarily defined as lasting about 47 seconds with an amplitude of about 128 microvolts, occurred in 29 of the 37 patients; in all but one of the remaining patients who showed no response to CO_2, the "non-build up" lasted 21 seconds with amplitude of the wave, 78 microvolts.

Shaefer (1691) has extensively investigated, and reported on, the toxicity of CO_2, particularly as related to submarines, and more recently to support systems in space vehicles. Experimental data from numerous human studies and some animal studies are reviewed, from which a concept of triple tolerance limits to CO_2 is derived. There has been demonstrated an inverse time - concentration relationship in continuous prolonged exposure to CO_2 in which time can replace concentration. Adaptation to CO_2 is defined as compensation of the respiratory acidosis induced by CO_2, described as of two phases determined by pH, CO_2, bicarbonate levels and electrolytes in blood and urine. Three levels of activities serving as tolerance limits are described:

1. At levels of 3% or greater CO_2, deteriorated performance and alterations in basic physiologic functions;

2. At 1.5% CO_2, basic performance and physiologic functions are unaffected, but adaptive processes are slow and could reach patho-physiologic states on greatly prolonged exposure; and

3. At 0.5-0.8% CO_2, probably no significant physiologic, psychological or adaptive changes occur.

This last level is not fully established. The triple tolerance limits concept is diagrammed below:

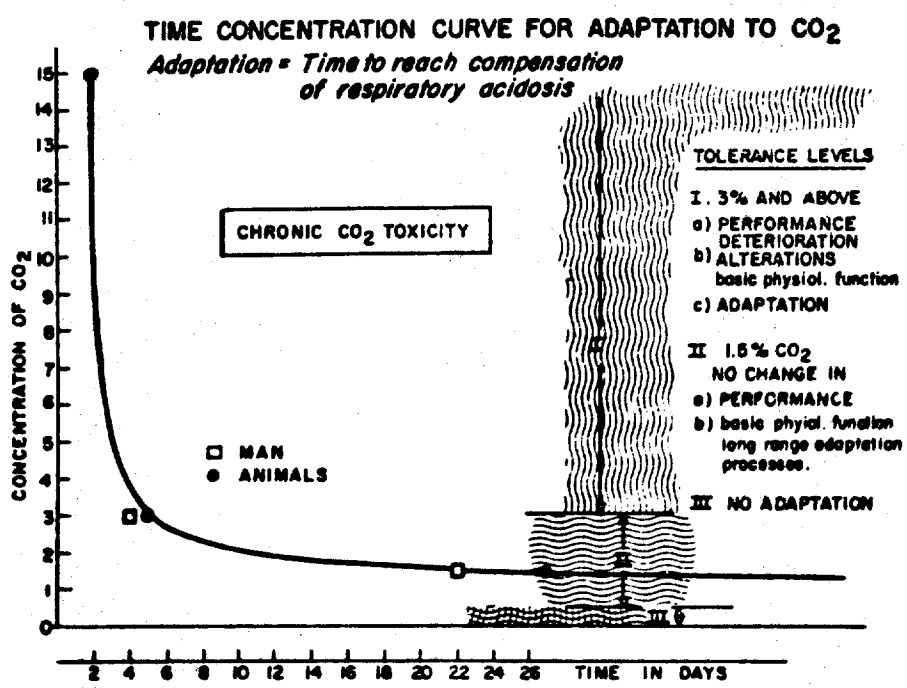


Fig. 3. Time concentration relationship in adaptation to increased carbon dioxide concentration based on experiments in humans and animals and tolerance limits for chronic carbon dioxide toxicity based on three different levels of activity.

Vogelenzang (2023) in 1962 described a case of mass poisoning of 30 persons with 7 deaths in a shipboard accident involving personnel in the engine room of an oil tanker. A fire extinguishing installation made up of several hundred bottles of liquid CO_2 was accidentally tripped, flooding the engine room with CO_2. It was determined that the CO_2 concentration rose to about 25%. Of the 30 persons in the engine room, 4 died immediately and 3 others either on route to the hospital or shortly after arriving. Nearly all others exposed to the CO_2 were taken out unconscious. Twenty-one were hospitalized, 11 only briefly. Numerous symptoms were observed among the patients, including loss of consciousness, retrograde amnesia, headache, vomiting, auricular fibrillation, cyanotic tongue, leucocytosis, albuminuria, etc. Several young men showed serious EKG disturbances, indicating myocardial damage. Autopsy of one of the fatalities showed typical symptoms of death by suffocation.

Harter (759) has reported on the effects of acute exposure (5 min.) to CO_2 (0.7.9%) on the EEG and reaction times of 5 human subjects. Alpha frequency and amplitude were recorded from the central and occipital-parietal areas of the scalp while subjects reacted by pushing a microswitch key with the right index finger. Closing the switch stopped counter and polygraph device monitoring the reaction time. Statistical analysis indicated that the percentage of CO_2 inhaled significantly influenced alpha frequency and reaction time; concentrations of 0-5.5% CO_2 accelerated alpha frequency and reaction time to a greater degree than did the 7.9% CO_2.

Therman (1934) reviewed in 1962 the neurophysiological effects of CO_2. Detailed studies of the direct influence of CO_2 have been performed on isolated parts of the nervous system, peripheral nerve fibers and sympathetic ganglia in vitro. Basically, CO_2 increases the slow electrotonic component of the total membrane potential which results in the following changes: an increased threshold of stimulation; an increased duration and height of the action potential; and decreased speed of nerve impulse conduction. Excitability is related to the slow electrotonic component, and variations in CO_2 tension result in specific excitability changes seen in a sustained lowering of the threshold of stimulation. A rapid fall in CO_2 tension produces "spontaneous" discharges of nerve impulse. The suggestion that CO_2 alters the pH in nerve fibers is not supported by altering pH in the absence of CO_2; more likely is the suggestion that the CO_2 effect results from the condensation of CO_2 and certain amino groups to form unstable carbamino compounds which participate in reversible biochemical equilibrium reactions.

Neurophysiological and neurochemical responses of the central nervous system to CO_2 were reviewed in 1960 by Woodbury and Karler (2121). Carbon

dioxide's most recognized function is as the principal buffering system of the body; changes in CO_2 pressure change the acid-base balance, and variations in pH can influence the rates of various metabolic reactions. Numerous substances are capable of influencing either the rate of CO_2 production or of its elimination and exert profound effects on the nervous system by altering CO_2 concentration in the brain, which is particularly sensitive to changes in the CO_2 concentration.

The effects of exogenous CO_2 on brain function are described under several headings: "neurophysiological" - brain excitability, EEG effects, spinal cord effects and CO_2 - withdrawal seizures; and "metabolic" - effects on electrolytes and acid-base balance and CO_2 and carbohydrate and amino acid metabolism. Drug effects which influence endogenous CO_2 metabolism in brain function, CO_2 release from brain, carbonic anhydrase inhibitors are discussed and include the effects of salicylates, DNP, acetazolanide, sulfanilamide, and congeners of acetazolamide. Various combinations of the foregoing drugs have been tested for enhancement of CO_2 production, or inhibition of its release from brain cells. The importance of the role of carbonic anhydrase in the CNS

$$(CO_2 + H_2O \longrightarrow \text{carbonic anhydrase} + H_2CO_3)$$

is discussed. The source and nature of CO_2 produced in the tissues, and the mechanism of the anticonvulsant action of CO_2 in the nervous system complete the review.

In an earlier paper (1949), Bain and Klein (75) had experimented with cats to determine the role of CO_2 in the CNS by assaying cerebral hemispheres for glucose, lactate, phosphates and pyruvate. The animals were paralyzed and maintained with artificial respiration; skulls were exposed for freezing of the brain following experimental treatment. Blood samples were taken, EEG and EKG data were recorded. Manometric measurements of blood gases were made, and blood pH noted. Inhalation of 10-30% CO_2 resulted in lowered lactate and pyruvate levels in the brain, in an inverse relationship to blood CO_2 levels, pH, O_2 and cerebral blood flow.

Little effect of CO_2 levels on glucose or phosphate concentration was observed.

Diagnosis and Therapy

The use of gas injection for contrast in clinical diagnostic roentgenology presents a possibility for gas embolism; Stauffer et al. (1860) discussed the hazards accompanying either deliberate introduction of air or O_2 in diagnosis in pneumoperitoneum, pneumothorax or angiocardiography, or accidentally - in surgical procedures, such as uterine curettage or that involving veins of neck or dural sinuses, etc. Pulmonary (venous) and arterial air embolisms are differentiated or characterized: in the former, air in the right ventricle produces an andible churning sound

("millwheel" murmur), in arterial embolism, serious blockage of coronary or cerebral vessels may occur.

Experimental studies in dogs have shown that serious circulatory disturbances resulting from venous air embolism may be agravated by the body position; in right decubitus or supine position, an air trap develops in the right ventricle, preventing circulation, and the pressure in the pulmonary artery rises. Intravenous air injection results in systemic blood pressure falling, accompanied by bradycardia and biphasic respiratory disturbances. Comparative studies of air and CO_2 injection intravenously into dogs has shown that doses of CO_2 equal to lethal doses of air are tolerated well, with no ill effects. Oxygen is almost equally hazardous as air when injected intravenously. It was suggested that a safe technique for selective CO_2 angiocardiography may well be developed for human subjects, due chiefly to its high solubility.

Pure CO_2 has been safely injected into animals and humans for diagnostic and therapeutic purposes, according to a brief report by Winters (2105) in 1960. The use of air or of oxygen has at times produced fatal or near-fatal accidents, and the author is recommending CO_2 as safe whether injected into systemic veins and arteries or left ventricle of the heart. The study of pericardial disease is described in which 50-100 ml CO_2 is injected into a large vein of the left arm of the patient, and x-ray exposure is minimized with maximum visibility of the heart and pulmonary parenchyma. The CO_2 bubble in the right heart disappears rapidly through solution in the pulmonary blood and by expiration.

Respiratory relief has been used as a simple, rapid treatment for specific phobias and phobic components of more complex neurotic conditions. Orwin (1429) has recently (1973) hypothecated that the satisfaction of an intense need to breathe, presented simultaneously with a phobic stimulus, would inhibit the anxiety previously provoked and that the inhibition would persist. An augmented respiratory relief is obtained by mixtures of CO_2-O_2; the range of 30 to 70% CO_2, with O_2 to 100% was determined to produce a marked respiratory response after maximum voluntary respiratory arrest. Ten phobias were rapidly removed by the augmented respiratory relief technique; two illustrative cases are described, one involving a spider phobia, and the other a severe obsessional compulsive neurosis with marked fear of fecal substances. It was speculated that the subcortical excitation occasional by CO_2 facilitates association of visceral relief with the phobic stimulus.

An early paper (1938) by Waters (2055) describes some of the difficulties attendant upon the surgical use of CO_2 as a depressant at high concentrations, and the dangers to individuals who may be hypersusceptible to CO_2. Three incorrect major premises as a basis upon which CO_2 is used as a

therapeutic agent were noted:

1. The idea that shock is caused by low CO_2 content of the blood and tissues following overbreathing;

2. The belief that all inhalation anesthesia implies overbreathing; and hence results in low CO_2; and

3. Faith that administration of CO_2 and O_2 mixtures periodically, reduces the incidence and severity of postanesthetic and other pulmonary morbidity.

The cautious use of CO_2 in therapy by trained personnel is urged.

Teratology

Haring (756) reported experiments exposing 71 pregnant young adults of Sprague Dawley rats to an atmosphere consisting of 6% CO_2, 20% O_2 and 74% N_2 for 24 hours during various stages of pregnancy varing from the fifth to the twenty-first day. Control animals were kept in normal atmosphere but otherwise similarly treated. After spontaneous or induced delivery of offspring, uteri were inspected, implantation sites counted, and mothers weighed. The offspring were examined and after visible malformations were noted, were sacrificed and various organs removed and prepared for microscopic examination. A total of 530 offspring were obtained from the test group and 159 from the 21 mothers of the control group. The data are summarized in the following tables:

Table 1

Experimental Data

	Control series	Test series
Number of rats bred	159	530
Number of male offsprings	80 (50.3%)	238 (44.9%)
Number of female offsprings	79 (49.7%)	292 (55.1%)
Average weight of newborn rats in mg.	5,275	6,276 (18.9% higher than control)
Average number of rats recovered per litter	7.6	7.4
Number of rats stillborn or dead immediately after birth	5 (3.1%)	41 (7.7%)
Number of rats with skeletal malformations	1 (0.6%)	58 (10.9%)

Table 2

Mean Weights

Day of mother's exposure to CO_2	Number of litters	Number of newborn	Mean weight of newborn in mgm
5th	5*	31	6,338 ± 6.47
6	3	26	6,292 ± 6.43
7	5*	40	6,277 ± 6.37
8	5	40	6,255 ± 6.31
9	5	45	5,748 ± 6.39
10	4	23	6,480 ± 5.88
11	6	44	6,013 ± 7.45
12	7	29	6,129 ± 6.27
13	4	36	6,237 ± 6.34
14	3	30	5,861 ± 5.83
15	6*	42	6,206 ± 6.27
16	4	31	6,134 ± 6.27
17	3*	23	6,215 ± 6.52
18	3	22	6,158 ± 6.31
19	3	22	6,331 ± 6.49
20	3	27	6,331 ± 6.46
21	2	19	5,862 ± 6.04
Total for test animals	71	530	6,276
Controls	21	159	5,275

*In one dam, the entire litter was resorbed.

Exposure of mothers to CO_2 increased the mortality rate among male fetuses (45% males, 55% females); controls were equally divided. The average weight of offspring in the CO_2 exposure was 19% higher than in controls. Stillborn offspring or dead immediately after birth, numbered 47 (7.7%) in test group, 5 (3.1%) in controls. Skeletal malformations (kyphosis or hydrocephalus) numbered 58 in test group, only 1 in controls. Twenty-four percent of the test animals showed cardiovascular defects; 86% had lung abnormalities; and in general, test animals had enlarged thymus.

Guinea pigs, in first and second months of gestation, were used by Giuntini and Corneli (654) to test the teratogenic action of CO_2 in mammals. Two groups of animals were placed in a sealed box: 40 guinea pigs, averaging 250 g body weight, were subjected to a CO_2 concentration of 0.48%; another group of 40 animals breathed 0.42% CO_2. Exposure times for the first group were 10 minutes/day for 20 days; the second group, 1 hour/day for a month. The animals were in the 20th day of gestation at the beginning of the exposure to CO_2. The results were as shown in the following table:

	27 miscarriages with 56 subjects apparently normal	
1st group	23 born on time, of which 18 were apparently normal	
40 guinea pigs with acute carbon dioxide poisoning	5 with flaccid paralysis of the posterior limbs, to a more or less evident degree	
Controls -- (10 guinea pigs) 27 normal births		
	22 normal births (46 well-formed feti)	
2nd group		
40 guinea pigs with chronic carbon dioxide intoxication	18 on time, with 39 microsomic births	24 with defects of the paws and 15 with normal limbs
Controls -- 10 guinea pigs with 23 normal births		

From the data it can be seen that CO_2 was clearly teratogenic to the offspring of guinea pigs exposed chronically to excessive CO_2, particularly at 0.42%. The embryonic abnormalities appeared chiefly in the posterior region, with flaws in extremities and some cases of paralysis. No skeletal malformations were seen. The authors suggested that those malformations (fetopathies) observed were attributable to a "neuro-muscular genetic component".

Cytostasis and Carcinogenesis

A theory was proposed by Loomis (1165) to explain cytostasis and malignancy in animal tissues, by a self-limiting growth in normal adult cells by inhibitory levels of free CO_2-produced by respiration. The growth of embryonic and malignant cells are not so limited by virtue of using adenosine triphosphate (ATP) generating systems (glycolysis) which do not generate CO_2. Thus, free CO_2 may be regarded as a physiological, nontoxic, reversible inhibitor of cell growth. This physiological control prevents normal, respiring cells from growing, but may be overridden in embryonic and cancer cells by the promotion of additional ATP through glycolytic mechanisms. Evidence to support the theory is presented from the various studies in lower animals (such as Arbacia) which indicate that increasing CO_2 pressure inhibits respiration, and lowered pressures lead to increased ATP available for growth. In general, it has been observed that the rate of glycolysis in embryonic and in malignant cells is far above normal adult tissue levels. A high titer of glycolytic enzymes is seen in embryonic cells but not in adult ones; some process which inhibits the synthesis of these enzymes at maturation of the cell

occurs. Knowledge of the mechanism of normal maturation could lead to some method of converting malignant cells to normal, and the CO_2 levels are of perhaps key significance.

A discussion of Loomis' theory by Kieler and Bicz (964) presents observations on the effects of CO_2 on aerobic glycolysis and respiration in tumor cells that lend support to the theory.

Biochemical Aspects

I. Breakdown

No relevant information found

II. Absorption - Distribution

A multicompartment theory of CO_2 stores in the body suggests that CO_2 is stored within body tissues as the bicarbonate and dissolved CO_2 at different levels of perfusion, metabolic rate and dissociation curves of CO_2. The low rate of perfusion per unit volume of muscle suggests muscle as a respiratory for CO_2 produced by other organs. When ventilation is stopped, the CO_2 content of venous blood should be lower than that of the arterial blood - the reverse of normal conditions. Heymann and Cherniack (809) tested the theory on nine anesthetized dogs; CO_2 and O_2 tensions, pH and hematocrits were measured in samples of femoral artery and venus blood. Half an hour of preliminary ventilation with 100% O_2 was followed by a cessation of ventilation. Measurements made at one or two minute intervals showed that after one minute of apnea, the CO_2 gradients were reversed from the earlier higher CO_2 tension of the venous blood, and at 6 minutes of apnea the average venous CO_2 tension was 13 mm Hg less than that of the arterial CO_2 tension, and CO_2 concentration was 3.3 vol.% less. It was suggested that for each minute of apnea, muscle stores about 3 ml CO_2 produced elsewhere, or about 4% of the total CO_2 produced by the body each minute.

Shephard (1768) in 1955 reported the results of studies on human volunteers under conditions of experimental hypercapnia to determine CO_2 accretion. Intake and output of CO_2 were measured by the use of Douglas bags; calculations of the net CO_2 accretion were based upon "balance sheets", with CO_2 income against CO_2 output and expressed as: Net CO_2 accretion equals the sum of 1) CO_2 uptake from inspiratory bag, 2) resting CO_2 output and 3) CO_2 output due to additional metabolic cost of hyperventilation, minus the sum of 4) CO_2 output in expiratory bags, and 5) apparent retention of CO_2 caused by technical factors, including diffusion.

Nine normal subjects were studied, two in detail; and 22 cases with congenital heart disease, representing four types: peripheral cyanosis, central cyanosis, increased R.V. pressure; and increased flow. Normal subjects after 30 minutes of breathing CO_2, show a total CO_2 retention of 1000-2000 ml; the ratio of extra-ventilation to CO_2 accretion has attained a steady value in most subjects, but maximum accretion has not yet been achieved. Similar findings characterized the subjects with congenital heart disease.

Small amounts of retained CO_2 were found in the pulmonary gas volume,

the lung tissues and the blood. The major proportion of retained CO_2 was distributed to other body tissues.

Enns (495) has described the mechanisms of gas transport in the body; O_2, CO_2 and the inert gases are considered. Bicarbonate ions play a role in red cell CO_2 capacity and transport similar to that performed by oxyhemoglobin in O_2 capacity and transport. Carbon dioxide is more soluble in water than is O_2 and is transported more rapidly in aqueous solutions by passive diffusion, in accordance with Fick's law. The transport is enhanced by bicarbonate ions; at pH 6.8, bicarbonate ions contain five-sixths of the total CO_2 in equilibrium solution. The exchange between dissolved CO_2 and bicarbonate ions is very slow, but greatly accelerated by carbonic anhydrase. The following figures show the relationships:

$$pH = 6.1 + \log \frac{[HCO_3^-]}{[CO_2]}$$

$$\frac{[HCO_3^-]}{[CO_2]} = 10^{(pH-6.1)}$$

FIG. 1. Simplified representation of ratios of dissolved CO_2 and bicarbonate ions at equilibrium.

I $$CO_2 + H_2O \underset{}{\overset{\text{ENZYME}}{\rightleftharpoons}} HCO_3^- + H^+$$

$$\nwarrow\!\!\searrow \qquad \swarrow\!\!\nearrow$$

$$H_2CO_3$$

II $$CO_2 + OH^- \rightleftharpoons CO_2 - E - OH^- \rightleftharpoons HCO_3^- + E$$

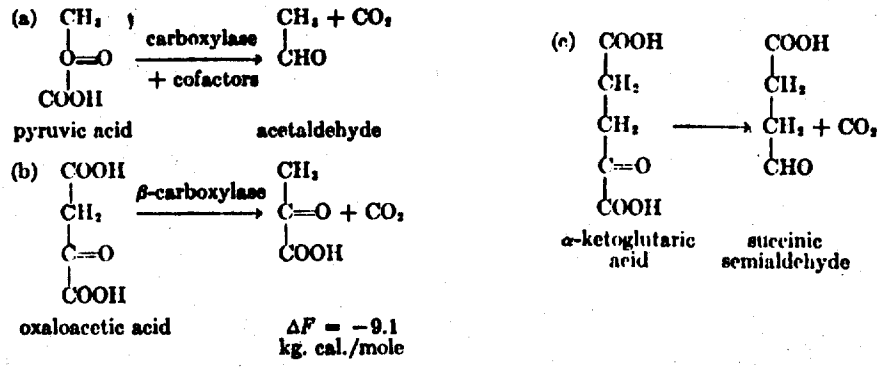
FIG. 2. Carbonic anhydrase and the interconversion of CO_2 and bicarbonate ions. In II, E denotes the carbonic anhydrase.

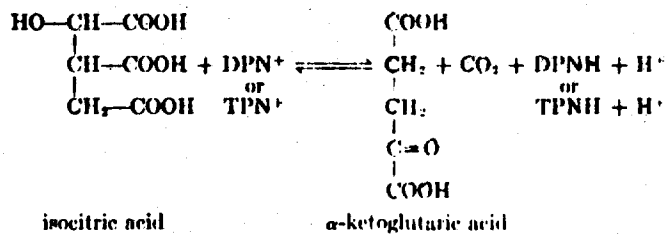
Human red cells probably have the highest known carbonic anhydrase activity, making more bicarbonate ions available for CO_2 transport.

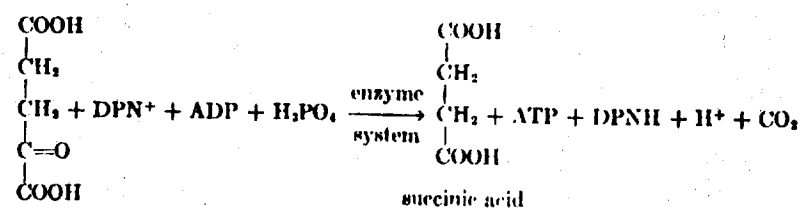
III. Metabolism and Excretion

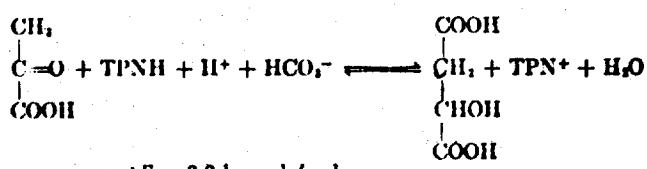
Goddard (661) has presented an excellent review of the biological role of CO_2. As recently as the 1930-40's, it was assumed that the sole metabolic role of CO_2 was in its utilization by green plants in photosynthesis and its production by other organisms in a irreversible oxidative

process. It has been found, however, that CO_2 is incorporated in a number of nonphotochemical reactions, such as a reaction with adenosine triphosphate and a hydrogen donor. In carbohydrate metabolism, CO_2 may be reversibly involved in several decarboxylation reactions, or in the reversible incorporation of CO_2 into malic acid or in similar reactions with isocitrate and alpha-ketoglutaric acids. In brief, CO_2 has become recognized as "a highly reactive cellular metabolite". Some of the reactions are shown below in diagrammatic fashion:

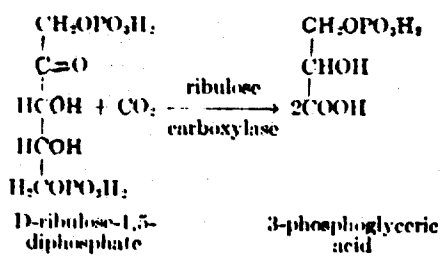








ΔF = 3.0 kg. cal./mole
TPNH = reduced triphosphopyridine nucleotide
TPN+ = oxidized triphosphopyridine nucleotide.



Drury et al. (457) used carbon-14-labeled bicarbonate, intravenously or intraventricularly injected into cats, to determine the rate of CO_2 elimination. Expired air, collected by tracheal cannula, was passed through a sodium hydroxide solution; the carbonate was precipitated as the barium salt and the radioactivity determined. Blood and tissue CO_2 was determined after sacrifice of the animals. The rates of elimination of labeled CO_2 after injection intravenously and intraventricularly were compared during the first minute after injection; the following tables show the results with regard to $C^{14}O_2$ exhaled, and the elimination from the body at successive minutes after injection:

TABLE 1. EFFECT OF SITE OF ADMINISTRATION ON PERCENTAGE OF INJECTED $C^{14}O_2$ EXHALED DURING THE 1ST MINUTE AFTER INJECTION

	I.V. Inj.		Inj. in Ascending Aorta	Inj. in Left Ventricle
1st 20 sec. after injection	8.01	8.64	.09	1.97
2nd 20 sec. after injection	2.14	1.57	.58	1.47
3rd 20 sec. after injection	1.50	.73	.56	1.06

TABLE 2. PERCENTAGE OF $C^{14}O_2$ IN THE BODY EXHALED IN SUCCESSIVE MINUTES AFTER INJECTION

Minute After Injection	Injected Intravenously	Injected Into Left Ventricle
2	1.87	2.94
3	1.40	4.45
4	1.27	3.85
5	1.28	2.00
6	1.08	2.00
7	1.03	1.47

The activities of CO_2 in various tissues relative to that of blood taken at the same time over 18 minutes following injection of the labeled carbonate are shown below; the relationships of blood flow to the tissue CO_2 content is also shown:

TABLE 3. RELATIVE ACTIVITIES OF CO_2 OF DIFFERENT TISSUES OF ANIMALS KILLED AT VARIOUS TIMES AFTER INJECTION OF $NaHC^{14}O_3$

	Relative Activities				
Time After Inj., min.	Blood	Muscle	Liver	Fat	Skin
1	100	28	88		
2	100	29	93	60	41
2.5	100	37	96		
5	100	58	74	64	
6	100	75	63		
13	100	96	93		
18	100	107			

TABLE 4. RELATIONSHIP OF BLOOD FLOW TO CO_2 CONTENT OF VARIOUS TISSUES

	Flow, ml/min/100 gm Organ Weight	% of Total Blood Flow*	Values for 1 kg Rabbit	
			Total CO_2 content of organ, mM†	% of total body CO_2
Brain	.54	3.6	.1	1.1
Heart	70	4.7	.05	.6
Liver	80	25.1	.5	5.7
Kidneys	430	25.4	.07	.8
Muscle (resting)	7	28.2	5	57.3
Skin	3.5	12.9	1.5	17.2
Blood & alveolar air			1.5	17.2

* These values are for all of the particular tissue in the body. Blood flow values are from H. D. GREEN. In: *Medical Physics*, edited by OTTO GLASSER. Chicago: Yr. Bk. Pub., 1950. p. 244, vol. II, *Circulatory System: Physical Principles*.

† Values for muscle from Irving *et al.*, reference 4. Other values are ours.

The results generally indicated that the mixing of injected CO_2 with the body CO_2 pool is not rapid; 65-75% of the CO_2 pool is in the tissues with a low blood flow rate compared to CO_2 content (muscle, skin and blood - lung air).

Winchell et al. (2101) undertook a study to provide information regarding the rates of exhange between various components of the body's CO_2-HCO_3 pools in normal male subjects at rest. Carbon-14-labelled bicarbonate was injected intravenously as a single bolus and expired $^{14}CO_2$ was monitored (total CO_2 excretion rates were determined) for a period of at least 2 hours. A mathematical treatment was employed in a compartmental model to analyze the $^{14}CO_2$ excretion data; the model is shown in the following figure:

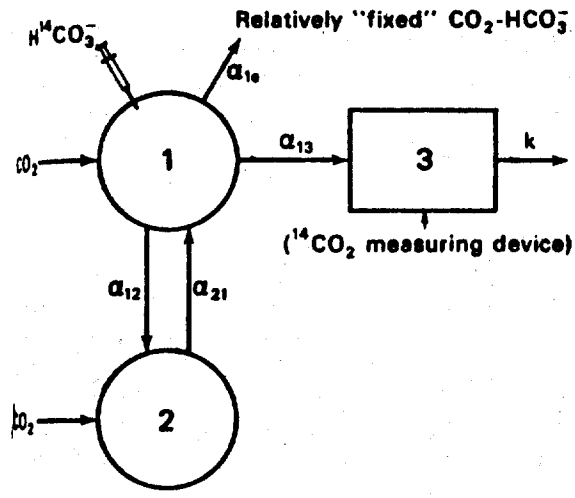


FIG. 1. Compartmental model used to analyze $^{14}CO_2$ excretion breath before intravenous administration of ^{14}C-labeled bicarbonate. Compartment 1 represents those CO_2-HCO_3^- pools that have equilibrated with blood CO_2-HCO_3^- within first 3 min after intravenous administration of labeled bicarbonate. Compartment 2 represents those CO_2-HCO_3^- pools which show significantly delayed equilibration with blood CO_2-HCO_3^-. Compartment 3 represents ^{14}C activity in measuring device. Relatively "fixed" CO_2-HCO_3^- represents that labeled bicarbonate leaving Compartment 1 which is not significantly recycled back into Compartment 1 during time of study.

It is currently (1970) thought that CO_2 passes across cell membranes rapidly; intravenously administered C-14-bicarbonate probably mixes rapidly with the CO_2-HCO_3 in blood and tissues with a high ratio of blood flow rate to tissue CO_2-HCO_3 content, such as abdominal and thoracic viscera, and having a total CO_2-HCO_3 content of 5.5 mM/kg body weight. In 13 normal subjects studies, an average fraction of 0.067/min of the first pool left to enter the second pool with a total CO_2-HCO_3 content of 7.9-11.0 mM/kg

body weight. The second pool is believed to consist of CO_2-HCO_3 contained in tissues with a small ratio of blood flow rate to tissue CO_2-HCO_3 content, such as muscle. A feedback of an average fraction of 0.046/min from second to first pool is assumed. Two other fractions of the first pool left the pool: 0.028/min expired in the breath; 0.005/min "fixed" somewhere in the body, possibly in part related to deposition in bone, and lost from the body by excretion in urine, feces and sweat. It is proposed that analyses of CO_2-HCO_3 kinetics in the body should aid in understanding the patterns of $^{14}CO_2$ excretion in the breath following administration of various C-14-labelled substrates in metabolic studies.

IV. Effects on Enzymes and Other Biochemical Parameters

Barbour et al. (86) reported on the effects of breathing 5-7% CO_2 on urine flow and mineral excretion in a number of human volunteers. The subject was either recumbent or sitting quietly during the administration of CO_2 and collection of urine. Urine flow was markedly increased by CO_2 in recumbent posture, but not in the sitting subject. During diuresis, the specific gravity of the urine fell; a slight rise in the excretion of total mols/min was attributed to urea. No changes in excretion/min of creatinine, sodium, potassium or chloride were observed. Glomerular filtration rate and renal plasma flow were not altered. The diuresis induced by CO_2 can be inhibited by pitressin and the probable mechanism of CO_2 diuresis is an inhibition of pitressin release. Hemodilution does not appear to be responsible for the diuresis; the plasma freezing point is not significantly altered by CO_2 inhalation.

V. Drug Interaction

No relevant information found

VI. Consumer Exposure Information

Solid CO_2 was one of the first ultra-cold materials to be used in the storage and transport of frozen foods. It has not been widely used, however, for rapid freezing of foodstuffs, due chiefly to the difficulty of achieving intimate contact between solids; in powder form, it can be mixed with powdered foods and rapid freezing attained (1718).

Carbon dioxide gas packaging has been used extensively in the United States for packaging coffee, bacon and cheese; the procedure has certain advantages over alternatives as vacuum and nitrogen gas packaging (1). The latter serve only to exclude oxygen and its chemical effect; CO_2 also inhibits the growth of many aerobic and some anaerobic microorganisms, not solely due to reduced O_2 levels, but presumably because of metabolic influences on the affected species. The high solubility of CO_2 in moisture and natural fats results in a tight, "snugged down" appearance in foods packed in clear, transparent plastic film; the package is neat, the color remains good, and it is attractive. In addition to coffee, bacon and

cheese, fish, biscuits, frankfurters, fresh red meat and dried foods are either presently CO_2-packaged, or are being studied for this type of preservation (1). Kraft et al. (1031) have described typical experiments comparing vacuum, and CO_2 packaging of bacon with respect to color retention, spoilage and "keeping" properties after opening. Generally, the investigations "point out the beneficial effects of use of CO_2 for extending keeping time of bacon and other cured meats".

The following tables were compiled from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on the frequency of eating, and from the USDA data on mean portion size of foods in each category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of the substance.

Table 2 reports the usage of CO_2 in foods; table 11 reports the annual poundage data. Table 13 reports the possible daily intake and total dietary, based on food consumption by total sample.

TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
CARBON DIOXIDE NAS 0060	01	BAKED GOODS(R)	*	.27190	.57920
	06	CHEESE(R)	*	.00010	.00050
	08	PROCSD FRUT(R)	*	.00624	.00624
	10	MEAT PRODS(R)	*	.20000	5.00000
	23	BEV TYPE I(R)	7	.70261	.93919
	24	BEV TYPE II(R)	*	1.44018	1.44084
	28	INIT DAIRY(R)	*	.12000	.36000

TABLE 11, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
CARBON DIOXIDE NAS 0060	12/ 15	13,005,040	24,026,553	26,720,021			26,720,021

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
CARBON DIOXIDE NAS 0060	01	BAKED GOODS(R)	*	0-5 MO.	9.244600	12.235500	19.692800
				6-11 MO.	69.062600	140.844200	147.116800
				12-23 MO.	148.185500	244.166200	315.664000
				2-65+ YR.	373.046800	554.132200	794.662400
CARBON DIOXIDE NAS 0060	06	CHEESE(R)	*	0-5 MO.	**********	.000100	**********
				6-11 MO.	.002700	.009700	.013500
				12-23 MO.	.007800	.022200	.039000
				2-65+ YR.	.009400	.023600	.047000
CARBON DIOXIDE NAS 0060	06	PROCSD FRUT(R)	*	0-5 MO.	.293290	.786240	.293290
				6-11 MO.	3.232320	8.049600	3.232320
				12-23 MO.	6.277440	12.461280	6.277440
				2-65+ YR.	7.381920	15.637440	7.381920
CARBON DIOXIDE NAS 0060	10	MEAT PROCS(R)	*	0-5 MO.	2.200000	5.800000	55.000000
				6-11 MO.	41.400000	111.600000	1035.000000
				12-23 MO.	60.400000	163.600000	1510.000000
				2-65+ YR.	156.800000	260.200000	3920.000000
CARBON DIOXIDE NAS 0060	23	BEV TYPE I(R)	7	0-5 MO.	16.862540	25.293960	22.540560
				6-11 MO.	159.492470	545.927970	213.190130
				12-23 MO.	300.814620	1141.741250	509.040980
				2-65+ YR.	730.714400	1951.147970	976.757600
CARBON DIOXIDE NAS 0060	24	BEV TYPE II(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	**********	1.440180	**********
				12-23 MO.	**********	2.890360	**********
				2-65+ YR.	468.058900	1359.529920	468.278000
CARBON DIOXIDE NAS 0060	26	IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	1.680000	2.760000	5.040000
				12-23 MO.	.960000	4.080000	2.880000
				2-65+ YR.	1.080000	1.800000	3.240000
CARBON DIOXIDE NAS 0060	ALL CATEGORIES **********		14	0-5 MO.	28.600520	44.115800	97.526640
				6-11 MO.	274.870090	810.031650	1403.598750
				12-23 MO.	596.645360	1509.151290	2343.901420
				2-65+ YR.	1737.091020	4142.471130	6170.361920

CARBON DIOXIDE

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Distillers Company (Carbon Dioxide) Limited

Food Manufacture 45 (9) 37-40.1970.

food PACKAGING
Abbey-'70
Bib No. 1

Carbon dioxide packaging has been somewhat neglected in the U.K. except for coffee but has advantages over nitrogen and vacuum packaging in certain applications. This survey also looks at suitable machinery

GAS PACKAGING
with carbon dioxide

Carbon dioxide gas packaging is the Cinderella of food packaging techniques in this country. Carbon dioxide has been used extensively in the U.S.A. for packaging coffee, bacon and cheese for many years, but in the U.K. primarily for coffee packaging. Its wider use in the U.K. has gathered momentum relatively slowly until recently, in part due to the lack of suitable gas packaging equipment. The advantages of CO_2 packaging compared to alternative processes such as vacuum and nitrogen gas packaging are possibly not fully appreciated by the food industry, but they should be of interest to packer, retailer and consumer alike.

Why carbon dioxide?

Carbon dioxide is an inert, bacteriostatic gas, commercially available as a low cost, high purity material, which is harmless both to humans and to foodstuffs in the context of food packaging. It is relatively little known to the food industry and general public, despite the fact that vast quantities of the gas are consumed annually in beer and mineral waters. This use, however, provides the answer to our question.

In beer, carbon dioxide performs two functions. First, by excluding air it protects the beer from the growth of harmful bacteria, moulds and wild yeasts, and thus lengthens shelf life. Secondly, its presence imparts a pleasant sparkling appearance to drink, improving sales appeal. Both reasons make CO_2 attractive to the packer and consumer of food products.

Microbiological aspects

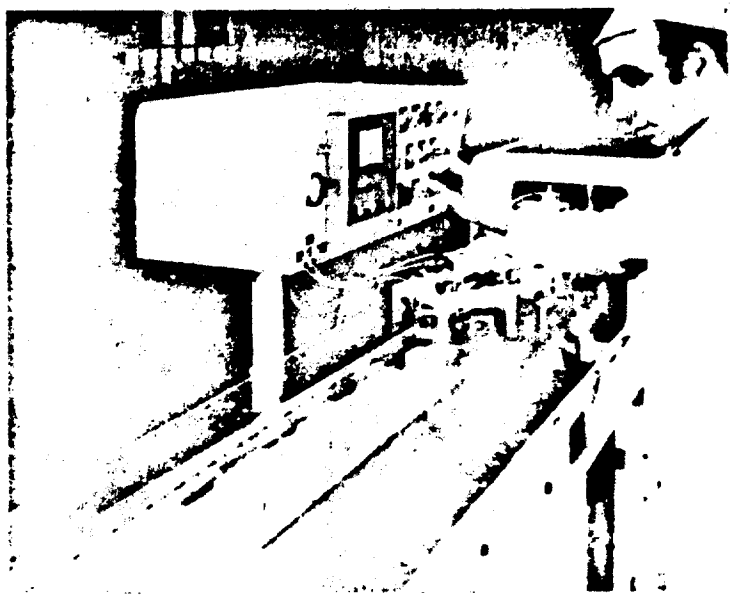
The shelf life of most perishable foodstuffs is limited by two factors — the chemical effect of atmospheric oxygen and the growth of food spoilage microorganisms. By introducing CO_2 into a gas-impermeable film surrounding a perishable foodstuff, air is purged from the pack which, when hermetically sealed, ensures that there is no oxidative degradation of natural oils, fats and vitamins due to atmospheric oxygen. There is thus no opportunity for the development of off-odours and flavours nor does the quality of the foodstuff deteriorate.

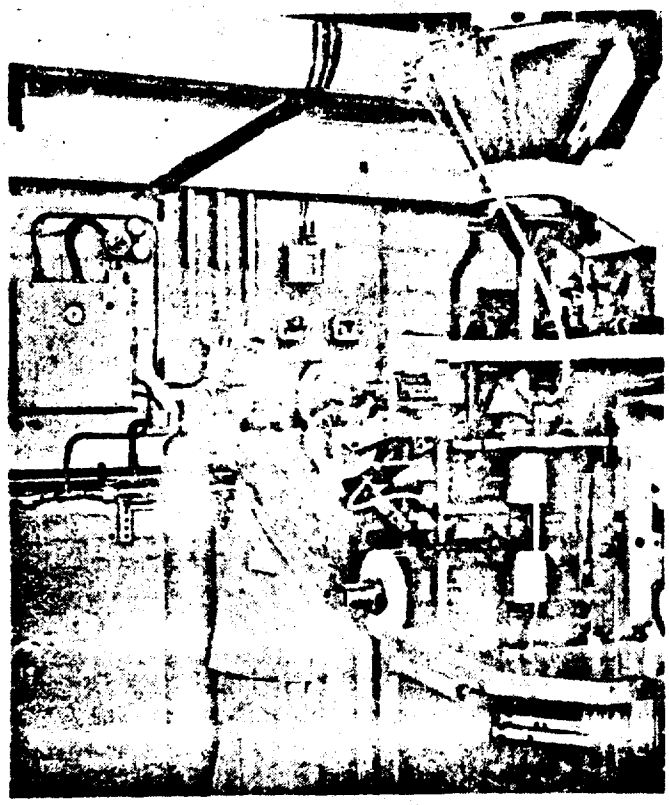
The presence of high concentrations of CO_2 also inhibits the growth of many aerobic and some anaerobic microorganisms thereby prolonging the storage life of the product. This action is not only a function of reduced oxygen levels, but is also believed to be due to the specific effect which CO_2 exerts on the metabolism of the affected species. Concentrations of CO_2 as low as 10 per cent have been shown to have a beneficial effect by reducing the growth rate of food spoilage moulds and bacteria, but generally speaking, the CO_2 level should be as high as possible. CO_2 gas packaging, therefore, offers additional protection compared to vacuum and nitrogen which are only effective by way of exclusion of oxygen.

Improved sales appeal

Appearance is of vital importance with a perishable foodstuff packed in a transparent plastic film. CO_2-packaged products benefit from the solubility of CO_2 in natural fats and moisture, which results in the "snugging down" effect characteristic of the CO_2 pack. A CO_2 pack has a relatively tight, vacuum pack appearance without undue compression. The pack is neat and tidy, movement of the product within the pack is prevented and there is no exuded moisture present (see under "Bacon" below). The colour of the packaged product—and "freshness" in the mind of the consumer—remains good and can even be enhanced in the case of some cooked and cured meats by the presence of a CO_2 atmosphere.

Gas packing of cheese at Dairy Produce Packers' factory





Eight-cylinder wall rack used in conjunction with a continuous automatic coffee packaging machine at G. Payne & Co.'s Croydon factory

Good appearance and longer life characterise these CO_2-packaged sweetcure bacon joints

PRODUCT CASE HISTORIES

The relevance of the properties of CO_2 applied to food packaging can be further highlighted by examination of products at present being packed under CO_2 atmospheres.

Coffee

CO_2 is widely employed in the U.K. in the manufacture of ground and instant coffee and for packaging both in tins and plastic film. Coffee evolves large quantities of occluded CO_2 when it is ground, resulting at the same time in the release of flavour and odour compounds. By employing an inert CO_2 atmosphere during processing and packaging, the loss of these oxygen sensitive components is minimised and product quality maintained. Shelf life is at least 6 months under reasonable storage conditions. The packs remain lightly compressed and of good appearance. There is no tendency to "balloon" since the partial pressure of the introduced CO_2 effectively prevents release of occluded CO_2.

The natural high specific gravity of CO_2 (1.5 times that of air) further helps to ensure that air is effectively purged from containers and the likelihood of further air entry is reduced. The use of alternative techniques, such as vacuum packaging and nitrogen gas packaging can result in loss of the flavour and odour components during the packaging operation and can give rise to "balloon" packs (the absence of a CO_2 partial pressure allows continual release of occluded CO_2), which have poor appearance and are difficult to handle without risk of puncturing.

Bacon

CO_2 packaging is employed as an alternative to vacuum for the automatic packaging of bacon. The CO_2 packs are superficially similar in appearance to vacuum packs but suffer neither from overcompression nor from the presence of visually unpleasant globules of moisture exuded as a result of the overcompression which is often experienced with vacuum packs. This is because CO_2 dissolves in the naturally present fats to give a lightly compressed pack which has a good appearance with the additional advantage that on opening the pack, the rashers of bacon can easily be separated — a problem not always appreciated by the packer but regularly encountered by the housewife. Colour also remains good and shelf life is at least as long as that obtained from the equivalent vacuum pack.

It has been shown that even vacuum packaged bacon does contain a small but significant quantity of residual oxygen. This enables bacteria to multiply until such time as they are prevented from further development by the CO_2 concentration which they themselves produce. It is considered that this initial growth of bacteria over the first one or two days of the life of the pack detracts from the ultimate shelf life and that the growth can be considerably retarded if high CO_2 concentrations are initially present.

One criticism levelled at the CO_2 gas pack is the difficulty in detection of "leakers". This problem is readily overcome by storing the pack in a cold store for a matter of a few hours which hastens the "snugging down" effect and hence the identification of puffy, leaking packs.

A high proportion of all packaged rashered bacon is still carried out on the particularly labour-intensive manual vacuum packaging machine. It is anticipated that over the next few years there will be a dramatic swing to highly automated bacon packaging lines, in many instances employing CO_2 gas flushing.

Cheese

A number of companies are gas packaging cheese on an automatic production basis for supermarket outlets. Cheeses most suited for CO_2 packaging are the harder varieties such as Cheddar and Leicester. Although there is very little work published on the effect of CO_2 on cheese it has been established that there is a critical level of CO_2 above which mould growth is rare. Practical experience indicates that the shelf life of gas packed cheese is at least equal to that achieved by a good vacuum pack.

Appearance is again similar to that of the vacuum pack, due to the absorption of some of the CO_2 by the natural fats but compression is not so severe allowing the packaging of some softer cheeses considered unsuited to vacuum.

Cakes

To achieve an adequate shelf life for bakery products distributed on a national basis, modifications to formulations are often required. The result tends to be a product with less free moisture — dryness is often associated with staleness in the mind of the housewife — resulting in generally less popular selling lines.

Mr. D. A. L. Seiler of the Flour Milling and Baking Research Association, Chorleywood, has carried out work which shows that the shelf life of cake packed under CO_2 atmospheres can be extended by 200-350 per cent. The multiplication of osmophilic yeasts in jam filled products has been shown to be reduced by atmospheres of 40 per cent CO_2 and above, with corresponding reductions in fermentation odour and flavour. Seiler has also shown that the growth rate of certain bacteria such as Staphylococcus aureus and Escherichia coli has been reduced in cream filled products stored under CO_2.

This work has been adapted for production application by a major U.K. manufacturer with considerable benefit and jam filled sponge cakes are now packed under CO_2 resulting in retail shelf life extension of more than three weeks. The shelf life extension has been achieved without major alterations to the formulation ensuring that freshness and moisture content remain unchanged. Distribution problems have been eased and complaint rates substantially reduced particularly during the summer months.

Fish

Small quantities of CO_2 are now being used for packaging kipper fillets mainly for export markets. Shelf life extension, particularly on the retailer's shelf, is the prime attraction. Fresh white fish has also been shown to benefit by shelf life extension when stored under CO_2 atmospheres, but no production application has yet been attempted.

Biscuits

CO_2 is at present used on a small scale for packaging higher-than-average fat content biscuits intended for export. They are sealed in gas impermeable films prior to final storage in air-tight tins.

Frankfurters

Work carried out in the U.S.A. by Ogilvy and Ayres has indicated that the shelf life of Frankfurters stored in 50 per cent CO_2 atmospheres is considerably increased and that oxidative rancidity becomes the limiting factor in storage. This work does not appear to have been investigated in the U.K. but could have an application in the packaging of several types of sausages.

Fresh red meat

There is considerable interest in the U.K. at present in the possibilities of gas packaging for fresh red meat. A number of companies are experimenting with carbon dioxide/oxygen/nitrogen mixtures using films of varying permeability in attempts to establish the conditions which will allow fresh red meat to be stored without loss of colour or quality for long periods at above freezing temperatures. Oxygen is essential for the red colour retention. CO_2 can also help to maintain the colour of fresh meat when employed at low concentrations, but is of particular interest for its bacteriostatic effects even in the presence of substantial concentrations of oxygen.

It is worthy of note that for a number of years chilled beef at 29-30°F has been shipped to this country in 10 per cent CO_2 atmospheres achieving periods of up to 70 days without spoilage. The technique has now largely been superseded by the shipment of beef in the frozen state.

Dried products

CO_2 gas packaging also has potential application for dried products including freeze-dried foods, dried milk powder and instant potato where the exclusion of atmospheric oxygen will prevent deterioration due to rancidity of fats. CO_2 has not so far been exploited to any extent in these fields.

MATERIALS and MACHINES

Having established that CO_2 packaging can be of benefit to certain products the next most important criteria are the choice of the correct packaging film to be used in conjunction with the most appropriate packaging machine.

Gas packaging films

Many of the problems encountered with gas packaging particularly during the early attempts were due to the inadequate film. Abrasion of coatings and delamination and cracking of films at chill temperatures helped to produce ineffective packs, in part due to lack of experience and in part due to unavailability of suitable films. The situation has now changed radically and a wide range of films, both laminates and coated varieties, are avilable and considerable experience has been gained in the choice of the right film for the job.

The basic requirement is for a film which is totally impermeable to CO_2, with correspondingly very low permeabilities to moisture and oxygen, at the same time retaining the required transparency, flexibility and heat sealability, although future research may well reveal that the permeability requirements are considerably more complex. At the present moment, any film suited to vacuum packing will be adequate for the retention of CO_2. Nylon, polypropylene and Saran-coated films and laminates are particularly suitable, polyethylene often being employed in laminates to achieve good heat sealability.

Most film converters and suppliers now offer satisfactory films and their recommendations should be sought in the light of the product to be packed and the machinery available.

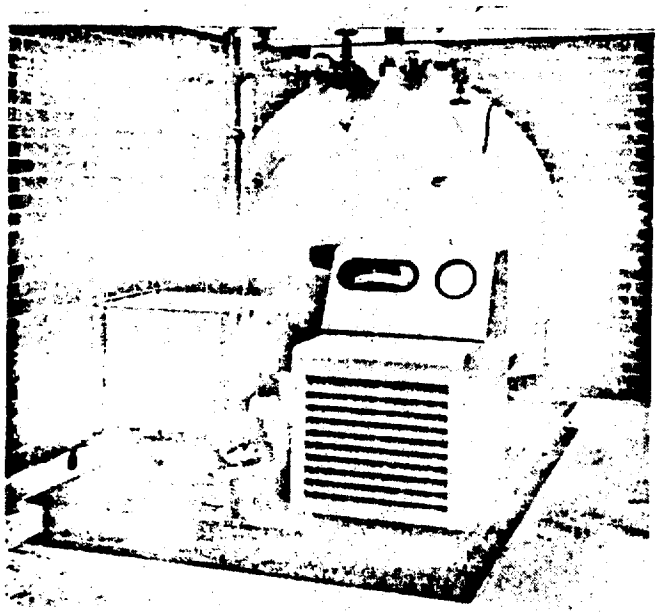
Packaging machinery

There is now wide choice of packaging equipment suitable for gas packaging including manual vacuum machines with gassing attachments, and specially designed vertical and horizontal high speed production machines.

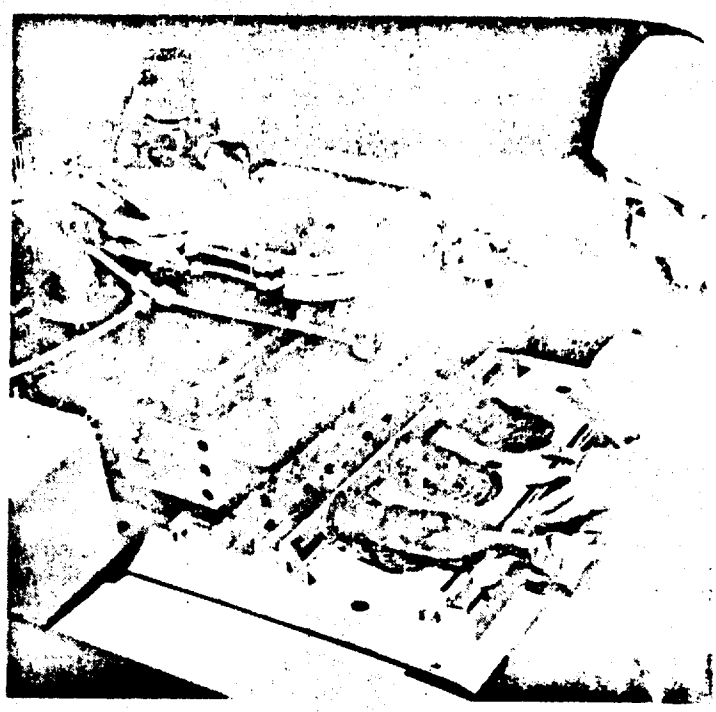
There is a wide range of manual vacuum packaging equipment on the U.K. market which can be readily adapted to employ CO_2 gassing by the addition of a simple gassing mechanism. The machines employ preformed pouches containing the product which are placed within the vacuum chamber. The vacuum is drawn and then broken by the introduction of CO_2 gas. The main advantages of this equipment are that quite large joints or catering packs can be gas packed with a high degree of sealing efficiency.

Manual vacuum/gassing equipment particularly suited to CO_2 is supplied by Jessett and Henry Ltd. (Clarovac range), Union Food Machinery & Equipment Ltd. (Autovac range), Soplaril (Great Britain) Ltd. (Swissvac range), and Foodtech Ltd.

There are two types of horizontal automatic machines available, known as the gas flush and thermoform types. The gas flush machines are confined at the moment to those manufactured by Hayssen. The Hayssen RT118E produces packs from a single web of film which is seamed along the centre, flushed with gas and crimp sealed at both ends by revolving heat-seal bars. The maximum product



Bulk liquid CO_2 storage vessel of 1¾ tons capacity on a manufacturer's premises



Vacuum packaging machine converted for CO_2 packaging of cured ham

height is approximately 3 inches. The RT318 is also specially designed for gas packaging particularly cooked meats and bacon. It employs a twin web feed and a four side seal. The height limitation is about ¼ inch. Both machines can also be provided with continuous oxygen monitoring equipment to ensure that the required packaging conditions obtain.

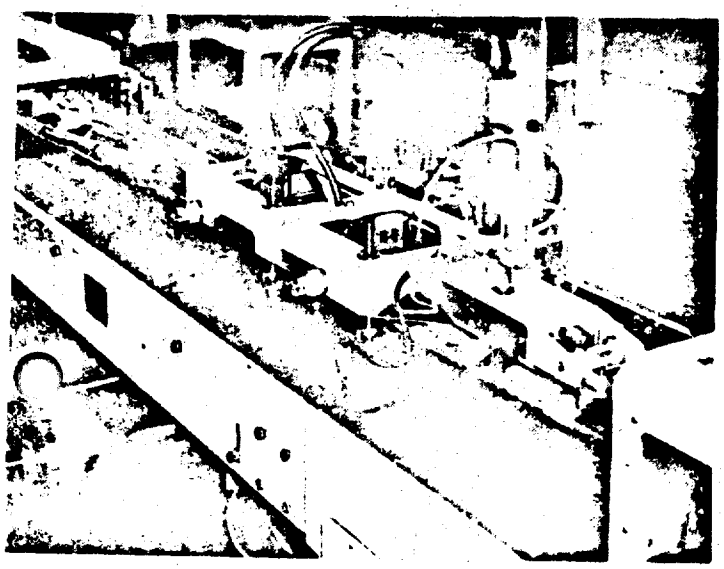
The Thermoform machines are basically vacuum machines with gas flush facility. The Multivac R68, the Robotor and the Soplaril Euroform 19/34 all employ twin webs, the lower web being thermoformed into trays to suit the product height. After loading, a vacuum is drawn which is broken by the introduction of CO_2 gas and the top web seal made. The product height can be several inches. A useful feature of these machines is that the packs are stationary when sealing is carried out permitting a high degree of sealing efficiency.

The Hayssen and Thermoform machines have similar production rates for given commodities. Overall operating costs are probably of the same order allowing for variation in labour costs, different film gauges and types and the cost of CO_2, although capital outlay and the period of amortisation tend to be greater with the Thermoform machines.

The vertical automatic machines, such as those supplied by Hamac Hansella and Hayssen, are employed with CO_2 almost exclusively for the packaging of coffee. A vertical "cylinder" of film sealed at the lower end is continuously formed from a single web and flushed with CO_2.

In the final analysis, the effectiveness of CO_2 packaging is only as good as the sealing efficiency of the packaging machines. Emphasis on correct choice of film and close attention to sealing bar temperature is essential. Operative training to ensure correct positioning of product in relation to the sealing operation is vital — one of the most common causes of faulty seals is that of product contamination in the sealing area.

Hayssen RT118E machine for the continuous packaging of rashered bacon



Carbon dioxide equipment

Carbon dioxide is supplied either from banks of cylinders or, for the larger user, from a refrigerated bulk storage vessel. In both instances, liquid CO_2 at a high pressure is converted to gas by electrical or steam vaporisers and is supplied to the packaging machine at a suitably reduced pressure. The CO_2 is of very high purity (identical to that used in breweries and in mineral water manufacture).

CO_2 gas packaging, although in its infancy in the U.K. is already applied to a wide range of commodities, offering a very attractive alternative to vacuum packaging and nitrogen gas packaging. By extending shelf life, gas packaging assures confidence in packaged product quality and by reducing complaint or return rates, customer satisfaction can be achieved with enhanced product and company image.

CO_2 is not a panacea and does not compensate for any inadequacies in respect of product quality or deviation from strict adherence to correct storage conditions before and after packaging. However, its use may well serve to enhance the acceptability of a product at the most important point in the chain from producer to consumer — the retailer's shelf.

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Problems associated with the determination of carbon dioxide by infrared absorption

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AMMANN, ELIZABETH C. B., AND R. D. GALVIN. *Problems associated with the determination of carbon dioxide by infrared absorption.* J. Appl. Physiol. 25(3): 333–335. 1968.—The infrared absorption of carbon dioxide (0–46 mm Hg) was measured in the presence of different diluent gases at a total pressure of 760 mm Hg. The 4.3 μ band of CO_2 was found to be affected by foreign gases in the order He, A < O_2 < N_2 < H_2. This effect is large enough to be considered in an analysis of carbon dioxide consumption or production by biological organisms in different gas environments.

interference of He, A, O_2, N_2, and H_2 in analysis of CO_2; gas mixing techniques; aerospace and man-in-sea research applications

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THE CONSUMPTION OR PRODUCTION of carbon dioxide by various biological organisms in gas environments other than nitrogen has recently come under investigation with the advent of manned space travel and deep-sea research. Several of these reports have indicated that the infrared (IR) absorption of carbon dioxide is altered in different diluent gases. Ammann and Lynch (2) found that IR absorption was decreased when carbon dioxide was in the presence of helium or argon as compared to nitrogen, while working with a unicellular alga. Adams et al. (1) also noted a lower output signal from the carbon dioxide analyzer when helium was substituted for nitrogen during their studies on man. Similar results have been observed in the field of anesthesiology. Common anesthetic gases such as cyclopropane and nitrous oxide were found to cause a discrepancy in the analysis of carbon dioxide by several workers (3, 6, 7). Earlier reports on the IR spectroscopy of gases in the field of physical chemistry revealed essentially the same information. It was found that the effect of adding either nitrogen or another diluent gas from 20 to 700 mm Hg to a constant pressure of an IR absorbing gas such as CH_4, N_2O, CO_2, or CO was to increase the absorption in a nonlinear manner (4, 5). The present experiments were carried out to determine quantitatively the change in absorption to be expected when carbon dioxide is in the presence of different diluent gases being considered for space travel and deep-sea research. Several concentrations of carbon dioxide were analyzed so that calibration curves could be drawn and compared. This information should be of value in the accurate determination of carbon dioxide in different diluent gases and, correspondingly, in the quantitative analysis of carbon dioxide metabolism by biological organisms in gas environments other than air.

Zero to 6% carbon dioxide was mixed with either nitrogen, oxygen, hydrogen, helium, or argon at a total pressure of 1 atm. These gases were obtained from the Airco Company and the National Welding Company. Carbon dioxide was not present as a contaminant in any of the tanks as measured by a Varian thermoconductivity gas chromatograph. Small amounts of nitrogen, oxygen, or argon were found to be contaminants in the other gas tanks, but in each case the impurities present were of such small concentration as not to affect the results.

The carbon dioxide mixtures were obtained by means of a Godart gas mixing pump, model NA 27/3, with an accuracy of ±0.025% carbon dioxide at 10% CO_2 in nitrogen. Small differences in mixing accuracy were experienced when helium, hydrogen, or argon were used in place of nitrogen or oxygen since the degree of fill of the piston is dependent on the molecular weight of the pumped gas. Table 1 shows the mixing accuracy which was obtained for each carbon dioxide gas mixture based on data and calculations supplied by the pump manufacturer. The deviations were not more than 0.1% at the most and were taken into consideration in the analysis of the data. All mixing was done at atmospheric pressure.

The mixing accuracy of the pump was verified by two means. The various carbon dioxide gas mixtures were analyzed with an aerograph thermoconductivity gas chromatograph (model A-90-P). Two columns, linked by a switching valve, were used to separate the gases. The columns contained 1% di(2-ethylhexyl)sebacate on charcoal and molecular sieve 5A. Helium was used as the carrier gas. The values for the concentration of carbon dioxide in the various diluent gases were found to be within the measurement accuracy of the chromatograph, which was ± 0.1% CO_2 at 6%. The carbon dioxide in nitrogen gas mixtures was also analyzed with a Beckman IR 215 infrared analyzer and was found to match exactly the calibration curve sent by the company.

The effect of various diluent gases on the IR absorption of carbon dioxide was studied with a Beckman IR 215 infrared analyzer having a 0–6% carbon dioxide range and operating in the 4.3 μ absorption band. Instrument response was measured with a digital multimeter (Electro Instruments, San Diego, Calif., model 881), and recorded with a Dura Mach 10 typewriter. Instrument accuracy was ± 0.025% carbon dioxide at 6%. All measurements were made at a flow rate of 150 ml/min and at 1 atm pressure. Samples containing 6% carbon dioxide in hydrogen or helium were also run on a Perkin-Elmer IR spectrophotometer with a 2.5–17 μ range.

The effect of the five diluent gases on the absorption of CO_2

TABLE 1. *Mixing error of Godart gas pump* for carbon dioxide in various diluent gases*

Diluent Gas	Concentration of CO_2 Obtained, %					
N_2	6.00	5.00	4.00	3.00	2.00	1.00
O_2	6.00	5.00	4.00	3.00	2.00	1.00
H_2	5.93	4.94	3.95	2.97	1.98	1.00
He	5.93	4.94	3.95	2.97	1.98	1.00
A	6.03	5.03	4.02	3.02	2.01	1.01

* Model NA 27/3.

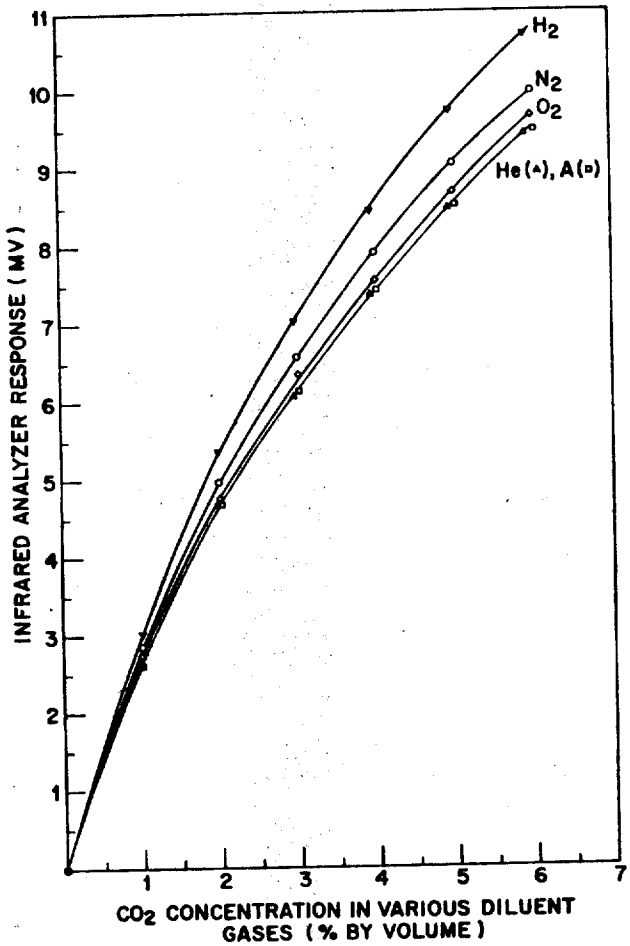

FIG. 1. Effect of various diluent gases on the infrared absorption of CO_2 as measured by a Beckman IR 215 infrared analyzer.

is shown graphically in Fig. 1. It will be seen that the 4.3 μ band of CO_2 absorption responds to the foreign gases in the order He, A < O_2 < N_2 < H_2. If it is assumed that the various diluent gases have no effect on CO_2 absorption, and measurements are made using an instrument calibrated solely with nitrogen as the background gas, then a measurement of 6% CO_2 will be in error by the following approximate amounts: +0.8% CO_2 for hydrogen (i.e., +13% error), −0.4% CO_2 for oxygen (−7% error), and −0.6% CO_2 for helium and argon (−10% error). The same proportional error is maintained throughout the range of 0-6% CO_2 concentration so

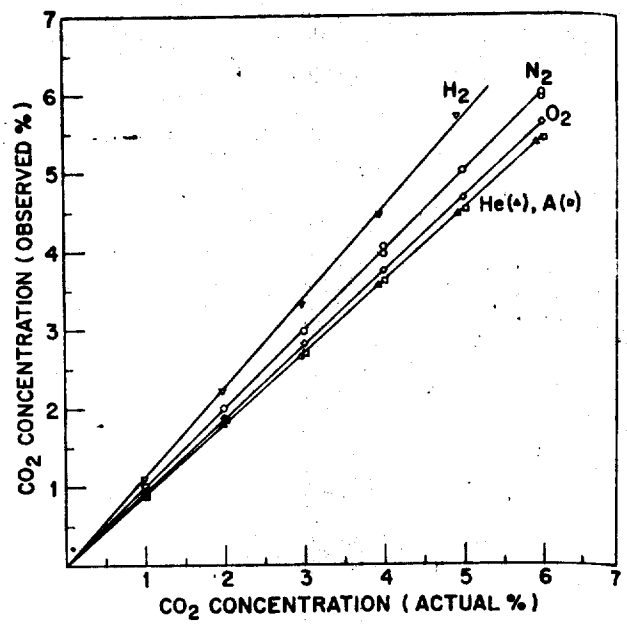

FIG. 2. Relationship between the actual percentage of CO_2 in various diluent gases and the observed percentage of CO_2 as determined with an instrument calibrated with CO_2 in N_2.

TABLE 2. *K values for various diluent gases where actual percentage of CO_2 in diluent gas equals K times observed percentage of CO_2 using an instrument calibrated with nitrogen as diluent gas*

Diluent Gas	K Value
He, A	1.11
O_2	1.06
N_2	1.00
H_2	0.87

that when the points in Fig. 1 are replotted to give the variation of "observed" percentage of CO_2 (using an instrument calibrated with nitrogen) against actual percentage of CO_2, the result is the set of straight lines shown in Fig. 2. We, therefore, have the following relation: actual percentage of CO_2 in diluent gas = K·observed percentage CO_2 where K is a consistent characteristic of each diluent gas (Table 2).

It was possible to calibrate the instrument with a single diluent gas and then use any other diluent gas with its correction factor to obtain the precise CO_2 value since the determination of each correction factor was found to be repeatable. Care should be taken to completely calibrate a particular IR analyzer in the beginning since the correction factor, at least with anesthetic gases, has been shown to vary with instrument design (7). The use of the Godart gas mixing pump facilitated all calibration procedures.

The state of knowledge regarding the effect of background gas on the IR absorption of carbon dioxide is not very complete at the present. No rule exists as to order of effectiveness. Crow and Daniels (5) found that the 4.5 μ band of nitrous oxide responds to foreign gases in the order He, A < O_2 < N_2 < H_2 from 25 to 250 mm Hg pressure which is the same order as determined in the present report for the carbon dioxide

band at 4.3 μ with 750 mm Hg of diluent gas and by Coggeshall and Saier (4) for carbon dioxide with 20–250 mm Hg. However, the latter workers noted that the 7.7 μ band of methane is affected in the order He < A < H_2, O_2, N_2. The absorption differences seem to depend on the IR absorbing molecule itself, the concentration of the absorbing molecule, the specific absorption band being used, and the concentration of the diluent gas. For a more complete discussion of the IR spectroscopy of gases, reference is made to Townes and Schawlow (9) and to Smith (8). It is sufficient to point out here that because of the above molecular interactions the effect of background gas on the IR absorption of carbon dioxide must be taken into consideration in an analysis of carbon dioxide consumption or production by biological organisms in different gas environments, including those relating to space and deep-sea research.

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FATAL POISONING FROM CARBON DIOXIDE REPORT OF FIVE CASES WITH TWO DEATHS*

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This paper describes the unusual circumstances that brought about carbon dioxide poisoning among five persons after they have gone down a well in Mapandan, Pangasinan, resulting in the death of two of them.

Domingo Bungato, 11 years, went down a well, using an improvised bamboo ladder, to get a pail that fell inside it. The well had a depth of 4.75 meters to the level of the water which was 0.90 meter deep to the bottom and its circular earthen sides has a diameter of 0.62 meter. Domingo was seen gasping upon reaching a level of about one foot above the water, then became motionless with his hands clasped about the ladder. Ignacio Bungato, grandfather of the boy, about 80 years, went to the rescue and in going down the same ladder, the boy fell inside the well with his face immersed in water. The old man, upon reaching the same level where the boy was seen gasping, fell inside the well with his face above the water. Cresencio Bungato, father of the boy then descended down the well and was able to tie a rope to the boy's body but the rope slipped on being pulled. Cresencio, meanwhile, was unable to go up. Amado Biacan went down the well then and was able to tie a rope to the boy who was hoisted up after staying inside the well for about two hours, with Amado remaining inside the well. Roque Bungato, uncle of the boy, then went to the rescue with a rope tied to his waistline and holding his breath as much as he could. He was able to tie a rope to Cresencio and Amado who were hoisted up and Roque, losing muscular power, was pulled from the well. The old man, left alone inside the well, as taken by catching his neck in a loop of a rope and pulling him up after a stay of about 4 hours inside the well.

Dr. Jose Giron, charity physician of the place, applied artificial respiration to Domingo Bungato for almost two hours in vain with Domingo presenting marked cyanosis of the lips, fingers and finger nails. Cresencio, Amado and Roque were semiconscious and restless after they were hoisted from the well. They were placed, with faces down, on top of empty gasoline drums and is so doing, water came out of their mouths. After a while, they were helped on their feet and taken to the house

* Read before the 26th scientific meeting of the Pangasinan Medical Society at Rosales, Pangasinan on July 30, 1950.

nearby. Cresencio, presenting mental excitement and clammy skin, was given a cardiac stimulant. Ignacio Bungato presented marked cyanosis of the fingers and finger nails and oozing of bloody fluid from the mouth.

The well, where the accident occurred, was constructed more than 60 years ago and was cleaned once a year without any unusual happening. Last May, however, Ignacio Bungato went down the well to clean it, but he was not able to stay long because of the intense heat inside the well. Let it be noted that a small distillery was established in a neighboring yard with a dumping pit for its waste product about 30 meters away from the well and by last December or 5 months before Ignacio Bungato felt intense heat inside the well, the dumping pit of the distillery was transferred to a place 7 meters from the well. The waste product of the distillery is the remains of a mixture of molasses and yeast undergoing fermentation, yielding a distillate containing less than 10% alcohol.

The nearness of the dumping pit to the well and the sandy nature of the soil created the suspicion that seepage of the waste product of the distillery to the well was most likely and the possibility of the presence of the end product of fermentation of molasses inside the well.

TEST AND LABORATORY EXAMINATIONS

1. Samples of air were taken by grab sampling and examined as follows:

Air Samples	*Depth Inside Well from Surf*	*Carbon Monoxide*	*Hydrogen Sulfide*		*Carbon Dioxide*		*Oxygen*
		By CO Detector	*By H_2S Detector*	*By Lead Acc. Pap.*	*By Orsa App.*	*By Pettenkofer Meth*	*By Orsat App.*
Sample I	3.65 M	Neg.	Neg.	Neg.	12.58%	12.5%	10.5%
Sample II	2.57 M	Neg.	Neg.	Neg.	10.5 %	6.8%	14.4%
Sample III	1.79 M	Neg.	Neg.	Neg.	Neg.	Neg.	15 %

2. Examination Of Other Samples.

Samples	*Source*	ALCOHOL		SUGAR
		Qualitative Analysis	*Quantitative Analysis*	*Qualitative Analysis*
Water	Inside the well	Positive	Not done	Negative
Mud	From bottom of the well	Positive	Not done	Negative
Waste product of Distillery	Dumping pit 7 meters from well	Positive	5.25%	Positive

The laboratory analysis of the samples taken showed that the end products of fermentation of molasses, which are alcohol and carbon dioxide, are present inside the well, which verified the suspicion of seepage of the waste product of the distillery to the well. The amount of carbon dioxide inside the well as analyzed by both Orsat's (Bureau of Mines) apparatus and by volumetric method of Pettenkofer, was found highest at a depth of 3.65 meters from the mouth of the well which is 12.5%. The water inside the well which was turbid and of septic odor and the mud at the bottom of the well showed the presence of alcohol by qualitative analysis.

DISCUSSIONS

Carbon dioxide is normally present in the air in the proportion of 0.03% by volume. It is a product of combustion, decay and fermentation. Its maximum allowable concentration in the air[1] is 0.1% or 1000 P.P.M.

Morris B. Jacobs[2] stated in Analytical Chemistry of Industrial Poisons that 5% of carbon dioxide causes laborious breathing, 12 to 15% causes unconsciousness soon and 25% causes death after several hours of exposure, high concentrations causing paralysis of the respiratory center and death by asphyxiation.

According to William D. McNally,[3] carbon dioxide acts locally producing a prickling or twitching sensation with a feeling of warmth followed by anesthesia if continued and remotely on the brain and spinal cord with stimulating effect at first, increasing blood pressure and reduction of pulse rate, then more intense respiration and ultimately paralysis. High concentration causes sudden death by suffocation; concentration not causing insensibility, produces loss of muscular power, nausea, profuse perspiration, mental excitement sometimes, etc.; 8.5% causes dyspnea and rise of blood pressure; up to 15% causes an increasing severity of symptoms which is not dangerous for an hour to animals and probably not to man; 25 to 30% causes depression, fall of blood pressure, decreased respiration and coma.

A burning candle was lowered inside the well and was put out at a depth of 1.9 meters from its mouth. Most flames are put out by 10% carbon dioxide.[3] Air containing 8 to 10% of carbon dioxide will put out a burning candle,[4] indicating that at the depth of 1.9 meters, there should be 8 to 10% carbon dioxide.

CONCLUSIONS

In the light of the laboratory findings and those of different authors as mentioned heretofore, we come to the conclusion that Domingo Bungato must have lost consciousness or muscular power upon reaching the level where the carbon dioxide content of the air inside the well was 12.5% and staying inside the well with his face immersed in water for almost two hours, he must have died by asphyxiation from drowning with signs of marked cyanosis of the lips, fingers and finger nails. Ignacio Bungato, likewise, must have lost consciousness or muscular power,

on reaching the same level and staying inside the well for about 4 hours plus the strangulation he must have suffered on being pulled up with a loop of a rope around his neck, must have died also by asphyxiation with signs of marked cyanosis of the lips, fingers and finger nails and oozing of bloody fluid from the mouth. Cresencio Bungato, Amado Biacan and Roque Bungato must have been overcome also by the high concentration of carbon dioxide on the same level and being rescued immediately, their recovery was rapid.

Follow-up of the survivors, 18 days later, showed that Cresencio complained of dizziness on exertion, slight headache, nausea at times, weakness and spasm of the eyelids while Amado and Roque complained of occasional weakness. After one month, all the complaints have completely disappeared.

According to Rutherford T. Johnstone,[5] treatment consists of artificial respiration, oxygen therapy and the patient usually recovers without any permanent disability.

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EFFECT OF CARBON DIOXIDE ON BRAIN GLUCOSE, LACTATE, PYRUVATE AND PHOSPHATES[1,2]

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CENTRAL nervous activity is considerably affected by procedures that cause changes within the carbon dioxide system of blood and tissue. For example, respiration of suitable concentrations of carbon dioxide induces hyperpnea, convulsions and anesthesia. Hyperventilation tends to induce relatively high-voltage, low-frequency cortical potentials and, in petit mal, typical seizure discharges (1). Conversely, petit mal seizures can be interrupted by increasing the carbon dioxide concentration of the inspired air (2). The total carbon dioxide content of arterial and jugular blood fluctuates abnormally prior to the onset of seizures (3). Repeated breathing of high concentrations of carbon dioxide appears to relieve certain neuroses (4). Moreover, breathing gas mixtures containing large (15–30%) amounts of carbon dioxide raises the convulsive thresholds to certain drugs and to electric shock, whereas it lowers the convulsive threshold to other drugs (5, 6).

At present there is insufficient information to warrant extensive consideration of the basic cellular mechanisms whereby the carbon dioxide system might affect central nervous activity. It appears that, due to relatively rapid rates of diffusion, the carbon dioxide and carbonic acid concentrations of the blood are the primary determinants of the hydrogen ion concentration of muscle (7). Generalization from this supports the impression that central control of respiration is more responsive to blood levels of carbon dioxide and carbonic acid than to the level of hydrogen ions (8). The central presence and localization of carbonic anhydrase (9) and the pathological variations in its concentration (10) suggest a special rôle for the carbon dioxide system in central nervous metabolism. The increase in cerebral blood flow (11) and consequent increase in oxygen tension of cortex (12) brought about by breathing increased concentrations of carbon dioxide and the decrease in flow (11) and oxygen tension (12) induced by hyperventilation are variables pertinent to considerations of central effects of the carbon dioxide system

In the present work, in order to gain information that might further rational consideration of the rôle of carbon dioxide in central nervous metabolism, the cerebral hemispheres of cats were assayed for glucose, lactate, phosphates and pyruvate following changes which were effected in several ways within the carbon dioxide system.

EXPERIMENTAL

Procedures. Cats were employed as the experimental animals. They were paralyzed with dihydro-β-erythroidine, maintained with artificial respiration, and the

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skulls exposed for eventual freezing of the brain. One hour after onset of paralysis the animals were subjected to particular experimental procedures at the end of which the brains were frozen by pouring liquid air on the exposed skulls. Arterial blood was taken in heparin at appropriate times. The electrical activity of heart and brain was recorded during the experiments with a six-channel Grass Electroencephalograph. The EEG leads were taken from screw type electrodes placed bilaterally over the center of the cerebral cortex. Blood gases were measured manometrically with the usual Van Slyke apparatus. The hydrogen ion concentration of blood was measured with the Beckman *p*H meter, model G, using Model 290J electrodes to prevent escape of carbon dioxide. The preparation of the animals and other methods of assay have been reported in detail (13, 14).

Lactate and Pyruvate. The effects of various experimental conditions upon the concentrations of brain lactate and pyruvate are indicated in table 1. The highest level of lactate was found in the animals having the lowest level of blood carbon dioxide (*exper. 1*) and the lowest level in those breathing carbon dioxide plus 20 or more per cent oxygen (*exper. 8–14*). Intermediate levels were found in animals not given carbon dioxide, but having blood carbon dioxide contents higher than in *experiment 1* (*exper. 2–6*). The pyruvate levels in the animals given bicarbonate, carbon dioxide or both were lower than in those given no special treatment (compare *exper. 1–4* with *5–15*).

The effect of breathing carbon dioxide on the concentrations of brain lactate and pyruvate was evident with 5 per cent (*exper. 8*), although not consistently (*exper. 7*), and was somewhat greater with higher concentrations. Administration during two minutes was sufficient to induce the lower levels of lactate and pyruvate (*exper. 10*).

The concentration of brain lactate bears some inverse relation to blood carbon dioxide levels (*exper. 1–5*); however, comparison of the lactate concentrations obtained when bicarbonate was administered (*exper. 5, 6*) with the concentrations found when carbon dioxide was given (*exper. 8–14*) indicates an exception to this apparent relation. The brain lactate level obtained when bicarbonate was administered alone (*exper. 5*) was higher than when carbon dioxide was also given (*exper. 6*). This, and the finding that administration of carbon dioxide alone resulted in the lowest lactate levels, suggests an inverse relation between the hydrogen ion concentration of blood and the concentration of brain lactate. However, the hydrogen ion concentration of blood cannot be the only determinant of brain lactate since the relatively high concentration of hydrogen ions in blood produced by injection of hydrochloric acid (*exper. 18*) was not accompanied by a particularly low level of brain lactate.

The concentration of blood oxygen may be concerned in the determination of the lactate level, since reduction of the oxygen content of the gas breathed to 10 per cent produced an elevation of lactate concentration (compare *exper. 9* and *15*). Breathing 10 per cent oxygen alone presumably would not affect the concentration of brain lactate (14).

Carbon dioxide in relatively high concentration did not prevent the increase in brain lactate expected to accompany a suitable degree of hypoxia (*exper. 16, 17*

TABLE 1. EFFECT OF CO_2 ON BRAIN LACTATE AND PYRUVATE[1]

EXPER. NO.	CONDITIONS	BRAIN		FINAL BLOOD		
		Lactate	Pyruvate	CO_2	pH	O_2 Sat.
1	Room air	2.8 ± 0.2 (2)	0.22 ± 0.09 (2)	30 ± 3 (2)	7.65 ± 0.20 (2)	0.97 ± 0.02 (2)
2	Room air	2.2 ± 0.5 (13)	0.22 ± 0.11 (12)	32 ± 3 (3)		0.98 ± 0.03 (3)
3	Room air	1.4 ± 0.3 (6)	0.20 ± 0.08 (4)	47 ± 4 (4)		0.86 ± 0.07 (4)
4	100% O_2; 10 min.	1.7 ± 0.3 (4)	0.21 ± 0.06 (4)	36 ± 6 (4)	7.33 ± 0.12 (4)	0.99 ± 0.03 (4)
5	I.v. bicarb.; room air	1.4 ± 0.2 (3)	0.12 ± 0.05 (2)	148 ± 50 (3)	7.84 ± 0.17 (3)	0.96 ± 0.03 (3)
6	I.v. bicarb.; 15% CO_2, 85% O_2; 10 min.	1.2 ± 0.1 (2)	0.11 ± 0.01 (2)	158 ± 46 (2)	7.42 ± 0.02 (2)	1.08 ± 0.03 (2)
7	5% CO_2, 95% O_2; 5 min.	1.8	0.19	44	7.07	0.98
8	Same as 7	0.9	0.13	46	7.29	1.00
9	15% CO_2, 85% O_2; 10 min.	0.6	0.12	70		1.00
10	20% CO_2, 80% O_2; 2 min.	0.8	0.07	61	6.99	1.00
11	20% CO_2, 80% O_2; 5 min.	0.7 ± 0.1 (3)	0.07 ± 0.01 (3)	58 ± 5 (3)	6.94 ± 0.03 (3)	1.00
12	20% CO_2, 80% O_2; 10 min.	0.4	0.06	56	6.92	1.00
13	20% CO_2, 80% O_2; 20 min.	0.8	0.06	62	6.95	0.95
14	30% CO_2, 70% O_2; 10 min.	0.6	0.09	60		1.00
15	15% CO_2, 10% O_2, 75% N_2; 10 min.	1.4 ± 0.05 (2)	0.11 ± 0.01 (2)	57 ± 7 (2)		0.37 ± 0.08 (2)
16	15% CO_2, 6% O_2, 79% N_2; 10 min.	9.6	0.26	57		0.25
17	100% CO_2	8.32		106	6.50	0.04
18	I.v. HCl; 7 min.	1.5		32	7.10	0.69
19[2]	15% CO_2, 85% O_2; 10 min.	1.7 ± 0.1 (3)	0.14 ± 0.03 (3)			
20	15% CO_2, 85% O_2; cyanide i.v.	5.6		38		1.02
21	Convulsions; room air	6.2	0.22 ± 0.11			
22	Convulsions; room air	5.0 ± 0.7 (2)	0.17 ± 0.07 (2)			
23	Convulsions 15% CO_2, 85% O_2	2.2 ± 0.6 (5)	0.18 ± 0.09 (3)	60 ± 4 (5)	7.01 ± 0.06 (3)	1.02 ± 0.050 (5)
24	Convulsions 15% CO_2, 10% O_2, 75% N_2	2.5 ± 0.1 (2)	0.10 ± 0.02 (2)			

[1] Where appropriate, 3 figures representing the mean, standard deviation and no. of animals (in parentheses) are given for each assay in a particular experiment. Concentrations of lactate and pyruvate are expressed in mM/kg. of tissue, and CO_2 content in volumes %. O_2 saturation signifies the ratio of the O_2 content of the blood as drawn to that after equilibration with room air. The data for lactate and pyruvate in *exper. 2* and *21* have been reported previously (13). *Exper. 1–3* differ in rates of ventilation used. In *exper. 5* and *6*, 60 ml. of 0.5 M sodium bicarbonate was injected intravenously during the period of time indicated. In *exper. 6* administration of CO_2 was begun immediately after initiation of bicarbonate injection. In *exper. 18*, 80 ml. of 0.05 M HCl was injected intravenously during the period indicated. In *exper. 20*, 0.8 mg. sodium cyanide/kg. body weight was injected immediately after the animal began breathing CO_2. Convulsions were produced by intravenous injection of about 15 mg. metrazol/kg. body weight. Brains frozen at end of 180 seconds of convulsive activity essentially like that recorded in (13).

[2] Carotids ligated immediately after the animals began breathing CO_2 mixture.

and *20*). The concentration of lactate found when the carotids were ligated during administration of carbon dioxide (*exper. 19*) was higher than when carbon dioxide was given alone. Therefore, it appears that cerebral circulation is involved in the effect of carbon dioxide on the concentration of lactate. It should be pointed out here that in the cat cerebral function can be maintained by the vertebrals alone.

The foregoing remarks about lactate apply to brain pyruvate, although in lesser degree.

Summarizing, it appears that the concentrations of brain lactate and pyruvate bear an inverse relation to the levels of blood carbon dioxide, hydrogen ions, oxygen and cerebral blood flow.

TABLE 2. EFFECT OF CO_2 ON BRAIN PHOSPHATES AND GLUCOSE[1]

EXPER. NO.	CONDITIONS	BRAIN					BRAIN/PLASMA GLUCOSE
		IP	PC	ATP	ADP	Glucose	
1	Room air	4.7± 1.8 (14)	2.2± 0.5 (14)	1.3± 0.5 (10)	1.1± 0.7 (10)	4.2± 1.1 (11)	0.36± 0.08 (11)
2	Room air	4.2± 1.3 (7)	2.6± 0.6 (9)	1.5± 0.6 (7)	0.9± 0.5 (7)	3.9± 1.2 (10)	0.38± 0.02 (10)
3	Bicarbonate	3.9± 0.9 (5)	3.0± 0.5 (5)	1.7± 0.2 (5)	0.7± 0.1 (5)	3.8± 0.6 (5)	0.43± 0.07 (5)
4	CO_2	4.3± 0.6 (5)	2.2± 0.1 (7)	1.4± 0.1 (7)	0.9± 0.1 (7)	4.9± 1.9 (10)	0.33± 0.05 (10)
5	Convulsions; room air	5.8	1.7	0.6	2.1	1.9	0.21
6	Convulsions; room air	6.0± 0.7 (2)	0.8± 0.3 (2)	0.9± 0.2 (2)	1.9± 0.6 (2)		
7	Convulsions; 15% CO_2, 85% O_2	3.6± 0.5 (4)	2.1± 0.2 (4)	1.4± 0.2 (4)	0.7± 0.2 (4)	5.5± 2.0 (5)	0.26± 0.07 (5)

[1] Concentrations are expressed in mM/kg. Portions of the data for *exper. 1* and *5* have been reported previously (13). The animals used in *exper. 2* were those of *exper. 1*, *3*, and *4*, table 1; in *exper. 3* were those of *exper. 5* and *6*, table 1; in *exper. 4* were those of *exper. 8–14*, table 1. The animals used in *exper. 7* were those of *exper. 24*, table 1.

Acid-soluble Phosphates and Glucose. The concentration of brain phosphates, glucose and ratio of brain to plasma glucose under various experimental conditions are given in table 2. The results for *experiments 2–4* were derived from data obtained in relatively similar individual experiments that differed in certain details. Comparison between the individual experiments was made but revealed no notable differences.

The data indicate that neither intravenous injection of considerable amounts of bicarbonate nor respiration of relatively high concentrations of carbon dioxide had any appreciable effect on the concentrations in brain of inorganic phosphate, phosphocreatine, adenosine phosphates or glucose.

Convulsions. Data indicating the effect of breathing carbon dioxide on the changes in brain accompanying seizures are given in tables 1 and 2. As shown by comparison of *experiments 2* with *19* and *20*, table 1 and other earlier work (13, 14, 16), convulsions are accompanied by an increase in brain lactate and by decreases

in the concentration of brain high-energy phosphates and glucose. (Compare *exper.* *1* with *5* and *6*, table 2.)

Comparison of *experiments 9* and *23*, table 1, shows that seizures occurring during administration of carbon dioxide were also accompanied by an increase in brain lactate. The percentage increase was about the same as found in air; however, the actual increase was considerably less. Further, the increase in lactate accompanying convulsions in animals breathing carbon dioxide and 10 per cent oxygen was less (compare *exper.* *15* and *22*) than in animals breathing room air.

Comparison of *experiments 4* and *7*, table 2, indicates that seizures occurring during administration of carbon dioxide, in contrast to the results in room air (compare *exper.* *1* and *2* with *5* and *6*), were not accompanied by appreciable change in the concentrations of high-energy phosphates.

Although the concentration of brain glucose in the convulsed animals breathing carbon dioxide was higher than in the controls (compare *exper.* *4* with *7*), the ratio of brain to plasma glucose was lower. Since, in the absence of rapid change in rate of metabolism or rate of supply of glucose, the ratio of brain to plasma glucose is relatively constant (*exper.* *1–4*) (17), this decrease in ratio may represent a decrease in concentration of brain glucose effected by the seizures.

Summarizing, breathing carbon dioxide practically prevents the decrease in high-energy phosphates accompanying seizures, limits the increase in lactate, and has little effect on the decrease in brain to plasma glucose ratios.

DISCUSSION

It is known that breathing carbon dioxide increases the rate of cerebral blood flow and that hyperventilation decreases the rate of flow (11) and the change in cerebral oxygen tension is in the expected direction (12). The rates of blood flow during administration of carbon dioxide, in the control state, and during hyperventilation are in arbitrary units about 4, 2, and 1 respectively (11). The concentrations of brain lactate in what roughly corresponded to those several states were about 0.7, 1.4, and 2.8 mM per 1000 grams respectively (compare *exper.* *8–14*, *3* and *1*, table 1). This nice inverse correspondence between probable rates of blood flow and concentrations of brain lactate, admittedly fortuitous, and the observation that ligation of the carotids limits the fall in lactate level accompanying administration of carbon dioxide suggest a major rôle for blood flow in determining the concentrations of lactate and, to a lesser extent, pyruvate in brain. Thus, it may be proposed that all of the present procedures that resulted in relatively low levels of brain lactate and pyruvate mediated their effect by producing a high rate of blood flow and, conversely, those resulting in high levels (except seizures) did so by effecting a relatively low rate of flow.

Blood flow might act by changing the effective concentrations of these substances in blood, e.g., an increase in rate of blood flow would be comparable to decreases in concentration of the substances in blood. From this, some relation between the concentrations in blood and brain would be expected. However, no consistent relation between blood and brain concentrations was found, and the available evidence indicates that markedly high levels of blood lactate have little, if any, influence on

the concentration in brain (18). The same low levels of brain lactate and pyruvate, induced by administration of carbon dioxide, were found in the presence of two-fold differences in blood levels. Animals breathing air and having high levels of brain lactate and pyruvate had blood levels lower than in animals breathing carbon dioxide and having, consequently, low concentrations of these substances in brain. Thus, the essentially mechanical aspects of blood flow hardly suffice as explanation of its relation to brain lactate and pyruvate.

The oxygen tension of cerebral cortex varies directly with blood flow (11). The finding that decrease in the concentration of oxygen in the carbon dioxide mixture breathed to 10 per cent limited the fall in lactate and pyruvate levels induced by the carbon dioxide suggests that the oxygen tension of brain may inversely affect the brain levels of the substances in question. In the case of lactate such an effect might proceed by varying the concentration of reduced diphosphopyridine nucleotide available for reduction of pyruvate, e.g., high oxygen tension would decrease the concentration of reduced nucleotide and consequently the rate of formation of lactate from pyruvate.

In addition, the level of pyruvate available for reduction might be reduced by increased efficiency of the Krebs cycle at increased oxygen tensions. There is a suggestion of this in the data since pyruvate drops with carbon dioxide and oxygen mixtures. It does not drop with 100 per cent oxygen but there is only a small increase in oxygen tension of the cortex under these conditions compared to carbon dioxide plus oxygen (6).

There is another related rationale for the observed changes in lactate under carbon dioxide inhalation. Brain is reported to have an unusually high rate of aerobic glycolysis (19). It has been shown in rat liver that one in eight carbons of newly formed glycogen comes from bicarbonate (20) and it was proposed that the glycogen was formed from carbon dioxide and phosphopyruvic acid. Moreover, it is known that carbon dioxide can be assimilated (21, 22). Presumably these reactions can also occur in brain since it has been demonstrated that carbon dioxide is incorporated into citrate in brain *in vivo* (23). All of these reactions would have the effect of limiting the amount of pyruvate available to accept hydrogen from the glycolytic mechanisms and might result in lowered lactates.

Craig (24) has reported that increase in hydrogen ion concentration and total carbon dioxide (from 1 per cent to 5 per cent) results in an increase in the rate of aerobic glycolysis in brain slices *in vitro*. Levels of carbon dioxide above 5 per cent had no further effect. The present work is not in obvious agreement with this report, but it should be noted that the level of carbon dioxide in the residual alveolar air is approximately 5 per cent (25). The exact carbon dioxide tension of the cortex in these artificially ventilated animals is not known although the value of 30 volumes per cent in the arterial blood is suggestive of values considerably above 1 per cent. It would appear that in the *in vitro* studies (24) changes were noted at values below normal carbon dioxide levels but not above.

There is little doubt that carbon dioxide has profound effects upon the levels of lactate, pyruvate and phosphates in the brain. The mechanism by which these effects are mediated remains obscure. The most probable explanation is that the

resulting increase in blood flow and oxygen tension enabled the brain to more nearly balance energy demands with oxidative processes and as a consequence less lactate was formed and high energy phosphate reservoirs were not depleted. The effects are especially marked during convulsions where energy demands are high. The other possibilities discussed above may contribute to the overall effect, but it is the authors' feeling that theirs is a minor rôle.

SUMMARY

The brains of normal and convulsing cats subjected to conditions designed to alter the carbon dioxide—bicarbonate—carbonic acid system were analyzed after freezing with liquid air. In normal animals, breathing 10 to 30 per cent carbon dioxide mixtures resulted in a lowering of lactate and pyruvate levels and had little effect on glucose or phosphate concentrations. In convulsed animals these conditions markedly limited the expected rise in lactate, pyruvate and inorganic phosphate and prevented the fall in high-energy phosphates. There was a rise in brain glucose but little effect was found on brain/plasma glucose ratios. The results are discussed with relation to possible mechanisms and the effects of carbon dioxide on the central nervous system.

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THE EFFECT OF BREATHING 5 TO 7% CARBON DIOXIDE ON URINE FLOW AND MINERAL EXCRETION.

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MOST investigators have approached the problem of water and salt metabolism by studying the effect of neural and humoral factors on the kidneys, which can be regarded as the effector and efferent part of the regulating system. Although certain aspects are still controversial, the part played by the glomeruli, tubules, suprarenals and pituitary gland in body water and salt regulation is gradually emerging. Less attention has been paid to the stimuli initiating water or salt retention or loss, the afferent system.

Verney's work (20, 33) has shown the role of changes in osmotic pressure of the plasma in altering water excretion and extracellular ion concentration but does not indicate how total body water and salt content are controlled. The need to postulate the existence of a receptor system sensitive to changes in body fluid volume as well as an osmoreceptor system has been known for some time (23), but the nature of the stimulus to its action remains completely unknown. Reasons have been given elsewhere why this receptor system is not in the kidney (5) and some indirect controlling system must be postulated. Borst (2) was the first to advance a possible explanation of its mode of action. He suggested that the regulation of total body water content took place through a primary regulation of the volume of the vascular compartment. Changes in blood volume altered the cardiac output by altering the filling pressure of the heart in the manner shown by Starling to hold for the isolated heart. The change in cardiac output caused the circulation to be greater or less than the needs of the tissues and this excess or deficiency of circulation was in some way the signal for salt loss or retention. Once salt was retained, water would have to be held with it because of the osmoreceptor, and *vice versa*.

Although Borst's hypothesis lacks certain proofs and may be incorrect in detail there is much to support his view that changes in the circulation play a part in regulating body water content. Oligæmic shock, dehydration, cardiac failure of both high and low output types and a number of other conditions all show similar patterns of water and salt retention and redistribution of the circulation. Elsewhere we have called this the reaction to circulatory inadequacy (5).

There are several possible ways in which changed circulation could affect a receptor system and initiate this reaction. The most likely seems to be alteration in oxygen supply or carbon dioxide removal from a tissue. This would account for the fact that cardiac failure of both high and low output types is associated

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with the same pattern of circulatory inadequacy (8). We have accordingly investigated the effects of breathing various mixtures of oxygen, nitrogen and carbon dioxide on water and ion excretion. The results of breathing 5 to 7% carbon dioxide (CO_2) and of hyperventilation are reported below.

Material and Methods.

To eliminate as far as possible the effects of emotion, most of the investigations reported were carried out on ourselves and certain observations were confirmed by the study of six other members of the medical staff. In addition, one patient suffering from diabetes insipidus was studied.

To distinguish any resulting diuresis from the normal diurnal variations in water and ion excretion (3) all but two observations were carried out in the afternoon after a standard breakfast and lunch. One of us (J.L.) took a standard unvarying diet through the day for several weeks during which the tests were being performed.

The subject was lying down or, in some studies, sitting quietly. Catheter collection of urine was not used because of the possible effects of emotion produced by the procedure, but the efficiency of bladder emptying was investigated (*see* later). Smoking was not allowed during the tests.

Five to 7% CO_2 mixtures in air were made up in 350 l. Douglas bags and breathed through a mouthpiece with a non-return valve. The period of carbon dioxide breathing was invariably preceded by a control period which lasted until a falling urine volume was recorded in 3 successive periods of urine collection.

Chemical methods were those previously reported (6). Clearances of para-amino-hippurate and inulin were performed, using the technique of continuous infusion (14). Urine specific gravity (SG) was determined by the Kagan falling drop technique (18) or by means of a standardised hydrometer.

Freezing point determinations were made as follows:—

Twenty-five ml. of blood were drawn from an antecubital vein without stasis, and 8 ml. of serum removed for measurement of the freezing point. Precautions were taken to ensure that there was no loss of CO_2 or evaporation of water.

Two copper-constantan thermocouples were connected back to back with a Cambridge d'Arsonval galvanometer of 20 ohm internal resistance. About two hours before analyses commenced, one of the thermocouples was placed in a test tube of ice inside a large thermos flask full of ice and left undisturbed until readings had been completed. The other thermocouple was placed in the serum, which was in a siliconed test tube 2·25 cm. in diameter. The serum was then supercooled to exactly −5·5°C. and freezing induced by tapping the side of the tube with a glass rod. (Silicone delays ice formation during supercooling.) The mixture of serum and frozen serum was placed immediately in ice at −4°C. and approximately 2 minutes later a galvanometer reading taken when the temperature in the mixture had reached a plateau. The frozen serum was then melted and the process repeated ten times for each sample.

The instrument was calibrated by taking readings with the thermocouple in a distilled water ice mixture and in solutions of NaCl of known concentration. The galvanometer scale could be read to 0·5 mm., equivalent to 0·0011°C..

Results.

Study of methods.

The efficiency of voluntary bladder emptying. It is assumed that during the control period glomerular filtration rate does not alter. Any fluctuation in creatinine excretion per minute will then be a reflection of the efficiency of bladder emptying. The overall coefficient of variations of creatinine excretion rate for all subjects investigated was ± 14·3%, and for A.B., G.B., and J.L. (the subjects on whom most investigations were carried out) was ± 7·1%, ± 8·5% and ± 5·4% respectively. These low values indicate efficient bladder emptying.

Control studies without breathing CO_2. Three of the subjects who were investigated while breathing CO_2 were also studied at the same time of day and while on the same diet while breathing room air in the recumbent posture. In 5 out of the 14 control studies the subject breathed air from a Douglas bag to simulate the conditions of the experiment and on two of these occasions the subject did not know the nature of the gas in the bag. The result of a typical control experiment is shown in Fig. 1.

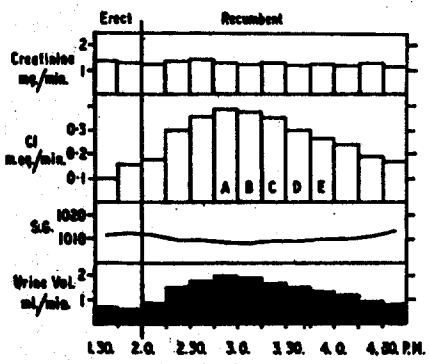

Fig. 1.

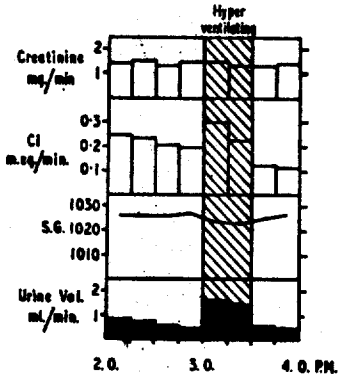

Fig. 2.

Fig. 1. Control study. The effect of lying down on the excretion of water, creatinine, chloride and on the urine specific gravity.
(For comment, *see* text.)

Fig. 2. The effect of voluntary hyperventilation on the excretion of water, creatinine and chloride and on the urine specific gravity.
(For comment, *see* text.)

The rise in urine volume and electrolyte excretion seen in the first four periods after lying down occurred almost invariably in both the control studies and in the periods in which CO_2 was breathed, but CO_2 was never administered in the experimental studies until the urine flow was falling again and had fallen steadily over three periods of urine collection. Subsequent increases in urine flow occurred only three times in the control studies and were never marked (*see* Fig. 4B). Data on the variability of excretion of urinary solutes in the control studies are shown in Tables III, IV and V.

Test of the freezing point method. To test the method, blood from 4 persons was taken before and 1 hour after drinking 1 litre of water.

Hæmodilution of 5·9%, 3·8%, 2% and 1·95% occurred in 4 subjects and the changes were highly significant when analysed by Student's " t " test. ($p = 0{\cdot}001$ in the first 3, and 0·01 in the fourth subject.)

The effect of voluntary hyperventilation. Four of the subjects were investigated under the same conditions as in the other experiments while overbreathing voluntarily to the point of producing and maintaining mild tetanic manifestations. The results of a typical experiment are shown in Fig. 2 and further data are shown in Table I and Fig. 4D.

The diuresis was associated with little change in urine specific gravity and a rise in the excretion of Cl..

The results of breathing 5 to 7% carbon dioxide.

Fig. 3 shows the design of an individual experiment. The period of breathing carbon dioxide mixtures varied in different experiments from 10 to 210 minutes, but was usually 30 to 60 minutes.

TABLE I.

The effect of voluntary hyperventilation on the excretion of creatinine and chloride.
(5 tests on 4 subjects.)

	Percentage excretion of mean control		
	Control; mean and S.E. of mean	Hyperventilating; mean and S.E. of mean	P
Creatinine	100 ± 1·76 (20 observations)	103 ± 3·05 (20 observations)	0·4
Chloride	100 ± 1·8 (20 observations)	130 ± 8·2 (20 observations)	0·001

Following the breathing of CO_2 there was a marked rise in urine flow but no significant change in the excretion of most other substances studied. A more detailed analysis on all subjects is shown below.

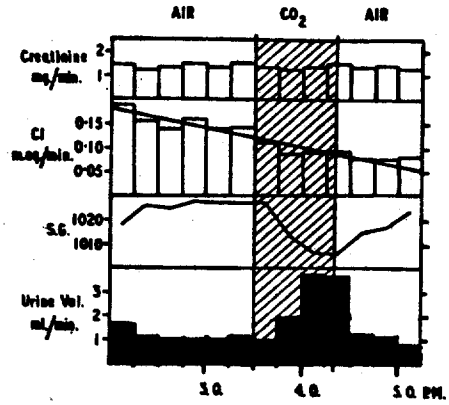

Fig. 3. The effect of breathing 5 to 7% CO_2 on the urine flow, creatinine and chloride excretion and on the urine specific gravity in a recumbent subject.
(For comment, *see* text.)

The effect of CO_2 on water excretion. On all occasions in which the subjects were studied while lying down, CO_2 caused a rise in urine flow. Fig. 4A shows the rate of urine flow at the height of the diuresis related to the flow immediately before the diuresis. The results of the control studies are plotted on the same scale. To express the results of the control studies in a comparable way (Fig. 4B), we assumed that once three periods of urine collection showed a progressive fall in urine flow (*e.g.*, periods A, B, C in Fig. 1) carbon dioxide would have been breathed in an experimental study. The urine flow in period D was therefore taken as the baseline and the urine flow in period E compared with it.

It will be seen that on each occasion on breathing CO_2 in the recumbent posture the urine flow rose to about threefold, while in the control studies there was usually a fall. Fig. 4C shows the relation of urine flow before breathing and after breathing CO_2 in the sitting position. Note the irregular and slight increase in urine flow in contrast to Fig. 4A.

The diuresis did not commence immediately the subject breathed CO_2 but was delayed consistently for a period of between 14 and 27 minutes.

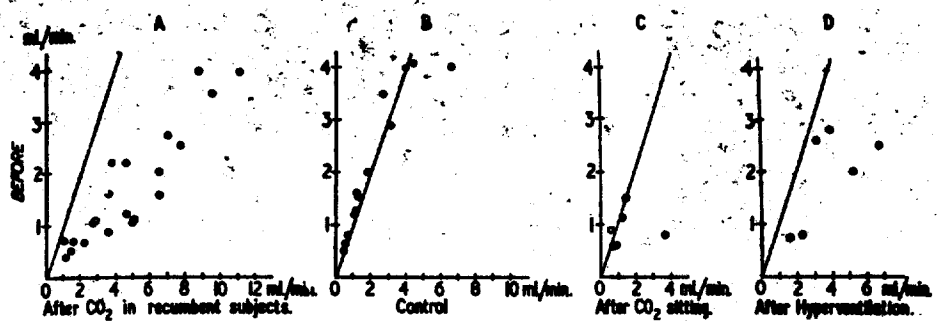

Fig. 4. The urine flow immediately before and during various procedures.
(*See* p. 4 for method of plotting.)

A. 5–7% CO_2 in the recumbent subjects.
B. Control studies (*see* p. 4 for method of plotting.)
C. 5–7% CO_2 in sitting subjects.
D. Voluntary hyperventilation in recumbent subjects.

The duration and extent of the diuresis induced by CO_2. On three occasions in 2 subjects examined in the recumbent posture the period of breathing CO_2 was prolonged to 3½ hours and during this time the urine flow reached a peak and then fell to approximately the basal level (*see* Fig. 5).

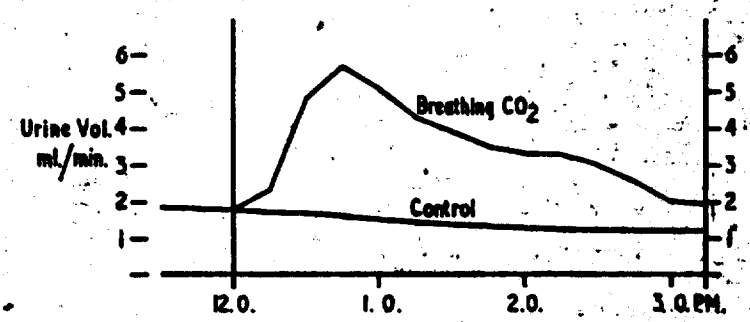

Fig. 5. The mean urine flow during a prolonged period of breathing 5–7% CO_2 in 3 subjects, compared with the mean control value.
(For comment, *see* text.)

The average amount of urine passed exceeded the expected figure over the whole period by 411 ml.. The expected figure was determined from the mean of the control studies on the same subjects.

The effect of breathing carbon dioxide on urine specific gravity. The increase in urine flow that follows breathing CO_2 was invariably accompanied by a fall in urine specific gravity (SG). The mean SG before breathing CO_2 was 1·018 ± 0·0016 and at the height of the diuresis was 1·006 ± 0·0006. In all but one of 14 control studies the SG rose by between 0·001 and 0·004 units. In one control study there was a fall in SG of 0·002 units.

In six of the studies the concentration of total mols in the urine was measured by the freezing point method. The results are shown in Table II and confirm the fall in specific gravity.

It will be seen that during a diuresis induced by CO_2 the molar concentration in urine may fall below that in plasma.

TABLE II.

Total m.mols per litre of urine before and at the height of diuresis induced by breathing CO_2 in the recumbent posture.

Immediately before commencement of diuresis m.mols/l.	At height of diuresis m.mols/l.
380	120
460	170
400	120
670	410
1000	200
520	160

The effect of CO_2 on the excretion of urinary solutes. To determine whether there was an alteration in the excretion of urinary solutes, the data from individual studies were plotted as in Fig. 3 and a best line drawn through the points in the control period by the method of least squares and extrapolated to the period of CO_2 breathing. Each individual reading was then expressed as a percentage of the reading derived from the line. The scatter of the control readings about the ideal line gave a measure of the variability in excretion in the control period, and the deviation and scatter of the readings in the experimental period could then be compared with the control period.

Deviations from the expected excretions expressed as percentages, and statistical data calculated from these figures, are presented in Table III.

The excretion of certain solutes was also checked by an independent method. One subject was on a constant diet under standard conditions of posture and was investigated at the same time of day on each of 7 days. Urine was collected at 20-minute intervals on each of 5 control and 2 experimental days. The excretion of solute per minute was estimated in each of these samples and the figure recorded in a table (Table IV shows one such table). Individual figures were then converted to a percentage (the figures in brackets in Table IV) of the mean output for the time of day over all the control days. Thus by expressing each individual reading as a percentage of the mean reading for the time of day at which it was collected the effect of the falling excretion of solute during the afternoon was eliminated and readings taken at different times of day could be compared with one another.

The samples of the experimental days were expressed as a percentage of the means of the control days. The significance of the difference between the means of the control (100) and the experimental results was then calculated in the usual way.

Table IV shows the results of this type of analysis in the case of chloride excretion, and Table V shows the results in the case of excretion of total mols and sodium.

It will be seen that there is no significant alteration in the excretion of creatinine, sodium, chloride or potassium as a result of breathing CO_2 but there was a significant rise in the excretion of total mols and urea. In two studies made to determine the point, increased urea excretion accounted for most of the increase in total mols excreted.

TABLE III.

The effect of CO_2 on the excretion of various substances.

Substance	No. subjects studied	Excretion % of expected value; control period Mean/S.E. of mean	No. observations	Excretion % of expected value during diuresis Mean/S.E. of mean	No. observations	Difference %	P	Difference detectable at P = 0·05 %
Creatinine	12	100 ± 1·76	66	98·6 ± 2·21	45	1·4	0·7>P>0·6	5·9
Na	4	100 ± 1·09	40	104 ± 6·3	31	4·0	0·9>P>0·8	50
Cl	8	100 ± 1·34	56	98·9 ± 2·54	36	1·1	0·7>P>0·6	5·4
K	3	100 ± 2·0	14	107·5 ± 3·62	12	7·5	0·1>P>0·05	8·6
Total mols	5	100 ± 2·16	29	134·2 ± 6·77	25	34·2	0·001>P	14·8
Urea	2	100 ± 2·77	17	119·9 ± 6·36	23	18·9	0·02>P>0·01	14·3
		ml./min.		ml./min.				ml./min.
Inulin clearance	4	133·2 ± 6·9	16	119 ± 6·64	9	14·2	0·2>P>0·1	20·8
PAH clearance	4	464·9 ± 25·0	15	438·8 ± 20·0	10	26·1	0·5>P>0·4	71

For method of analysis of data, *see* text.

The effect of CO_2 on glomerular filtration and renal blood flow. Over short periods, changes of creatinine excretion are believed to reflect changes in glomerular filtration rate (GFR). From Table III it will be seen that there was no significant difference in creatinine excretion between the control period and the period following breathing of CO_2. A difference of as little as 5·7% in GFR would have been detected at the 0·05 level of probability. This conclusion was also checked by inulin clearances

TABLE IV.

Chloride excretion. Comparison of control days breathing room air and days breathing CO_2.

Time p.m.	Control day 1	2	3	4	5	CO_2 1	2	Mean control days
12.0–12.20	390	350	320	380	340	290	370	368
	(106)	(95)	(97)	(103)	(92)	(79)	(100)	(100)
12.20–12.40	310	310	230	350	330	290	340	306
	(101)	(101)	(75)	(115)	(108)	(95)	(111)	(100)
12.40–1.0	280	265	280	320	320	210	195	292
	(96)	(91)	(96)	(110)	(110)	(72)	(67)	(100)
1.0–1.20	180	230	175	340	250	220	185	235
	(77)	(98)	(75)	(145)	(107)	(94)	(79)	(100)
1.20–1.40	175	190	170	190		190	145	181
	(97)	(105)	(94)	(105)		(105)	(80)	(100)
1.40–2.0	160	190				220	130	175
	(91)	(109)				(126)	(74)	(100)

Grand mean and S.E. of all readings on control days = 100 ± 2·72.

Grand mean and S.E. of all readings on CO_2 days = 90 ± 4·96.

Difference 10% P = 0·1.

TABLE V.

Excretion of Na and total mols, comparing control days with days on which CO_2 was breathed.

Substance	Excretion % Control days Mean S.E. of mean	CO_2 days	P
Na	100 ± 3·26	94 ± 9·8	0·55
Total mols	100 ± 3·48	159 ± 13·7	0·001

Method of analysis as in Table IV.

in 4 subjects and no significant alteration in GFR was detected (Table III). Clearances of para-amino-hippurate were also determined in 4 subjects and there was also no significant alteration in this function (Table III).

Fig. 6 summarises these findings.

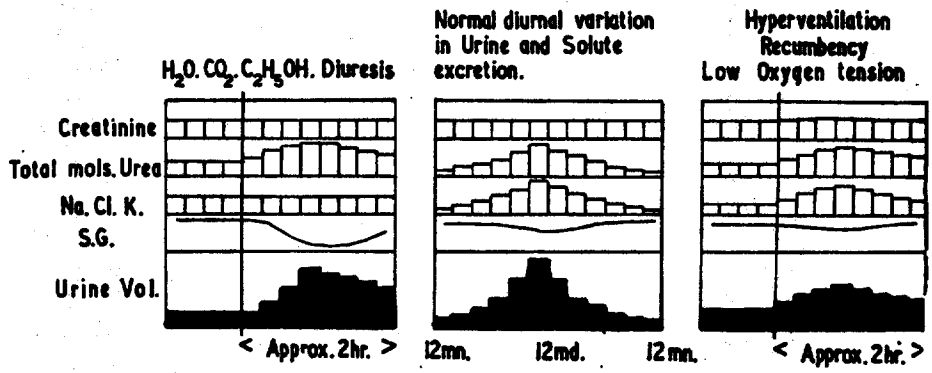

Fig. 6. A diagrammatic comparison of different types of diuresis.

The inhibition of the CO_2 diuresis by means of pitressin.

Two subjects, A.B. and G.B., each drank over a litre of water and then when a diuresis was established were given an injection of pitressin, 1 and 2 units respectively. Once the expected fall in urine flow had occurred they then breathed a carbon dioxide mixture as before. In neither subject did this evoke any diuretic response or fall in urine specific gravity.

One patient, M.E., suffering from diabetes insipidus, was also investigated while he was under the control of his usual dose of pitressin snuff. He, too, showed no increase in urine flow or fall in urine specific gravity.

Freezing point depression of plasma.

The diuresis induced by CO_2 has all the characters of that following the drinking of water (*see* this page). It was, therefore, necessary to show whether CO_2 produced a hæmodilution and inhibited pitressin secretion through the osmoreceptor in the usual way. This is theoretically possible for the following reason. Proteins are believed to contain between 5 and 15% of bound water and the amount of water bound diminishes as the isoelectric point of the protein is approached. CO_2 would be expected to cause a fall in blood pH and so could theoretically release protein bound water and cause a fall in plasma osmolarity. This fall in osmolarity could not be detected by means of determination of plasma specific gravity or chemical determination of any of the components of plasma because these would not be altered. It could only be determined by freezing point, vapour pressure or osmotic pressure estimation. The first method was chosen and samples of plasma before and after breathing CO_2 for half an hour studied. In one subject (J.L.) the plasma was taken after breathing CO_2 for 3½ hours. The results are shown in Table VI.

It will be seen that there was no consistent significant change in plasma osmolarity despite the fact that a change of as little as 0·6% would have been detected at the 0·05 probability level. In Verney's experiments a change of osmolarity of 1·8% was required to affect urine flow (33). The specimen of blood drawn after breathing CO_2 for 3½ hours (Subject J.L.) showed a significant concentration. At the time it was taken the urine flow was still greater than the control value, indicating that

the CO_2 diuresis can take place despite hæmoconcentration and probably actually produced hæmoconcentration.

TABLE VI.

The effect of breathing CO_2 on plasma molarity.

Subject	% change in molarity	% change detectable at 95% confidence level	P
L.B.	− 0·8	1·34	0·25
A.B.	+ 0·8	1·62	0·31
N.J.	+ 0·41	1·05	0·42
T.R.	+ 0·93	0·62	0·008
J.R.	− 0·57	0·50	0·03
B.E.	+ 0·33	0·73	0·3
L.D.	− 0·74	0·49	0·008
J.L.	+ 1·1	0·52	<0·001
Mean	+ 0·184		

Standard error of the mean 0·289%.

Discussion.

The literature on water and salt excretion is somewhat confused by different authors having different interpretations of the same terms. In the following discussion we use the definitions below:—

"Diuresis": an increase in urine flow above that found in a control period.

"Water diuresis": a diuresis not accompanied by an increase in urine electrolyte excretion in unit time.

"Hypotonic," "isotonic" or "hypertonic water and salt diureses": diureses associated with absolute increases in electrolyte output per unit of time. In isotonic diuresis the concentration of electrolytes in urine is the same as in plasma. Osmotic diuresis is the diuresis which results from an increased molar load filtered.

A diuresis following the breathing of 5% carbon dioxide was reported by Davies, Haldane and Kennaway (9). This diuresis has also been noted by Ullmann (32) and by Prime (24). Hartmann, Orskov and Rein (15) and Springorum (28) found that 10% CO_2 caused an antidiuresis, but their findings are not strictly comparable in that they were investigating anæsthetised animals and using higher concentrations of CO_2 than we did.

The diuresis following the breathing of 5–7% CO_2 is not the result of an increase in glomerular filtration rate, for we were unable to demonstrate any significant alteration in the output of creatinine or in the clearance of inulin under its influence (p. 7) and Hood (17) actually found a reduction in inulin clearance when patients breathed 6% CO_2.*

* A reduction in GFR and RPF occurs in emotional disturbances and Hood's findings are probably due to the fact that he investigated patients unused to the procedures involved.

The increased urine flow must therefore be due to a decreased tubular reabsorption of water. It does not have the characteristics of an osmotic diuresis for we did not show any significant increase in the load of solutes (plasma solute concentration and filtration rate being unchanged). Furthermore, at the height of the diuresis we sometimes found the concentration of total mols in the urine to be below that in the plasma (p. 6). The increased excretion per minute of total mols accompanying the diuresis (p. 7) was almost entirely due to urea and was never as high as that in a comparable osmotic diuresis. Increased urea excretion accompanies any diuresis and is probably due to diminished back diffusion of urea resulting from the lowered concentration of urea in the tubules and the increased speed of urine flow.

The diuresis following the breathing of CO_2 was not accompanied by an increased excretion of sodium, chloride or potassium (pp. 7 & 8) and this serves to distinguish it from the normal increase in urine flow that occurs in the middle of the day (1), from the diuresis that occurs on lying down (1,22) and from the diuresis that accompanies hyperventilation (29, 31 and p. 3) or the breathing of mixtures of lower than normal oxygen tension (31). The carbon dioxide diuresis is a water diuresis and has, in fact, all the characteristics of that which occurs after the ingestion of water.

The increased urine flow is almost certainly not due to insensitivity of the kidney to pitressin, for it was completely prevented by 1 unit of pitressin and did not occur in a patient suffering from diabetes insipidus (p. 9).

The diuresis following water ingestion or injection is probably due to inhibition of pitressin secretion as Verney has shown and it is likely that carbon dioxide has the same effect. In the case of " water " diuresis the increase in urine flow does not occur until some time after hæmodilution has occurred and this delay is believed to be due to the time taken for destruction of the pitressin already circulating (20). The same delay is seen in onset of the carbon dioxide diuresis (p. 4). This makes it unlikely that CO_2 increases the rate of pitressin inactivation.

In the case of " water " diuresis, hæmodilution is believed to inhibit pitressin release through its action on the supraopticohypophyseal system (SOHS) but this cannot be the cause of the postulated diminution of pitressin release on breathing CO_2 for we found no significant hæmodilution and indeed on two occasions the diuresis persisted despite significant hæmoconcentration (p. 10). A similar state of affairs probably follows the ingestion of alcohol (13).

The CO_2 diuresis is therefore probably an example of an effect produced on the SOHS by a stimulus which is not osmotic. The following are other examples of a " water " type of diuresis probably not due to hæmodilution:—

(1) Hinkle *et al.* (16) report that a " water " type of diuresis occurs in man under emotional stress and reviews previous reports of this phenomenon. Emotion almost certainly played no part in our studies, for the subjects were trained and under no emotional stress.

(2) Sieker, Gauer and Henry (27) report that a " water " type of diuresis occurs on negative pressure breathing. It is certain that our findings are not due to breathing against an appreciable negative pressure for the non-return valve on the Douglas bag had a low resistance and furthermore diuresis did not occur in control studies when air was breathed from the same bag. It is of course possible that the same mechanism may be involved in the diuresis, for breathing of CO_2 causes hyperventilation and consequent changes in intrathoracic pressure.

There are also examples of antidiuresis probably due to non-osmotic stimuli causing increased release of pitressin. A number of drugs including morphine (10) nicotine (7) and barbiturates (11) are known to cause antidiuretic hormone release.

Physical stimuli can also have these effects. Brun, Knudsen and Raaschou (4) have shown that pitressin is released in increased amounts during the vaso-vagal faint. Rydin and Verney (25) showed that a water diuresis in dogs could be inhibited by stimulation of afferent nerves, by loud noises, by exercise or by pain. Exercise and pain have the same effect in man (34). Drury, Henry and Goodman (12) report an antidiuresis that is probably due to antidiuretic hormone release on positive pressure breathing.

The common factor in all these probably non-osmotic effects on the SOHS is not easy to find. The fact that the carbon dioxide diuresis is less marked or absent in the sitting subject (p. 5) is of interest. Strauss, Davis, Rosenbaum and Rossmeisl (30) showed that isotonic expansion of extracellular fluid volume caused water and salt diuresis in the recumbent subject but not in the sitting subject and Lewis *et al.* (21) report alterations in chloride excretion when a venous occluding cuff was applied to the neck of sitting subjects. Using a slightly different procedure involving control studies not used by Lewis *et al.* we have been unable to confirm their findings. Both groups have suggested that this difference between sitting and lying might indicate that the receptor responsible for the homeostasis of the compartments of body water may lie in the "cephalad" part of the body. It is equally possible that it may lie in the heart or lungs or even below the diaphragm, for it is impossible to produce an isolated effect on the circulation anywhere in the body without producing secondary changes elsewhere.

Sieker, Gauer and Henry suggest that there may be receptors in the thorax which are sensitive to changes in pressure or volume of blood in the intrathoracic veins and that stimuli from these affect hormone release. The facts would appear to be consistent with this hypothesis and the effect of breathing CO_2 lends some support to their suggestion. On the other hand it is equally possible or indeed more likely that some indirect effect is occurring secondary to the changed intrathoracic pressure. If a rise in intrathoracic blood volume or in pressure in the intrathoracic venous system alone initiated the responses we would expect a water diuresis in congestive cardiac failure. These changes in intrathoracic pressure or volume must of necessity be associated with alterations in circulation and blood content in other parts of the body and the afferent side of the receptor system might be elsewhere. For example it could be postulated that it is in the head and that it is sensitive to changes in cerebral blood flow, for an alteration in cerebral blood flow is known to occur in many and may occur in all the circumstances above (19, 26).

Even if changes in intrathoracic pressures or volumes or some other change consequent on these do cause changes in urine flow, it is unlikely that this mechanism can play any important role in the water retention of œdematous states, as the diuresis of both negative pressure breathing and CO_2 and the antidiuresis of positive pressure breathing are not associated with any significant change in sodium metabolism. The elucidation of the reason for the differences between CO_2 diuresis which is not associated with increased sodium excretion and the diuresis of anoxia or hyperventilation which are accompanied by increased sodium excretion would be a considerable advance.

SUMMARY.

Breathing 5 to 7% carbon dioxide (CO_2) causes a marked increase in urine flow in the recumbent normal subject but no regular change in the sitting subject.

During the diuresis induced by CO_2, urine specific gravity falls and there is a slight rise in the excretion of total mols per minute. Urea contributes most of this increase and there is no significant change in the excretion per minute of creatinine, sodium, chloride or potassium. The glomerular filtration rate and renal plasma flow are not altered.

The diuresis ordinarily induced by CO_2 can be inhibited by pitressin.

The freezing point of plasma is not significantly altered by breathing CO_2. Thus hæmodilution is not responsible for the diuresis. Indeed the diuresis may persist despite hæmoconcentration.

The probable mechanism of the CO_2 diuresis is an inhibition of pitressin release.

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A COMPARISON OF THE ACUTE AND CHRONIC TOXICITY OF CARBON DIOXIDE WITH ESPECIAL REFERENCE TO ITS NARCOTIC ACTION[1]

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It is well established that CO_2 exerts a narcotic action on certain functions of the central nervous system when this gas is inhaled in moderately high concentrations (1, 2, 3, 4). This effect may be due to the increase in hydrogen ion concentration resulting from the uncompensated respiratory acidosis and the condition is somewhat analogous to the cerebral depression and coma which commonly occur coincidentally with an accumulation of acid metabolites. The available evidence indicates that this narcotic action is associated with, and probably results from, a reduction in cellular oxidations, a concept which would receive theoretical support from the known inhibition of many oxidative enzymes with increase in hydrogen ion concentration. Since the literature regarding the narcotic action of CO_2 is scanty and the conclusions vague, the problem is deemed worthy of further investigation.

METHODS. In most experiments the albino rat served as the experimental animal although a few rabbits and dogs were used. The acute and chronic toxicity was determined by placing animals in a closed circuit system of 1100 liters capacity, consisting of a 600-liter animal chamber, a 100-liter spirometer, and two tanks of 200 liters each. The atmosphere in this system was circulated by a small blower; the concentration of O_2 was maintained at approximately 21%. In chronic experiments the concentrations of CO_2 and O_2 varied from the stated levels as much as 2 to 3% during any 24-hour period.

The oxygen consumption of the rat was determined in an airtight 6-liter glass chamber suspended in a water bath maintained at 28°C. The brass cover contained, in addition to openings for a thermometer, a mercury manometer and a sampling outlet, two large stopcocks through which gas mixtures from the large system could be circulated continuously. Access to a small wire animal cage suspended from the cover was obtained through a 3-inch opening with an airtight rubber seal. Gas entering the small chamber from the large system was saturated with water vapor at 28°C. as it passed through a large brass coil and shower type humifider, both of which were suspended in the bath. Circulation within this chamber, when it was isolated from the large circuit, was obtained with a small electric fan placed entirely within the chamber. In making the determination, the rat, fasted 18 hours, was placed in the chamber one hour before the run. The gas mixture containing the desired amount of CO_2 and O_2 was then circulated through the small chamber until a uniform atmosphere was attained (15 minutes), a gas sample was taken, and the ports closed. One-half hour later another gas sample was taken, the ports opened, and the gas from the large system allowed to circulate for at least 15 minutes before the process was repeated.

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Both the gas samples were analyzed in the Van Slyke manometric apparatus, and calculations were made of the quantities (S.T.P.) of CO_2 produced and O_2 consumed.

The effect of exposure to 11% CO_2 on the acid-base balance of the blood was determined on arterial blood samples collected from rats anesthetized with 45 mgm. per kgm. of sodium pentobarbital. One hour after injection of the drug, and after exposure to the desired atmosphere for the stated intervals, the blood samples were obtained by aortic puncture after laparotomy. The animals remained in the same atmosphere during the sampling procedure. The blood samples were analyzed by the micro method of Shock and Hastings (5) and the pH corrected for rats' blood at 38°C. Control determinations in room air were made by the same general procedures.

The rate of disappearance of bromsulfalein from the blood of rabbits was used as a criterion of the effect on liver function of acute and chronic exposure to CO_2. The method used was the modification reported by Tatum, Nelson and Kozelka (6). In the acute experiments the rabbits were exposed to 10–15% CO_2 for 2 hours before the test, while in the chronic experiments the rabbits had been in 15% CO_2 for one week. The animals were maintained in the CO_2 atmosphere during the test period.

RESULTS. *A. Toxicity of Carbon Dioxide. 1. Following sudden exposure.* The acute maximal tolerated concentration of CO_2 for our albino rats lay between 10 and 20%. All animals survived 10% CO_2 indefinitely, an occasional one succumbed in 15%, but 80% of rats placed in 20% CO_2 died within 4 days. None survived in 25% CO_2 for more than 36 hours, whereas 50% CO_2 was uniformly lethal within 6 hours. In all cases the oxygen concentration was maintained at 21%.

The rats were narcotized immediately when placed in concentrations of CO_2 above 30 per cent. A detectable grade of depression occurred with concentrations as low as 20% although the animals were not deeply narcotized. Death from carbon dioxide was always associated with evidence of pulmonary injury. The lungs were found to be edematous and filled with a sanguineous exudate when examined at autopsy. In the higher concentrations similar changes also occurred in the exposed mucous membranes. In the rabbit, transient convulsions were produced by concentrations of 25% CO_2 and above, followed by a marked depression.

2. During prolonged exposure. Ten rats were placed in the large animal chamber, supplied with food and water, and the concentration of CO_2 raised immediately to 10% where it was maintained for 30 days. The oxygen concentration was maintained between 19 and 21%. The usual respiratory response to excess CO_2 was the only objective sign observed, since the rats appeared normally active, and young born under these conditions were apparently normal. However, a considerable weight loss occurred, ranging from 14 to 27%. This loss of weight is believed to be due primarily to a reduction in food intake since, as will be discussed later, animals kept at higher concentrations ate sparingly or refused food entirely. Although no studies were made to determine the possible rôle of dehydration, it is perhaps significant that diuresis occurred during acute exposure to CO_2. In view of the good general condition of the animals when they were removed, it is believed that this experiment could have been much prolonged without death of the animals.

Blood studies were made on five of these rats at weekly intervals by Miss

Ethel Thewlis and Dr. O. O. Meyer, of the Department of Medicine, who found a marked reticulocytosis as the only significant change. These results on the rat differ from those obtained on the rabbit and dog by other investigators (7, 8, 9) since we observed no significant change in hemoglobin levels or in the number of leukocytes or erythrocytes.

In another experiment the CO_2 was allowed to accumulate gradually over a 5-day period until a concentration of 20% was reached. The rats were maintained at this level for 6 days and the concentration was then allowed to increase to 25% during the next 3 days. One animal died after 4 days at this level. At this time the concentration was reduced to 20% since the survivors were greatly depressed and a serosanguineous exudate appeared on the exposed mucous membranes. A similar situation occurred when another attempt was made to maintain the concentration at 25%. In this instance, the concentration was reduced to 23% and maintained for 10 days longer—a total of 29 days survival in concentrations of CO_2 varying between 20 and 25% (oxygen, 19 to 21%). The rats ate sparingly at CO_2 concentrations above 10% and food was refused above 23%. During the 34-day experiment, 50% of the original body weight was lost. It is doubtful if this experiment could have been continued much longer with survival of the animals.

When this group of animals was removed to room atmosphere they became very irritable and developed moderate tetany with episodes of mild clonic convulsions. This state lasted for 12 hours but no permanent effects were observed. The animals gained weight rapidly and had recovered completely within a few days. The action of these animals is in direct contrast to that observed in rats removed after 5 hours of acute exposure to 25% CO_2. The latter were much more depressed during exposure and no convulsive manifestations were evident on removal; in fact, the depression produced by a given concentration of CO_2 was always greater upon sudden exposure than during prolonged exposure.

The toxicity of CO_2 is approximately the same for certain other laboratory animals as it is for the rat. Rabbits were depressed very significantly by a concentration of 27% CO_2 built up slowly over a period of 72 hours. A sanguineous exudate appeared on exposed mucous membranes but no convulsions were noted on removal. Dogs were partially narcotized by a concentration of 23% CO_2, even when this level was reached by slow accumulation over a 5-day period. No convulsions occurred when the dogs were removed to room air.

B. Effect of Carbon Dioxide on Oxygen Consumption. 1. Following sudden exposure. The oxygen consumption of rats determined at various intervals after exposure to 11% CO_2 is shown in table 1. Numerous control runs in air and in an atmosphere of 11% CO_2 and 21% O_2 indicate that the method is accurate to $\pm$ 3%. The values for the oxygen consumption obtained in these runs checked well with data in the literature obtained by other methods (10), although the results were slightly higher since the rats were kept at 23° to 25°C.

The oxygen consumption diminished immediately after exposure to 11% CO_2 and continued to decrease during the first several hours, reaching a minimum in between two and one-half and five hours. In some animals the oxygen consump-

tion was reduced to 75% of the control value at this time, and with continued exposure a gradual increase in oxygen consumption toward the control level occurred, until at 24 hours the latter was usually regained and in some instances exceeded. In one experiment in which a rat was exposed to a higher level of CO_2 (20%), there was a much greater decrease in the oxygen consumption during the first few hours, and on continued exposure for 48 hours the oxygen consumption did not regain the basal level but remained 15 to 20% below it.

2. During prolonged exposure. The oxygen consumption of rats determined at several concentrations of CO_2 during prolonged exposure is shown in figure 1. The CO_2 content of the atmosphere was allowed to increase at a constant rate of 1.5 to 2% per 24 hours and was maintained at each concentration 24 to 48 hours before the determination of oxygen consumption was made. No change in oxygen consumption was observed until the concentration of CO_2 was greater than 10%. It was reduced to approximately 80% of normal by 15% CO_2 and to

TABLE 1

Oxygen consumption following sudden exposure to CO_2 at 28°C.*

RAT	CONTROL	½ hr.	1½ hr.	2½ hr.	3½ hr.	4½ hr.	15 hrs.	24–72 hrs.	MAXIMUM REDUCTION
									%
1	81	73	68	70		59	73	80	27
2	76					67		84	12
3	74	75	67	63	67	71	82		15
4	88	82	75	71	67	67		73	24
5	92	84	79	73	79	84		106	21
6	75	64	60		57	63	60		24

Rats nos. 1–5 in 11% CO_2.

Rat no. 6 in 20% CO_2.

* Values expressed as cubic centimeters of oxygen consumed per 100 square centimeters body surface per hour.

Surface area calculated by Lee's formula: $s = 12.44 \times (w)^{2/3}$

71% of normal by 20% CO_2. During the 19 days of this experiment a 25 to 30% weight loss occurred. Since no significant loss in weight or reduction in oxygen consumption was noted until the concentration of CO_2 exceeded 10%, it is possible that the reduction in oxygen consumption which occurs during exposure to 15 and 20% CO_2 may be related in part to the diminished food intake.

C. Acid-base Balance of Blood Resulting from Acute and Chronic Exposure to Carbon Dioxide. The nature of the shift in the acid-base equilibrium resulting from both sudden and prolonged exposure of rats to CO_2 is shown in table 2. Since pentobarbital depresses respiratory activity, a respiratory acidosis characterizes all of the control determinations made in room air. These abnormal control levels were considered adequate for comparative purposes since we were interested primarily in the degree to which the rat could compensate for the acidosis produced when exposed continuously to 11% CO_2 for a

period of 2 weeks. It will be noted that the plasma pH of rats which have been exposed to 11% CO_2 and 21% O_2 for 17 days is in the same range as that found after one-half hour exposure even though the total CO_2 content is greatly increased. These results indicate that acclimatization, in so far as it occurs, is not due to a reëstablishment of a normal level of pH.

D. Effects of Various Concentrations of Carbon Dioxide on the Duration of Action and Toxicity of Pentobarbital. In the determinations of the acid-base balance of the blood during exposure to CO_2, it was noted that high concentrations of CO_2 in the atmosphere prolonged the action of pentobarbital. This action of CO_2 was therefore investigated in the following experiments.

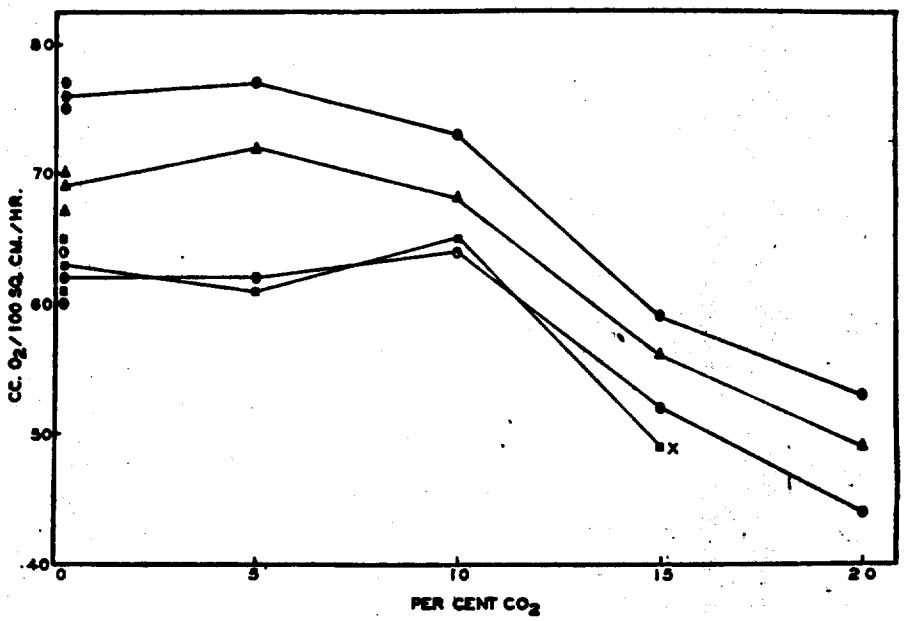

FIG. 1. OXYGEN CONSUMPTION DURING PROLONGED EXPOSURE TO CO_2 AT 25°C. Surface areas calculated by Lee's formula: $s = 12.44 \times (w)^{2/3}$. *X*—Died at 19% CO_2.

1. Following acute exposure to CO_2. Eighty rats were given 40 mgm. per kgm. of pentobarbital sodium intraperitoneally and divided into four groups of twenty animals each. One group served as a control and the other three groups were placed immediately after injection of the drug into atmospheres containing 5, 10, and 20% CO_2, respectively. The animals were considered to have recovered when they could assume and retain the upright position. The data are expressed in figure 2 as number of animals recovered plotted against time. The few deaths that occurred were in animals with preëxistent respiratory infections. Since no deaths occurred in animals without respiratory infections, the rats that survived may be treated statistically as homogeneous groups. Whereas 5% CO_2 had no significant effect on recovery time ("t" be-

tween 0 and 5% = 0.529), a definite delay occurred with 10% CO_2 ("t" between 5 and 10% = 3.83) and a marked prolongation was noted with 20% CO_2 ("t" between 10 and 20% = 4.5). Two other series of rats, not shown in figure 2, gave results which were qualitatively similar although the exact time relationships were not identical.

TABLE 2

Acid-base balance of blood of rats exposed to 11% CO_2*

TIME IN CO_2	PER CENT CO_2	V_c	pH	VOLUME PER CENT CO_2
0	0	30	7.36	84.0
		42	7.21	82.8
		42	7.26	78.4
Average		38	7.28	81.7
½ hr.	12	38	7.09	91.4
		43	7.09	91.4
		38	7.08	89.6
Average		40	7.09	90.8
4 hrs.	11	40	7.25	114.3
		45	7.10	100.1
		37	7.07	96.7
		37	7.09	104.7
Average		40	7.13	104.0
20 hrs.	11	44	7.14	120.3
		45	7.17	120.3
		40	7.39	145.0
		42	7.16	123.6
Average		43	7.22	127.3
17 days	11	39	7.10	145.8
		40	7.14	146.2
		41	7.09	128.5
		38	7.19	142.8
Average		40	7.13	140.8

* Anesthetized with sodium pentobarbital 45 mg. per kg. intraperitoneally.

The increased sleeping time could result from an interference with the mechanism of detoxication of the barbiturate or from an additive or potentiating action of the drug and CO_2. To obtain evidence relating to this question, three groups of twenty animals each were injected as outlined above. Group A served as a control and groups B and C were placed in an atmosphere containing 20% CO_2. After six hours, when 60% of group A had recovered, group B was removed from the CO_2 while group C was allowed to remain in the chamber. The mean re-

covery times of the groups were as follows: Group A, 5.6 hours ($\sigma = \pm 1.5$); Group B, 8.3 hours ($\sigma = \pm 1.4$); Group C, 13 hours ($\sigma = \pm 3.5$). Assuming that all the effects of CO_2 are completely and rapidly reversible, sixty per cent of group B should have recovered immediately if detoxication of the barbiturate had proceeded at the same rate as in group A. However, since only 15% of group B recovered immediately and the mean recovery time of the remaining animals in this group was two to three hours longer than in group A ("t" between B and A = 5.9), it seems clear that during the period in which the animals had been in CO_2 the detoxication of the barbiturate was proceeding at a slower rate.

The depressant action of CO_2 is shown clearly in the further prolongation of sleeping time in group C ("t" between means of B and C = 4.75), since none

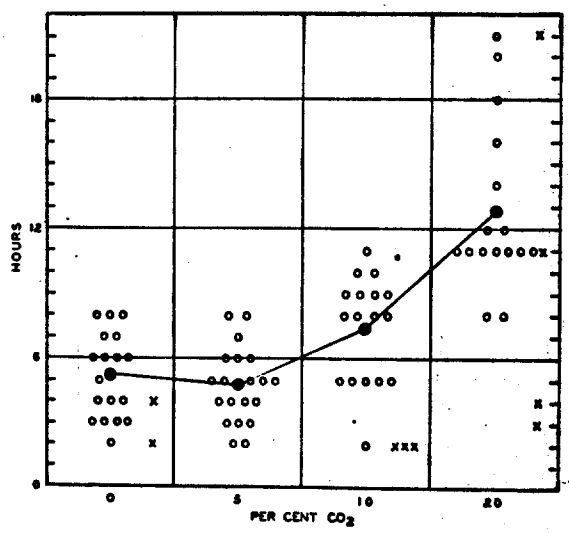

FIG. 2. EFFECT OF CO_2 ON DURATION OF ACTION OF PENTOBARBITAL (40 mgm. per kgm. intraperitoneally). O = recoveries; x = deaths; ● = average sleeping time.

of this group recovered until two hours after group B had been removed, and some individuals of the group were still narcotized when removed after twenty-one hours.

Another experiment of a similar nature was performed except that the animals were given 75 mgm. per kgm. of pentobarbital, which is approximately the LD_{50} (11). Contrary to what might have been predicted from the results of the experiments with smaller doses, no additive toxicity could be demonstrated, even with 20% CO_2, although the recovery time of the survivors of these experiments was prolonged in CO_2 as in the experiments cited above. The mortality percentages in the four groups (20 animals each) were: control—60%; 5% CO_2—30%; 10% CO_2—35%; 20% CO_2—55%.

A 25% prolongation of sleeping time was observed in 3 rabbits given 55 mgm.

per kgm. of amytal intravenously, and immediately placed in an atmosphere containing 20% CO_2, the mean sleeping time increasing from 3.75 hours ($\sigma = \pm 0.3$) to 4.75 hours ($\sigma = \pm 0.5$). Although too few animals were used for the results to be significant in themselves, these facts concerning the intravenous administration of the barbiturate support the findings on the rat and indicate, furthermore, that the prolonged sleeping time observed in the rat is not due to delayed absorption of the drug from the peritoneal cavity.

2. During chronic exposure to CO_2. After exposure to 10% CO_2 for seven days, 16 rats (group C) were given pentobarbital (40 mgm. per kgm.) and replaced in the same concentration of CO_2. The sleeping time was compared with that observed in two control groups of 16 rats each, group A receiving the barbiturate only, and group B placed for the first time in 10% CO_2 after the barbiturate had been administered. The mean sleeping time of group A was three hours ($\sigma = \pm 1.4$), while the mean sleeping times of groups B and C were 4.9 hours ($\sigma = \pm 2.3$) and 5.1 hours ($\sigma = \pm 2.4$), respectively. Since the "t"s between the means of groups A and B and A and C were 2.48 and 2.74, respectively, and no

TABLE 3

Effect of CO_2 on liver function

	NUMBER OF ANIMALS*	AVERAGE RETENTION OF DYE AFTER			
		5 min.	10 min.	15 min.	20 min.
		per cent	*per cent*	*per cent*	*per cent*
Controls	6	45	23	11	0
After sudden exposure to CO_2	5			23	15
After chronic exposure to CO_2	3			17	11

* The same rabbits were used for all determinations.

significant difference exists between the means and variances of B and C, it seems clear that no tolerance to this narcotic action of the 10% CO_2 had been acquired by previous exposure for one week when tested by this method.

E. Effect of Carbon Dioxide on Liver Function. In order to obtain further evidence relating to the mode of action of CO_2 in prolonging the action of pentobarbital, bromsulfalein liver function tests were made on six rabbits during both sudden and prolonged exposure to concentrations of 10 to 15% CO_2. It will be noted (table 3) that all the dye had been removed from the blood of the controls within twenty minutes after injection. However, after acute exposure to CO_2, 15% of the dye was still present in the blood twenty minutes after the injection, and during chronic exposure 11% was still present. It is therefore apparent that such concentrations of CO_2 interfere with liver function and that there is little acclimatization to this action of CO_2.

The interference with liver function could result from a direct depression of the activity of the liver cells or from an interference with the blood flow through that organ. To obtain evidence relating to this question, 56 rats were given 45 mgm. per kgm. of pentobarbital sodium intraperitoneally and divided into

two groups of 28 rats each. One group served as a control and the other was placed immediately after injection into an atmosphere containing 10% CO_2. One-half of the rats in each group then received 15 mgm. per kgm. of sodium nitrite subcutaneously at hourly intervals until a total of 60 mgm. per kgm. had been given. It was found that the nitrite alone had no effect on the sleeping time (average: 5½ hours ($\sigma = \pm 1.4$) with or without nitrite). However, the nitrite practically abolished the usual CO_2 effect on the duration of the narcotic action of pentobarbital, reducing the sleeping time from an average of 10 hours ($\sigma = \pm 1.8$) to only 7 hours ($\sigma = \pm 2.5$). Since the prolongation of sleeping time in the presence of 10% CO_2 ("t" between 0 and 10% CO_2 = 7) has been reduced by the presence of a vasodilator to an amount that can not be considered significant ("t" between 0% CO_2 and 10% CO_2 plus nitrite = 1.77), these results indicate that circulatory changes play the major rôle in prolonging the action of pentobarbital.

Discussion. The results on the acute toxicity of CO_2 for short periods of exposure are in accord with the observations of Prausnitz (3) who demonstrated that laboratory animals are not affected significantly by acute exposure to 10% CO_2, and that a short exposure to 25 to 30% CO_2 produces a deep narcosis without appreciable after-effects. We have found, however, that concentrations of 20% and above are usually lethal within 96 hours of continuous exposure when the animals are placed immediately in these concentrations of the gas. However, concentrations of 20 to 25% CO_2, when gradually attained, are tolerated for nearly thirty days, indicating that some degree of acclimatization may be acquired to the higher concentrations of CO_2.

This acclimatization is not due to a reëstablishment of a normal pH, a fact which is in accord with the results of Miller (7) who demonstrated that dogs exposed to 1.5 to 5% CO_2 for one to four weeks developed a mild uncompensated acidosis. In his experiments, the CO_2-combining power was reduced, indicating the existence of a metabolic acidosis.

Gesell and others (12, 13) report that short exposure to moderately high concentrations of CO_2 decreases oxidations, and therefore oxygen consumption. The data presented here support these observations since the oxygen consumption is reduced by CO_2 concentrations of 10 to 20% during the first few hours following sudden exposure. However, in the rat, the process of acclimatization is associated with a reëstablishment of the normal oxidative level since the total oxygen consumption returns to the normal level within 24 hours of exposure to 10% CO_2. Moreover, if the concentrations of CO_2 are attained gradually, no reduction in oxygen consumption occurs until definitely narcotic concentrations are obtained (15% and above).

If we accept the current theories which relate narcosis to diminished oxidations in the central nervous system, it is necessary to assume that certain tissues of the body are more susceptible to the depressant effect of CO_2 on oxidations than are those of the central nervous system, since 10% CO_2 reduces the total oxygen uptake to 75% of normal, yet fails to induce a significant degree of narcosis. Furthermore, it seems probable that it is these same tissues which are involved

primarily in the process of acclimatization, since 10% CO_2 effects the same prolongation of sleeping time after pentobarbital in the unacclimatized as in the acclimatized rat (one which has regained a normal level of total oxygen uptake during prolonged exposure to CO_2).

This increased duration of action of pentobarbital during both sudden and prolonged exposure to 10% CO_2 may be related to a decrease in liver function. Results obtained by the bromsulfalein test support the view. Since this increase may be practically abolished by the administration of a vasodilator, sodium nitrite, and since the inhalation of CO_2 produces an acidosis with a resulting vasoconstriction which is only slightly, if at all, compensated after prolonged exposure, it is most probable that both the increased sleeping time after pentobarbital and increased retention of the dye are due to a reduced blood supply to the liver. Moreover, this suffices to explain the failure of 5% CO_2 to affect the duration of action of pentobarbital, since Grollman (14) has shown that CO_2 does not affect the circulatory system significantly until a concentration of approximately 6% is reached.

SUMMARY

The acute and chronic toxicities of carbon dioxide have been compared and certain factors concerned in its narcotic action have been investigated.

1. The maximal tolerated concentration of CO_2 for the albino rat after sudden exposure is approximately 15%. However, when the final concentration of CO_2 is attained gradually over several days, the maximal tolerated concentration is approximately 23%. Likewise, a greater degree of depression is produced by sudden exposure to a given concentration of CO_2 than by prolonged exposure to the same concentration, indicating that the rat is capable of a certain degree of acclimatization.

2 Following sudden exposure to 11% CO_2, there is a temporary decrease in the total oxygen consumption of the rat. This reaches a level 15 to 25% below normal within two to four hours and slowly returns to the normal range within twenty-four hours of continued exposure. When the rat is placed in atmospheres with gradually increasing concentrations of CO_2, the oxygen consumption remains within normal limits until concentrations of CO_2 greater than 10% are attained.

3. During exposure to 11% CO_2 for thirty days, the plasma pH falls to a level as low as 7.09 in the first half hour and remains below 7.15 during the remainder of the period. The CO_2 content of the blood gradually increases to 130 to 148 volumes %.

4. A marked reticulocytosis occurs in the rat during prolonged exposure to 11% CO_2, but no corresponding increase in the number of leukocytes or erythrocytes is observed.

5. The sleeping time of normal rats anesthetized with pentobarbital is prolonged in atmospheres containing 10 or 20% CO_2. Similar results are obtained with rats previously exposed to 10% CO_2 for one week. Evidence is presented which suggests that CO_2 delays the detoxication of pentobarbital by diminishing the blood flow through the liver as a result of the splanchnic vasoconstriction.

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Soft Drink Flavours Their History and Characteristics

Part II. Lemonade

GEORGE B. BEATTIE

The first number of this series dealt with a drink, cola, which is generally considered to be typically American. Our present subject, lemonade, has, on the contrary, been regarded for many generations as the leading British soft drink, though its supremacy was seriously threatened in pre-war days by 'grapefruit squash' and is now challenged by 'orange' flavours, which, incidentally appear even to be overtaking cola for the first time in the States.[2] In the UK, the word is used in three senses, first, for still admixtures of lemon juice and sugar, not often produced commercially in pre-packed form, second, for the essence-flavoured, citric-acidified sweet carbonated drink in bottle which constitutes a best-seller in the mineral water maker's repertoire and third, as a general term for effervescent drinks, whatever their flavour, this being most noticeable in Scotland where the French practice of terming all sweet carbonated drinks 'limonades' is paralleled.

In Britain, according to the terms of the *Soft Drinks Order*,[3] ready-to-drink beverages containing fruit juice (apart from comminuted fresh orange beverages) must contain certain minima, 3% by volume for 'lime juice and soda', 'barley citrus juice drinks', and 5% by volume in all other cases. According to the *SD Code of Practice*[4] appertaining to the *Labelling of Food Order*,[5] however, the suffix '-ade' is taken to indicate a 'flavoured' type of ready-to-drink beverage and although the Code has no legal status, the absence of juice in UK lemonade is regarded as in no way invalidating the description. This is in sharp contrast to US nomenclature where '-ade' is invariably regarded as indicating a drink with a substantial amount of fruit juice, and some States have demanded as high percentages as 30 or so for such ready-to-drink '-ades'.

HISTORY

Linnaeus considered the lemon and the citron to be essentially the same, a theory supported by many later workers, including Hooker and Brandis.[6] The lemon tree is a native of NW India, but no evidence exists to date the introduction of the fruit to Europe with certainty. Ancient literature speaks often of the citron but never of the lemon and some students have supported the idea that in those days the terms were largely interchangeable. From certain rather vague allusions, lemons seem to have been known in Italy towards the close of the fifteenth century,[7] and Gerard[8] speaks of 'in the shoppes, limones' in Elizabeth's time, as though they were quite a common article for household use in England by then. Lemon juice acquired a massive medicinal reputation in the early seventeenth century when Commander Anson reported its excellent anti-scorbutic properties, but Woodall[9] had written in similar terms a hundred years before and Ronsseus[10] as early as 1564 had set out categorically the values of both lemon and orange juice in combating the scourge of scurvy at sea.

Just when any form of lemonade was first proposed is anyone's guess, but such a concoction, too, would appear to have originally been a medical brainwave, one French physician in 1660 attributing its invention to 'an Italian from Florence' who 'had the happy idea of converting the beverage to ice': presumably the first 'ice lollies' ever. Wm. Kirkby,[12] however, states that French physicians were already using the beverage at the beginning of the seventeenth century. The same authority says the drink was well known in Charles II's time in Britain, and the first English use of the word is believed to be by Thomas Killegrew[13] in his play, 'The Parson's Wedding', 1663. In Paris a company of Lemonadiers was formed as early as 1676, operating under Government-granted patents. This company continued its operations for well over a century and appears to have dispensed the chilled 'Florentine' style of lemonade, Kirkby[16] referring to an English 1771 cartoon of a French Limonadier complete with tank on his back, this vessel having a pipe and tap projecting under the man's left arm, while he held a dispensing cup in his right hand. Rather curiously, the Americans revived this portable supply plan a few years ago to boost sales at sports meetings.

Early reference to the temperance qualities of the drink were made by *Arbuthnot*[14] and the great Dr. Johnson[15] was a marked addict, frequently threatening physical violence against unfortunates who did not make his potions sweet enough or castigating sundry caterers for their unhygienic methods. The first known definition of composition is by Blancard[17] in 1684—'Limonada, Limonade—A kind of mixture or julep made with the juice of lemons, water and sugar', and in 1747, James[18] advised the use of citron fruit in preparing a suitable blend. Gillespie,[19] fell into the error of supposing lime juice was equally efficacious antiscorbutically as lemon juice, and this may have originated the popular practice of blending lime and lemon in concocting summer drinks.

ENTER CARBONATION

While attempts had been made artificially to carbonate water from 1560,[20] no commercial progress was made until after Priestley[21], had concluded his experiments and published his history paper on the subject in 1772. The early carbonated beverages, however, were all alkaline solutions, and apart from a reference by Henry in 1881 to the use of a draught of lemonade as a 'chaser' to 4oz of Dr. Bewley's Julep[22]—a kind of soda water—and a process for mixing fresh lime juice with a carbonate[23] to produce a kind of effervescing 'lemonade', 1767, the idea of utilizing fruit juices or flavours in conjunction with carbonated water does not seem to have dawned until well into last century. A US publication[24] in 1913 attributed the invention of carbonated lemonades to a Philadelphia druggist, Townsend Speakman, who was said to have added flavours and sugar at the instance of a Dr. Syng Physick. The foremost

US trade historian,[25] however, has since unearthed the facts that while this discovery is supposed to have been made in 1807, Speakman died of yellow fever in 1793 and the doctor and he probably never even met! So far as America is concerned it is much more likely that a man named Smith produced the first 'lemonade' about 1828 by expressing juice from fruit and mixing it with sugar syrup, the syrup then being diluted and carbonated.

Matters are no clearer in Britain. Bateman is often credited with being the first to produce carbonated lemonade and similar drinks, the slight evidence of this being a reference to 'saccharine drinks' in his 1932 patent specification,[26] but Grattan & Co. of Belfast advertised and engaged the services of the local town crier to announce that 'this amazing drink' would be on sale in June 1825.[31] True, there was some 'technical hitch' but the first supplies were made towards Christmas of the same year. Powdered lemonades appear to have been introduced to the US about 1800, but following Scheele's discovery of citric acid or as it was commonly and charmingly dubbed, 'concrete acid of lemons', 1784, recipes for 'Portable Lemonade' soon abounded, one of which quoted by Kirkby[27] comprised 1oz citric acid, 4 lb sugar and essence (ess. oil) of lemons, 2 drachms. About the same time, domestic cookery books seem to have found that the flavour of juice lemonade could be fortified by the addition of the rind,[28] while an alcoholic lemonade based on fermented honey, and some phosphatic types appeared. Liquid lemonades using citric acid, peel tincture and capillaire (a sugar syrup flavoured by orange flower water) are mentioned in *Gray's Supplement* of 1821.

Wm. Kirkby,[27] who was chief chemist to Jewsbury and Brown for many years attributes the first effervescing lemonade to that firm's originators, Jewsbury & Whitlow, some time between 1826 and 1833, quoting an early Manchester publication,[29] and he adds that effervescent lemonade was originally sold as 'a new and different article' and did not contain any juice, the acidity being derived from citric acid and the flavour from lemon oil. He alleges that it was only the availability of citric acid which made such carbonated lemonades possible, and very probably previous attempts to carbonate juice types had led to disaster through early juice spoilage, as the carbonators of the time would almost certainly whisk in a great deal of atmospheric air.

There is no evidence that either Paul or Schweppe, the initial commercial pioneers, produced sweetened drinks either in Geneva or London, but the present writer imagines that attempts to carbonate lemonade and similar drinks were made by the turn of the century if not before. Certainly, there is at least as much evidence in the patent specification of Wm. Hamilton[30] of Dublin, dated May 4th, 1809, that sweetened and flavoured drinks were then quite well known in the carbonated form as one finds in Bateman's much later specification[26] for he describes his carbonator as 'a new mode of preparing soda and other mineral waters . . . acetous, saccharine and aromatic liquors, using various solutions of salts and combinations of spirits, vegetable acids, aromatic oils, sugars . . . etc.'

Probably one reason why we don't hear much about carbonated lemonade and similar drinks for a long time is that the solutions frothed so badly when charged with gas in the carbonators of the time that filling was unmanageable. We moderns are inclined to regard what is known as pre-mix carbonation as something very new, but from a perusal of old specifications and descriptions, it is clear that in the early years of the nineteenth century, 'syruping'—the addition of a small amount of concentrated flavoured syrup to each bottle on a separate machine before filling up the vessel with carbonated water—was quite unknown. As late as 1840, Hayward Tyler, who had taken over the Bramah carbonator from Wm. Russell, the 'Bramah' being developed from the Hamilton machine, patented a dual purpose machine 'intended to make both soda water and aerated lemonade'.[32] One pump was used only for lemonade and was designed to prevent premature frothing of the pre-mixed solution, a detachable inner vessel which could readily be taken out for cleaning being also provided to fit inside the condenser, so that soda water made later would not carry any residual lemon flavour.

WHAT IS LEMONADE?

Confining ourselves to the carbonated, bottled variety of the drink—in Britain, juice-containing lemon drinks are usually described as 'squash', 'crush', 'squeeze' or by some other similar term—*examination of early UK specifications shows very clearly that from the first, carbonated lemonades were juiceless productions, acidified by citric (sometimes tartaric) acid, rind oil or 'essence' and sweetened with sugar, the degree of carbonation being difficult to evaluate as although high pressures, sometimes up to 120 p.s.i., are suggested. These old machines were so wasteful that often enough the carbonation in bottle probably did not reach a quarter of that figure. However, a moderately high degree of carbonation was always indicated.* In 1938, Kirkby[25] suggested as a standard for carbonated lemonade, 7% sucrose, citric acid, rind essence modified to the taste of the individual maker and suitable effervescence. In the present writer's view, the sucrose percentage is, excepting for a 'dry' lemon, too low, and should be at least 8.75% w/w. Fairly early specifications show that 'lemonade' was often used as a general term. In one[33] which covers the use of tartaric acid instead of citric acid, 'raspberry lemonade', 'orange flower lemonade', 'Algerian lemonade, citronade and orangeade' are all mentioned, recipes citing various essential oils, being appended. In another,[34] where the flavours are provided in 'lozenge' form, lemonade is said to be flavoured 'with citron, orange, etc.' But the real brainwave is an 1865 one,[35] where it is seriously stated that aerated lemonade can be greatly improved by adding one-eighth of its volume of alcohol or spiritous liquor either during manufacture or when bottling. The ideas of an inventor who followed the next year[36] are comparatively tame (*i*) the use of a cold extract of tea in making the lemon flavoured syrup, (*ii*) the addition of phosphates.

After such freak variations, the sensible reply of the chairman of the judges at the last important soft drinks competition[37] to be held in this country, is refreshing. He told a reporter who asked 'What is lemonade?' that it was 'a well-balanced lemon-flavoured drink'. In this connection, J. H. Toulouse[38] between the wars examined hundreds of American lemon drinks. Probably the US 'lemon and lime' drink which virtually dated from the inauguration of '7-Up' is the nearest to a typical British 'lemonade', but Toulouse chiefly concerned himself with what were then called 'lemon sodas' and 'lemon sours', roughly corresponding to our own ordinary 'sweet' lemonade and our 'dry' lemonade respectively. Dr. Toulouse found that at that time there were really three different types of US lemon drinks. 'Lemon soda', 'lemon sour' and 'lemon dry', though the actual names given by makers varied.

The first group had sugar contents from 9.29 to 12.09% w/w, with acidities of from 36.5 to 45.6 grains per US gallon (approx.[41] to 55 grains per Imp. gal) with carbonation from 2.8 to 3.25 volumes Bunsen. 'Lemon sours' ran from about 10.5 to 12.0% w/w sucrose, with 62.5 to 70 g.p.g. (75 to 85 g.p. Imp. gal) with carbonation from 2.9 to 3.0 volumes. 'Dry lemon' has an average sugar content of 9.6, acidity 69.1 g.p.g. (US)—equal to approx. 83 g.p. Imp. gal—with carbonation averaging to 3.32 volumes Bunsen. A British worker[39] has compared

Toulouse's figures and also some post-war US lemon drinks with typical British lemonades, his chief findings being that the latter were not so sweet but appreciably more acid and carbonated more fully. British sweet lemonades most usually contained 2 to 2¼oz citric acid per gallon syrup, dosed 1½fl.oz per half pint = 131 to 146.5 grains per Imp. gal final goods; sugar content being most usually 6 lb per gallon syrup = 8.75% w/w. (Exceptional samples examined by the writer have been found to exceed 10.5% or even reach 11% w/w.) 3.5 volumes Bunsen was regarded as the average carbonation, but the present writer has found samples as low as 2.5 and as high as 4.75 volumes in bottle; 3.5 can be regarded, nevertheless, as a very suitable rate.

For dry lemons, UK ideas on carbonation generally run higher, 4.5 being quite common with 4.0 volumes often looked on as the minimum. Sugar contents as low as 5% (with no other sweetener) have been encountered but our UK worker referred to above,[39] found 7 to 8% usual, with most acidities between 131 and 181 grains per Imp. gal, the acid rates being lowered slightly where saccharin replaced some of the sucrose. The present writer prefers for a 'dry lemon' to employ a sucrose content of at least 8.75%, at which up to 200 g.p.g. citric acid can be used without undue harshness with 4 to 4.5 volumes carbonation, and specially compounded dry lemon essences which include some lime and green peel tincture, rather than reducing the inherent sweetness and consequent palate fullness.

LEMON FLAVOURS

For many years bottlers used distilled or hand-pressed essential oils of lemon, modified by limes, orange, citron, etc., and all manner of crude techniques were used, one of the most grotesque, which persisted long, being the 'dry mix',[40] in which the oils were added to the sugar in its dry state in a rotatable vessel which was driven for anything up to an hour in order to mix the flavour and sugar, the flavoured sucrose being then converted to solution by addition of hot water. The plan was supposed to prevent separation of the oil—used at the rate of 1 to 2fl.dr per gallon syrup—but just why, one cannot imagine. Many early soft drink makers made syrup by the hot process, adding the oil while the syrup was hot and constantly stirring until it cooled sufficiently for bottling, the theory being that the unwanted hydrocarbons volatilized, leaving the valuable flavouring principles behind! The oil was often mixed with powdered citric acid, this also being thought to aid solubility.

A more feasible plan was to make a paste, using light carbonate of magnesia,[41] of the oil, which was then usually stirred into warm syrup, the flavoured solution being 'aged' in a covered vessel for anything from two or three days to a month. While this was quite good with ginger ales, it frequently led to lemonades having an earthy off-taste and sometimes showing floating precipitates. After the ageing period the syrup was filtered through a magnesia-lined fabric bag and only then were acids, colours and often bouquets, added. About 1860, the discovery was made that while absolute alcohol dissolved all the essential oil's constituents, a weaker alcohol of controlled strength took up most of the flavouring principles but left many of the terpenes and sesquiterpenes behind, it being a simple matter to separate them. Thus, the first crude 'soluble essences' were evolved. It was also found that using comparatively weak alcohols for maceration or percolation of thinly pared rinds, the flavouring principles could be won with very little of the unwanted 'passengers'. This discovery had several important aspects. First, the bottler was provided with a flavour which dissolved in syrup easily and did not 'ring' in the bottled finished state. Second, even when the product contained 'high air', no off-tastes were usual, as these were mainly due to the oxidation of the hydrocarbons—now eliminated. Third, the essence maker could produce individual blends by (*a*) charging his percolators and maceration vessels with proportions of citron, lime, orange, bergamot, shaddock, etc., peels as well as lemon, and (*b*) incorporating further additives in the form of treated natural oils and later, various fine chemicals.

The next stage was the production of first, terpeneless oils, then sesquiterpeneless oils, originally by fractional evaporation, to which vacuum methods were soon applied, and ultimately by means of sundry 'cold' processes. By terpeneless oil fortification of soluble essences, much more highly concentrated flavours were rendered available and were in regular use even by 1900. Various artificial lemon oils or essences, mainly based on citral from lemongrass oil, have been devised, a typical example being a German one[42] containing in addition to citral, geraniol, geranyl acetate, linalol, linalyl acetate, citronellol, phellandrene, limonene, octyl aldehyde and nonyl aldehyde. For those wishing to delve further into this phase of our subject the following references may be consulted.[43]

There is little standardization of nomenclature with respect to soluble lemon essences in the essence trade, numeral qualifications quite meaningless to the uninitiated sometimes being used, but in general, terms such as 'strong' or 'pungent' may be regarded as indicating a lime-fortified type, though there are exceptions, while 'zest', 'peely' or 'green' indicate that a fair amount of peel tincture is present, the last-named denoting that attractively peculiar tang which comes from the use of a certain amount of tincture derived from the rinds of unripe fruits. Words such as 'mellow', 'soft', 'sweet', 'luscious' may or may not indicate the incorporation of orange or citron, and 'blended' can mean almost anything. This lack of standardization is rather unfortunate as it sets at naught many published formulae.

BLENDING

In manufacturing a lemonade, there are three basic factors to be studied: (1) Formulation of a well-balanced taste basis or sweetness, acidity and carbonation inter-relationship. (2) Use of an appropriately blended lemon essence with or without certain minor additives such as a small amount of lime juice. (3) Employment of a suitable bouquet for the class of lemonade. The first factor we have already discussed. Turning to the second, although H. S. Redgrove[55] once claimed to have made a fine lemonade without using any of the natural constituents of the lemon fruit at all, the flavour foundation should obviously be a true lemon one, whether secured entirely from an ordinary or terpeneless oil in alcoholic solution or from the peel by some tincture process, or a blend of the two.

From that starting point, the manner of building up a good blend depends on the class of lemonade we are trying to devise. Even in the selection of the basic lemon flavour, however, some care needs to be exercised, and although it is admittedly a gross over-simplification—we can say that where a dry or sour type is desired, a basis with a peely or green peel note of prominence is distinctly helpful. That does not mean that when we are aiming at a 'sweet' lemonade of considerable palate fullness, the peely tang is best omitted. Far from it, but the green peel note must be faint if used at all.

If we require pungency and dryness, lime is the best qualifier of the main lemon flavour, and probably the most pungent lemonades of all are those based on green lemon tinctures with both lime juice and soluble essence of limes (W.I.) added. Older recipes for the more pungent classes of lemonades frequently call also for citron, and some for shaddock (grapefruit) but while these additions impart individual and some-

what curious flavours, it is doubtful whether they assist the pungency of the preparation. Practically all the other available additives tend to give greater mellowness and palate fullness. Orange is the classical example, but remembering that we are not as yet discussing bouquet, we can say that the influence of the most commonly employed varieties of blending agents are as under:

1. *Sweet Orange.* Gives a 'sweet' note, eliminating any harshness and enhancing palate fullness.

2. *Bitter orange.* As 1 but to a lesser extent, the orangy character being more readily identified.

3. *Tangerine or Mandarin.* Imparts an even sweeter and softer note than 1 with some resemblance to the effect produced when a bergamot/neroli bouquet is used. (Examination of the respective constituents will soon show why.)

4. *Bergamot.* This ingredient broadens out the palate appreciably, giving greater lusciousness. If too much is used, the impression is that of 'fanciness' and complaints of 'scenty taste' have even been ventilated. Synthetic 'bergamots' are *not* advised!

5. *Citron.* Citron essences are frequently blends, but the true citron preparations based on tinctures are intended in the older formulae. They increase fullness but with what I personally do not regard as too desirable a tang, and from organoleptic tests, I am inclined to think that most consumers regard a touch of citron as an 'off-taste' rather than an enhancement.

6. *Grapefruit.* Acts somewhat like bitter orange but must be used sparingly.

7. *Neroli.* When used as a principal additive and not as bouquetting ingredient, the effect is rather like that of bergamot, but even more care is needed to avoid the charge of a 'perfumed taste'. Employed in strict moderation, with or without bergamot, this additive can transform an undistinguished lemon base into a most attractive blend. He may be quite wrong, but the writer prefers certain synthetic preparations to either the 'Portugal' or 'Bigarade' natural oil-derivations.

8. *Citronella.* Some bottlers use this agent, but in the writer's view it is not very suitable, and carelessly employed can convert a good preparation into a bad imitation of hair oil!

9. *Citronellol.* Much more attractive, imparting a slight rose flavour and enriching the lemon flavour appreciably.

10. *Jasmine.* Should be regulated to the bouquet ingredient status, though it is sometimes used as a more principal additive.

11. *Geraniol.* With or without Citronellol, this agent adds considerably to palate fullness but if too much is used, the entire lemon character wanes and the rose tone becomes far too aggressive.

12. *Octylic alcohol.* Again a common bouquetting ingredient, but used as a main additive it seems to emphasize the lemony character of the beverage while increasing palate fullness.

13. *Nonylic alcohol.* This agent has more of an orange/neroli/rose note, and as a bouquet is the complete one in itself. As a main additive it needs some care but helps out when used in conjunction with orange or bergamot or both.

14. *Vanilla.* Here we have the best palate enricher of all, but unfortunately it has such a strong flavour of its own that only minute proportions can be employed if a 'cream soda' flavour is to be avoided.

15. *Various spices.* Ginger, cloves, nutmeg, pimento and mace all occur in various formulae, separately or in blends, and in some districts, the natives expect lemonade to be mildly spiced. Such additives, nevertheless, should be avoided unless local preferences tend that way!

16. *Lemongrass.* Greatly reinforces the 'strength' of the lemon taste but with an unwanted undertone.

BOUQUETING

Some people hold that lemonade should be flavoured by lemon only, not even a suspicion of any other essence being used, even as a bouquet, but these good folks have probably never even tried the effect of a *judicious* addition of bergamot or neroli. One has to agree that laying on bouquets 'with a trowel' can ruin the inherently best of lemonades. The main bouqueting groups for lemonade are (*a*) the 'Eau de Cologne' group, (*b*) the 'alcoholic' or 'vinous' group, (*c*) the 'floral' group, particularly 'rose' bouquets, (*d*) the 'pungent' group, including the spices, (*e*) the 'lemon-like' group.

On the first Ruemele[49] contributed an interesting general paper in this journal not long ago, and a contemporary[50] has discussed the use of these 'eaus' as general beverage bouquets comprehensively. The oldest bouquet of this class was orange flower water but its biological instability led to its replacement by a spiritous solution of neroli, etc. For a sweet 'white' lemonade, the following bouquet of this class is excellent: bergamot, 20 parts; neroli, 15 parts; cedrat, 15 parts; balm, 15 parts; mandarin, 10 parts. For a sweet coloured lemonade: bergamot, 35 parts; bigarade neroli, 20 parts; citronellol, 10 parts; rose, 5 parts; aldehyde C,[12] 5 parts.

The second group is designed to impart a 'champagnic' basis, various synthetic imitations of wine flavours, from 'sherry' and 'champagne' to 'muscatel', also oil of cognac being the foundation. These flavours are often combined with orange, bergamot, neroli, etc., and for a 'dry lemon', here is a typical admixture: bitter orange, 30 parts; neroli, 20 parts; petitgrain, 10 parts; sherry flavour, 20 parts; cognac flavour, 20 parts. The floral group is theoretically extensive but in practice, the ease with which a 'perfumed taste' can be imparted restricts the list. The neroli types and rose flavours, natural and synthetic are the principal agents, with jasmine, lime flowers, jonquil, carnation and geranium sometimes augmenting them in *very* minute proportions. The 'pungent' group may be divided into two; bouquets based on lime, green lemons, lime flowers and very sparingly, rosemary and lavender and those derived from spices, of which the principal are cinnamon, cassia, cloves and nutmeg, all used in trace amounts. Finally the lemonlike group has the following chief members, lemongrass (also sometimes used as an extra additive in larger amounts to give a stronger but coarser 'lemon' flavour), balm, verbena, lemon thyme and, of course, special varieties of lemon citron, and lime peel tinctures. *On the whole, probably the safest form of bouquet is the bergamot/neroli based one with a touch of muscat flavour where what is termed a 'champagnic finish' is required.*

FORMULAE

Many of the published textbooks of formulae are singularly unrewarding, but some practical working recipes have been given of late years in a beverage contemporary, for sweet lemonades,[51] dry lemons,[52] lemon-lime drinks[53] and 'fancy' lemonades.[54] In some formulae small amounts of lemon and lime juices figure, but in the vast majority of cases all flavouring media—apart sometimes from blended bouquets which are added separately—are skilfully combined by the expert essence supplier to form an easily used blended but highly concentrated soluble essence, usually of from ½ to 3fl.oz per gallon syrup strength, the 1fl.oz type being the most popular of all.

The essence-flavoured lemonades naturally lack ascorbic acid, which can be readily added if required, but while this plan was warmly advocated and debated during the war,[56] it is

seldom if ever practised in the UK, although some lemon and lemon-lime drinks are now regularly vitamin-fortified in the United States.

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[56]Controversy between H. S. Redgrove, S. G. Kendrick and G. B. Beattie, *ibid.*, 1941, Jan. to May issues.

Flavours from the USA

Virginia Dare Extract Co., Inc. of 882 Third Avenue, Brooklyn, NY 11232, USA, have forwarded news of their special products. Currently, there is much interest in the US in Dominol Cloudy Complex Base 193-B-9, produced by their subsidiary, Dominion Products. Dominol is a replacement for brominated vegetable oil, which is facing imminent severe restriction, possibly even a ban in the USA. Sales of this product have increased dramatically in the past two months as a result of these rulings. Unlike other proposed substitutes, Dominol is composed entirely of ingredients permitted under the present federal standard of identity for soda water. We have received technical bulletins on Imitation Delicious Apple, No. 15, Imitation Ripe Banana, No. 16, Flavours for Non-Dairy Products, No. 23, Imitation Blue Cheese, No. 24, and Imitation Onion Flavour, No. 25. Technical Bulletin No. 21 deals with Cloud SD. This is a spray dried product, which is used to impart a uniform cloudy appearance in beverages. Cloud SD contains—gum acacia and other gums, limonene and BHA (antioxidant). About 1oz is sufficient to impart a cloudy appearance to 5gal beverage, but the amount may be varied to obtain the degree of cloudiness desired.

Technical Bulletin No. 220 revised, lists the laboratory procedure for converting Dominol Cloudy Complex Base 193-B-9 into a Ringless Cloudy Citrus Emulsion (Non-Brominated), used at the rate of 1oz per gallon of syrup. It is interesting to note that the particle size of the finished emulsion should be 1μ or less.

Technical Bulletin No. 221 lists the procedure for converting Dominol Cloudy Base Complex 193-B-9 into 100gal of Ringless Cloudy Neutral Emulsion (Non-Brominated), used at the rate of 3/4oz per gallon of syrup. This is a neutral non-brominated cloud which can be used as a base by adding concentrated oils or essences to produce the desired ringless cloudy flavoured emulsion.

Europemballage now hold 87.6% Interest in Thomassen and Drijver-Verblifa NV

On April 9th, 1970 Europemballage Corporation announced from Brussels that its public offering of March 16th, 1970, has resulted in the acquisition of 77.3% of the shares of Thomassen and Drijver-Verblifa NV, a packaging company in the Benelux countries.

After having obtained the 10.3% of Thomassen and Drijver-Verblifa held by Continental Can Company, Inc., Europemballage Corporation's total interest will therefore represent 87.6%.

In addition to its share in Thomassen and Drijver-Verblifa NV, Europemballage will have an 86% interest in Schmalbach-Lubeca-Werke AG, a packaging manufacturer in Germany. Negotiation regarding the participation of Metal Box in Europemballage are continuing.

Europemballage is a truly multi-national company which will enable the national companies to promote the growth of the packaging industry throughout the European economic community. By introducing a wider range of packaging innovations and providing more comprehensive services to customers and consumers it should fulfil many of the aims and objectives of the Common Market.

New Chairman for SPP Group

Mr. G. T. Shoosmith has become chairman of SPP Group, following a reorganization of the board, and Mr. J. A. Nutt and Mr. J. F. Hall-Craggs have been appointed joint managing directors. Mr. Leslie Davies has resigned as chairman and managing director.

Mr. Shoosmith is the director of Booker McConnell responsible for the engineering companies. Previously he was chairman and managing director of Plenty & Son of Newbury, which in January 1969, merged with Sigmund Pulsometer Pumps of Reading and Gateshead (a subsidiary of Booker McConnell) to form SPP Group, a company manufacturing a wide range of pumps and other fluid-handling equipment.

Mr. Nutt and Mr. Hall-Craggs were previously assistant managing directors of SPP Group, Mr. Nutt having originally been commercial director of Sigmund Pulsometer Pumps and Mr. Hall-Craggs marketing director of Plenty & Son.

Clemons-'37
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THE HISTORY OF THE THERAPEUTICS OF CARBON DIOXIDE WITH SPECIAL REFERENCE TO THE CARBON DIOXIDE CANCER TREATMENT

BY

E. JAY CLEMONS, M.D.

ABSTRACT.

This history, with special reference to the carbon dioxide cancer treatment, gives the ancient record, the part played in ancient religion, the pioneer therapy, and the early anesthetic use of carbon dioxide—together with freezing and heat-abstraction therewith. Thus, proof that carbon dioxide has been therapeutically employed throughout the ages with most gratifying results is brought forward.

THE ANCIENT RECORD.

The application of carbon dioxide for therapeutic purposes has been traced to very early times. In fact, Dioscorides and Plinius spoke of the stone of Memphis in 45 B.C. They recorded that this stone was pulverized, dissolved in vinegar, and applied to the surface of the body in order to produce anesthesia.

The appearances of mineral springs attracted the attention of the early philosophers. They made various tales current, relative to the marvels of certain springs, by relating supernatural properties of their effervescing waters.

Strabo related that the springs of Hierapolis imparted red colour to roots of trees and shrubs. He said that their juices, when they were mixed with water, produced the purple liquor which was used for dyeing wool. Philostratus, when writing about the sanguinary battle which the Greek army fought with Telephus, on the banks of the Caicus river, stated that the wounded soldiers, who resorted to Agamemnon's spring, were all restored. According to Herodotus, a spring in the country of the Ichthyophagi prolonged life beyond one hundred and twenty years. A certain spring in Chios caused insanity, according to tradition. Another one in Magnesia improved the voice of singers. Those waters at Lethe and Mnemosyne are often mentioned in classical literature. The former spring brought about oblivion. The latter one restored memory.

The Book of Genesis recorded the fact that Anah, the father of Esau's wife, discovered some thermal springs in the desert. In one book of Kings, mention was made of the spring near Jericho which made the ground barren. The prophet Elisha threw salt into it and said: "There shall not be from thence any more death or barren land."

From the New Testament we learn that the Jews used thermal waters before the time of Christ, as it is recorded that "There lay a great multitude of impotent folk, of blind, halt and withered, in the porches of lake Bethesda, by the sheep-market of Jerusalem, waiting for the moving of the waters; and, whosoever first after the troubling of the waters stepped in was made whole of whatsoever disease he had."

THE PART PLAYED BY SPRINGS IN ANCIENT RELIGION.

Mineral springs played an important part in religion of the ancients, as the disciples of Æsculapius erected temples to the God of Medicine in vicinities of mineral springs. These monuments were not only provided with theatres and places of amusements, as our fashionable watering-resorts, but also with hospitals and medical schools for instruction of students.

The Castalian spring had a temperature of two degrees Fahrenheit. In this spring Pythia had to bathe, before she ascended the tripod within the steaming cave of Apollo's oracle at Delphi, where copious eruptions of carbon dioxide occurred. Here, from the short and incoherent sentences, which the priestess uttered in paroxysms, the prophecies were drawn.

THE PIONEERS IN CARBON DIOXIDE THERAPY.

Hey, Withering, Percival, Dobson, Warren, McBride, Ingenhousz, Beddœs, Henry, Lee, Rotheran and White made systematic use of carbon dioxide for therapeutic purposes, before Faraday succeeded in liquefying the chemical in 1823. Twelve years later Thilorien solidified carbon dioxide. However, it was not therapeutically used, in this form, until 1899.

Percival reasoned that, since carbon dioxide has antiputrid properties, it should be used for the treatment of malignant ulcerations. In fact, he confirmed his thought in the Infirmary of Manchester upon Dr. Withering's service where he demonstrated that suffusion of a cancerous wound with carbon dioxide gas caused a disappearance of purulent matter, relieved pain and gave a better aspect to a wound. He wrote that palliation in so frightful and hopeless a disease should be considered a very precious acquisition.

Dobson, who likewise used carbon dioxide in gaseous form for the treatment of cancer, did not report that he effected a cure, but that pain was relieved and the appearance of wounds improved.

The analgesic effect of carbon dioxide was soon established after Beddoes learned from John Ingenhousz of its remarkable property of almost instantaneously quieting very severe pain such as that produced by vesication, Ingenhousz having discovered that, during the time that a blistered finger, with its epidermis removed, was exposed to atmospheric air, the pain was severe. This pain became more intense, when the member was in an oxygen-

bath. But, that pain immediately subsided after the finger was held within a container of carbon dioxide gas.

Beddoes applied the gas to malignant ulcerations, thus relieving pain and diminishing discharge. Subsequently, Ewart likewise reported that he treated a case of cancer of the breast. He stated that, upon the next day after the application of the gas, the ulcerated surface had a better appearance. The pain which had been very severe disappeared. The fetid odour became less noticeable, and the wound healed within two and a half months.

Soon after he published his report, Ewart wrote to Beddoes that, while the wound had healed, it reopened soon after the carbon dioxide suffusions had been discontinued. However, the ulcer had a less hideous appearance, and the pain had been controlled by the use of carbon dioxide gas.

Ten months later, Ewart again wrote to Beddoes and informed him that the woman was still alive, but that the recurrent ulcer had not cicatrized as the primary one had. However, the relief from pain had continued and the absence of odour was strikingly noticeable.

Such results which were obtained in other countries were too important not to be considered by the French practitioners of that time. In fact, everywhere in France experiments with carbon dioxide were made in order to verify that which had been learned.

The carbon dioxide gas treatment of cancer thus became popular throughout France, its popularity being foremost at the Academy of Science and Art of Dijon where great scientific activity developed, and where facts were established showing the beneficial effects of the gas upon all kinds of ulcerations.

J. L. Targioni of Florence reported to this academy the detail of a case of cancer of the breast which had been treated by carbon dioxide in gaseous form. The pain was relieved, the bad character of the suppuration subsided, and the general condition of the patient improved markedly. In fact, one-half of the cancerous ulcer had cicatrized.

In Paris case-reports were also published by the Royal Society of Medicine, proving the efficacy of the remedy. Then, the commission, whose chairman was Lalouette, confirmed that carbon dioxide gas, although it did not cure cancer, did relieve pain and acted upon ulcers in a beneficial way by modifying secretion and causing, to a certain extent, cicatrization.

At a later period, Demarquay, who wrote the history of the therapeutic use of carbon dioxide in gaseous form, reported analogous cases wherein long continued suffusions of malignant growths with carbon dioxide gas ameliorated the sufferings of cancerous individuals.

The Early use of Carbon Dioxide Inhalation.

Dating from the end of the eighteenth, and the first quarter of the nineteenth centuries, inhalation of carbon dioxide was employed for treatment of tuberculosis. Such treatment, in fact, became exceedingly popular, and it was widely accepted, as in most of the European countries numerous recorded case-records confirmed that inhalation of air containing a high percentage of carbon dioxide effected an improvement in, and sometimes a cure of, pulmonary tuberculosis.

Edmund Weill spoke in the Academy of Science at Paris. He gave an account of experiences with carbon dioxide inhalations. The two to five minute sittings consumed from two to five liters of the gas once or twice a day. He reported that no unpleasant symptoms were experienced. The coughing spells were relieved and prevented. In fact, patients were soon rested and rejuvenated.

Owing to the significance of the transport of an excess of carbon dioxide in the blood-stream, it is not difficult for one to explain how such results were thus obtained. However, the disastrous consequences of intravenous injections of carbon dioxide, which were subsequently employed, might have placed the use of the gas in disrepute.

The Early Anesthetic use of Carbon Dioxide.

At an annual dinner of the Royal Society of Medicine, the president, Lord Dawson of Penn, referred to the very important discovery made by Mr. Thompson, the curator of the Historical Medical Museum. Mr. Thompson produced records which proved that, in 1825, preceding the introduction of ether in the United States in 1845, and of chloroform in Scotland in 1847, a physician by the name of Hickman induced anesthesia in animals by inhalation of carbon dioxide and nitrous oxide, and performed painless operations upon them. Afterwards, he brought his results to the attention of the English and French professions, and tried in vain to persuade them to institute the same form of anesthesia in man. However, Brown-Sequard did demonstrate the anesthetizing effect of carbon dioxide on mucous membrane by having animals breathe through tracheal openings, thus excluding entrance of the gas into the lungs. In this manner Brown-Sequard discovered that carbon dioxide induced local anesthesia of mucous membrane independent of its absorption by the blood.

Large Bowel Inflation with Carbon Dioxide.

While a recommendation that carbon dioxide be introduced into the rectum was published in 1863, Von Ziemssen in 1883, was the first physician to describe experiments that established its value for diagnostic

purposes, his method being to develop the gas within the large bowel by introducing bicarbonate of soda into the rectum, then water, and finally, tartaric acid.

Having the large bowel distended, a doctor is enabled to diagnosticate by means of inspection, palpation, precussion, auscultation and the flat-plate. But, while it must be conceded the inflation of the large bowel with air, as practiced by Ewald, Runeberg, Oser and others renders essentially the same service, carbon dioxide is preferable because it is safer.

Bowel inflation demonstrates the facility with which carbon dioxide is absorbed from mucous membrane. This rapidity may be judged from the shortness of the time that is required for a carbon dioxide meteorism to become reduced by absorption, it being evident that this gas enters the venous portion of the blood-stream, after it is introduced into the large bowel.

Ephraim, who came to be very enthusiastic about the use of carbon dioxide in medicine, reasoned that, whenever a quantity of carbon dioxide gas entered the bowel, it was taken up by the red corpuscles within the venous blood-stream, and carried to the lungs in which excessive diffusion of gases took place, the result being an increased accumulation of oxygen within the red-corpuscles of arterial blood. Thus, the presence of the increased amount of oxyhemoglobin in arterial blood was, in Ephraim's opinion, the main factor in the curative effect of carbon dioxide.

Early Therapeutics of Rectal Inflation.

An old book upon "Arzneimittellehre" of which the last edition appeared in 1863 recorded that Kuster and Perkin treated ulcers of the rectum, especially those of dysentery, by inflation of the lower bowel with carbon dioxide gas. Afterwards, nothing appeared in print until the article which was written by Achilles Rose was published in 1883.

The article that Rose wrote appeared in the December issue of the Annals of Anatomy and Surgery, giving the account of a case of dysentery that had been treated with gratifying results. As Rose wrote, "Although carbon dioxide may not destroy the ameba, it is of distinct service for the alleviation of the distressing symptoms of the disease." It is thus interesting to note that physicians of India have insisted for years that patients suffering from dysentery drink water which has been charged with carbon dioxide gas.

Bergeon, who had been using carbon dioxide inflation of the rectum for pertussis, published articles and spoke before medical societies. But he failed to make converts until he sat beside Professor Girod at a dinner party. The professor at once became interested because he had been a sufferer from whooping-cough for months. Having tried numerous remedies in vain, Professor Girod then employed the procedure, and, much to his astonishment, was immediately relieved.

Bergeon's instructions were to inflate the rectum after each paroxysm of coughing, provided that there had elapsed a three-hour-period since the previous inflation in the day. Thus, as a rule, three inflations were administered during the daytime. In obstinate cases, however, nightly inflations were also employed.

In 1897, Dr. Joseph O'Dwyer gave the carbon dioxide treatment for whooping-cough a trial in the New York Foundling Asylum. The results were published by himself and Norton in their treatise that appeared in the fourteenth volume of the Twentieth Century Practice.

Of the one hundred and fifty cases of pertussis in the O'Dwyer and Norton series, one hundred and forty-three patients improved markedly. In fact, by the end of the second or third day, vomiting ceased, whoop disappeared and paroxysms reduced to two or three a day.

The following year Achilles Rose treated a large number of children in the same asylum who were likewise afflicted with whooping-cough. He recorded that carbon dioxide inflation of the lower bowel with carbon dioxide gas was of marked benefit to individuals having pertussis.

Freezing of Tissue with Solid Carbon Dioxide.

In 1899, the author obtained a case of an epithelioma of the face through the courtesy of Professor Harsha at the College of Physicians and Surgeons of Chicago. Then, in the laboratory of Dr. William A. Evans, this malignant growth upon the face was frozen with solid carbon dioxide.

After this time, Dr. William Allen Pusey employed carbon dioxide in solid form with most gratifying results. Following his report in 1905, numerous workers in this country and abroad tried it. The consequence has been that this method of treatment has come to be very well-recognized where freezing of tissue has been indicated.

Abstraction of Heat with Solid Carbon Dioxide.

In 1920, the author also conceived that the abstraction of heat from a portion of the body with carbon dioxide in solid form would relax scar tissue because oedema changed non-resistant, fibrous tissue into that of a soft and yielding state. However, not only was fibrous tissue thus changed, but malignant neoplasms had their metabolism interfered with to the extent so that they could be subdued.

In fact, cautious experimentation demonstrated that fibrous tissue, such as that of benign rectal stricture, changed to a soft and yielding state, after abstraction of heat, and, in like manner, the metabolism of cancer was interfered with to such a degree

that that malignancy was made amenable to its eradication by surgery.

Proof of this Historical Review.

This historical review proves just how well the words of John Kanakl apply to the subject under consideration, he having said in the year 1709: "It is to be hoped that, at this time, no person with common sense will deny that past history is the most important, and the most valued support of the whole of medicine because, by thorough knowledge of the history, we learn to arrive at the true pathologico-etiologic conception, and consequently, we find the necessary, and reliable indication for the use of a curable measure."

Thus, we learn from this history that carbon dioxide has been therapeutically employed throughout the ages with gratifying results.

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Rate of Elimination of Labeled Carbon Dioxide From the Body[1]

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ABSTRACT

When C^{14} labeled bicarbonate is injected intravenously about 10% of it is exhaled within 30 seconds by the lungs before it is mixed with body CO_2. Labeled CO_2 that has escaped elimination by the lungs is mixed with body CO_2 in a complex manner. The CO_2 in those tissues with a high blood flow equilibrates very rapidly with that of the blood. Resting muscle and skin need at least 5 minutes for equilibration with the CO_2 of the blood. Calculations of body CO_2 pool based on the assumption of complete instantaneous equilibrium between blood and tissues are not valid. The manner of elimination of labeled CO_2 produced in metabolism is complex and does not simulate that following intravenous or intraperitoneal injection of labeled bicarbonate.

RATE AT WHICH labeled carbon dioxide is eliminated from the body is of great interest to the student of intermediary metabolism, since it is the end product of oxidation in the body of C^{14}-labeled foodstuffs. Because of the body bicarbonate pool it would not be eliminated by the lungs at first at the same rate as it is produced by the tissues of the body. Several workers recently have attempted to determine the size of this pool by following the elimination of tagged CO_2 from the lungs after intraperitoneal or intravenous injection of a known amount of radioactive bicarbonate assuming the body is an ideal compartment in which CO_2 is immediately and thoroughly mixed. Such a method might give only approximate duplication of the elimination of radioactive carbon dioxide produced in the cells. The bicarbonate that is introduced into the body intravenously or intraperitoneally goes first to the lungs before equilibration with the carbon dioxide of the tissues, whereas labeled carbon dioxide produced from metabolism in the cells would be diluted by the carbon dioxide already present in the tissues before entering the venous blood to be taken to the lungs. Furthermore this method assumes a rapid uniform distribution of the tagged injected carbonate with the untagged carbon dioxide throughout the body. This would hardly be so since equilibration would take place rapidly in organs with large blood-tissue perfusion rates like the liver and heart, whereas tissues like skin, fat and resting skeletal muscle with low blood-tissue perfusion rates would be slow in attaining equilibrium. The work reported in this paper was planned to determine the magnitude of these effects and if possible to obtain more exact information that may be used in estimating metabolic production of CO_2 based on the rate of its elimination by the lungs.

Kornberg *et al.* (1) followed the disposal of C^{14}-labeled bicarbonate by the cat after a single injection. In 4 hours about 80% of the injected carbonate had been eliminated by the lungs, 1% was in blood, muscles and viscera, 6% was in bone and about 3% had been incorporated into urea. The relatively large amount of residual activity present in bone was due to the slow turnover rate of this tissue which has been shown by others (2) (3). Our concern was with the tissues having much higher mixing rates since it would be these that would affect the rate of elimination of CO_2 produced by oxidation in the usual metabolic studies lasting 8 hours or less.

In this paper we will use the expression 'metabolic $C^{14}O_2$' to indicate $C^{14}O_2$ produced

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TABLE 1. EFFECT OF SITE OF ADMINISTRATION ON PERCENTAGE OF INJECTED $C^{14}O_2$ EXHALED DURING THE 1ST MINUTE AFTER INJECTION

	I.V. Inj.		Inj. in Ascending Aorta	Inj. in Left Ventricle
1st 20 sec. after injection	8.61	8.64	.69	1.07
2nd 20 sec. after injection	2.14	1.57	.58	1.47
3rd 20 sec. after injection	1.50	.73	.56	1.06

by the oxidation of C^{14}-labeled fuels in the body in order to distinguish it from $C^{14}O_2$ injected as bicarbonate to study mixing of CO_2 in the body. We use the term 'CO_2 content' to include CO_2 in solution and that chemically bound as bicarbonate or carbonate.

METHODS

The problem was studied in two ways. In one the rate of elimination of labeled CO_2 was followed during several short periods after the single intravenous injection of a definite amount of radioactive bicarbonate. In some experiments the bicarbonate solution was injected into the left ventricle. For intraventricular injection a fine plastic tube was run down from the right carotid into the left ventricle and tied in place 2 or 3 hours before the experiment. At the same time a cannula was tied into the trachea from which the expired air could be passed through an absorber containing sodium hydroxide solution to collect the exhaled CO_2. The carbonate was precipitated as the barium salt and the radioactivity was determined on planchet samples of infinite thickness.

The problem was also studied by injecting tagged bicarbonate and then after a given interval killing the animal and determining the specific activity of the CO_2 of the blood and individual tissues. This method reveals the manner in which the tissues, important for this study, take up the circulating labeled CO_2.

Analysis of Tissues. As soon as the animal was anesthetized by injected Nembutal, the abdomen was opened and blood was aspirated from the abdominal aorta. Tissues were then taken and frozen in liquid nitrogen. For extraction of the CO_2, the tissues were homogenized in a modified Waring Blendor which was a closed system with a large ground glass jointed top having inlet and outlet tubes for aeration and an inlet tube for introduction of liquids. After the tissue was ground up lactic acid was added and the CO_2 was aerated over into a barium hydroxide solution where it was precipitated as barium carbonate.

RESULTS

Amount of Labeled CO_2 Eliminated Before Body Mixing When It Has Been Given by Intravenous Injection. This can be determined by comparing the rate of elimination after intravenous injection with that after injection into the left ventricle. Table 1 gives the results of such an experiment. It may be seen that radioactive CO_2 is eliminated at a very high rate during the first few seconds after intravenous injection but that this rate rapidly declines so that at the end of the first minute it is about the same as that after injection into the arterial blood. In the dye dilution method for the determination of cardiac output the intravenously injected dye proceeding from its first passage through the lungs reaches negligible concentrations in the arterial blood in 30 seconds. Bicarbonate may be expected to be somewhat delayed since the lung pool for it would be larger because of the alveolar CO_2 which is in equilibrium with the blood bicarbonate. However it appears most likely that practically all of the intravenously injected bicarbonate has been pumped into the arterial system within 1 minute. In studying the mixing of labeled CO_2 in the body after intravenous injection one should discount material eliminated in the 1st minute. It is surprising to find so much $C^{14}O_2$ eliminated in the first 20 seconds after intraventricular injection. It probably results from some of the injected material getting to the lungs before much dilution by blood or tissues. This could pass to the lungs by way of the bronchial arteries or by short circuits through the heart.

Changes in Rate of Elimination of Labeled CO_2 After the First Mixing in the Body. Table 2 shows the fraction of body $C^{14}O_2$ that is eliminated in successive minutes after injection. The amount in the body for the successive periods is calculated by subtracting from injected radioactivity the activity that has previously been eliminated. The elimination for the first minute is not given although it was used in estimating the radioactivity

TABLE 2. PERCENTAGE OF $C^{14}O_2$ IN THE BODY EXHALED IN SUCCESSIVE MINUTES AFTER INJECTION

Minute After Injection	Injected Intravenously	Injected Into Left Ventricle
2	1.87	2.94
3	1.40	4.45
4	1.27	3.85
5	1.28	2.00
6	1.08	2.00
7	1.03	1.47

remaining in the body. It is apparent that the injected bicarbonate continues to be diluted (or distributed in a greater volume) for at least the first 7 minutes after injection.

Relative Activities of Blood and Tissue CO_2 at Various Times After Injection of a Dose of $NaHC^{14}O_3$. Table 3 shows the activities of the CO_2 from different tissues relative to that of the blood at the same time at various times after injection. It is apparent that muscle and skin are slow in taking up the labeled material from the blood whereas liver appears to take it up quite rapidly. The relatively higher figure for muscle at 18 minutes indicates that this tissue is sluggish in releasing the labeled CO_2 to the blood later on.

It may be noticed that although the relative activity of liver CO_2 is high even at the end of 1 minute it always remains less than that of the blood. This does not necessarily indicate an inadequate perfusion rate for this tissue. If the passage of CO_2 through the tissue cells requires an appreciable gradient the concentration of metabolic CO_2 (unlabeled) would be greater in the tissue than in the blood.

DISCUSSION

Our results show that the mixing of CO_2 in the body is a complicated process and is fairly slow in some tissues. It appears to proceed in the different tissues in a manner that is fairly predictable on the basis of known values for blood flow and CO_2 content for each tissue. The CO_2 concentration of tissues follows roughly that of the blood, the concentration per liter of tissue water being about the same as that in the plasma (4). In table 4 are given values for blood flow and CO_2 content of the important tissues of the body in which CO_2 mixes relatively rapidly. These may be divided into two categories: the first, consisting of brain, liver, kidneys and heart, has a high ratio of blood flow to CO_2 content; the other, comprising resting muscle and skin, contains most of the body CO_2 but has low tissue blood perfusion rates. The tissues of the first category also have high metabolic rates so that when a labeled fuel is given, tagged CO_2 produced by them would be little diluted by CO_2 already present. The labeled CO_2 from these tissues would therefore go to the lungs without being diluted by the large pool of CO_2 in muscle and skin. Some 10% of this CO_2 would be removed in the first passage through the lungs. Of that remaining in the blood some 60% would pass through the highly vascular tissues and again go to the lungs without muscle-skin dilution. Such recycling would give a higher rate of elimination of metabolic CO_2 at first than would be expected if it were first thoroughly mixed with the body pool.

Our results indicate that the mixing of injected CO_2 with the body CO_2 pool is not rapid. Sixty-five to seventy-five per cent of the CO_2 pool of the body is in tissues with a low blood flow rate compared to their CO_2 content. These tissues take up $C^{14}O_2$ sluggishly from the blood and would yield it equally

TABLE 3. RELATIVE ACTIVITIES OF CO_2 OF DIFFERENT TISSUES OF ANIMALS KILLED AT VARIOUS TIMES AFTER INJECTION OF $NaHC^{14}O_3$

Time After Inj., min.	Relative Activities				
	Blood	Muscle	Liver	Fat	Skin
1	100	28	88		
2	100	29	93	60	41
2.5	100	37	66		
5	100	58	74	64	
6	100	75	63		
13	100	96	93		
18	100	107			

TABLE 4. RELATIONSHIP OF BLOOD FLOW TO CO_2 CONTENT OF VARIOUS TISSUES

	Flow, ml/min/100 gm Organ Weight	% of Total Blood Flow*	Values for 1 kg Rabbit	
			Total CO_2 content of organ, mM†	% of total body CO_2
Brain	54	3.6	.1	1.1
Heart	70	4.7	.05	.6
Liver	80	25.1	.5	5.7
Kidneys	430	25.4	.07	.8
Muscle (resting)	7	28.2	5	57.3
Skin	3.5	12.9	1.5	17.2
Blood & alveolar air			1.5	17.2

* These values are for all of the particular tissue in the body. Blood flow values are from H. D. GREEN. In: *Medical Physics*, edited by OTTO GLASSER. Chicago: Yr. Bk. Pub., 1950, p. 244, vol. II, *Circulatory System: Physical Principles*.

† Values for muscle from Irving *et al.*, reference 4. Other values are ours.

sluggishly when the partial pressure of $C^{14}O_2$ is higher in them than in the blood. It follows that the method of calculating the body carbonate pool from the curve of specific activity of respired CO_2 after injection of a dose of labeled bicarbonate can never give accurate values. The magnitude of the pool so calculated would be too low during the period of uptake by these 'sluggish' tissues and it would be too high during the period when they would be giving up stored labeled CO_2 to the blood.

Kornberg *et al.* (1) report that after intravenous injection of labeled bicarbonate the tagged carbon is excreted in the respired CO_2 at a rate that corresponds to an exponential curve having three components with half-lives of 2.85, 29.6 and 582 minutes. They did not subdivide the respiration period of the 1st minute after injection so did not observe any component due to the elimination by the lungs before mixing with any body CO_2. The half-life of this must be similar to that of the dye concentration after injection in cardiac output determinations which for the rabbit is about 10 seconds. The 2.85-minute component of Kornberg *et al.* might be a composite of this very rapid one and one produced by taking up labeled CO_2 by the pool in the sluggish tissues (total time about 10 min.). The component having a half time of 29.6 minutes could correspond to the period during which the sluggish tissues yielded their stored tagged material. The long component of 582 minutes would depend on processes that we did not study such as bone deposition, recirculation of carbonate in digestive secretions and oxidation of compounds in which CO_2 had been 'fixed.'

Our results indicate that the manner in which metabolic $C^{14}O_2$ is eliminated depends considerably on where it is produced. If it is produced by tissues having high blood perfusion rates it would be eliminated more rapidly at first than in an ideal body in which there was rapid and complete mixing of CO_2. If the metabolic $C^{14}O_2$ were only produced in muscle that was relatively inactive the elimination would be slower than in the ideal body. If the metabolic $C^{14}O_2$ is produced in all tissues at rates proportional to their total metabolic rates elimination would simulate the former situation, since the tissues with high blood perfusion rates have high metabolic rates and the metabolic $C^{14}O_2$ would be produced chiefly in them.

It is evident that it would be impossible to calculate with any accuracy the rate of oxidation of a labeled fuel having a changing specific activity even though this is known at all times. The preferred experimental procedure would be to produce a steady state which may be attained by maintaining the labeled fuel at a constant concentration and constant specific activity for several hours. In such a situation with certain fuels the metabolic $C^{14}O_2$ is produced at a constant rate and the manner in which it would be eliminated would be most closely simulated experimentally by injecting labeled bicarbonate into the left ventricle at a constant rate. The chief difference between the two conditions is that of the gradient of $C^{14}O_2$ between blood and tissues. When $C^{14}O_2$ is produced in metabolism the gradient would be from tissues to blood and when it is injected the gradient is from blood to tissues. It is quite possible that the movement of CO_2 through the tissues may involve a considerable gradient.

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Gas transport and the red cell

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THE GASES to be considered are oxygen, carbon dioxide, and inert gases. As the cell is a transport vehicle, we will deal with those properties of the cell which are involved in transport of gas by it. Relative gas capacity of the cell, transport inside the cell, transport into the cell, and transport out of the cell will be considered.

Because these terms will be used, passive diffusion and facilitated diffusion are defined as follows. Passive diffusion is diffusion of dissolved gases in accordance with Fick's law. Facilitated diffusion is also diffusion in the direction of the chemical potential gradient of the gas, but involves an additional path, thus increasing total diffusion transport.

OXYGEN

The carrier capacity of the red cell is greatly increased by the binding of oxygen by hemoglobin. The oxygenation of hemoglobin at different equilibrium partial pressures of oxygen (hereafter called oxygen tension) has been measured for many types of hemoglobin and the kinetics of the binding of oxygen molecules to the four sites have been analyzed by many authors.

The binding of oxygen to hemoglobin also furnishes facilitated oxygen transport as was demonstrated by Scholander (6). At low oxygen tension this facilitated transport may greatly exceed the passive diffusion transport. Oxygen transport inside the red cell is therefore the sum of passive diffusion of dissolved oxygen and facilitated transport by hemoglobin.

Transport of oxygen into the red cell is by passive diffusion through the cell membrane and then by passive diffusion and hemoglobin facilitated transport toward the cell interior. Hemoglobin aids the oxygen charging of the cell not only by providing greater oxygen capacity but also by increasing transport to utilize the capacity more rapidly.

Discharge of oxygen from the red cell is essentially the reverse of the charging process.

Several attempts have been made to explain the facilitated transport of oxygen by hemoglobin. The most prevalent suggestion is that the oxygen is carried by diffusing hemoglobin. I have recently measured the diffusion coefficient of ^{59}Fe-tagged hemoglobin under the experimental conditions used by Scholander (6) to measure the transport, in millipore filters, and obtained a value which fits this concept exactly. Several years ago I estimated oxygenation gradients in conducting hemoglobin solutions and obtained some values at higher oxygen tensions which do not fit this concept (2). I then suggested that the available data were compatible with transport of oxygen by hemoglobin collision exchange. What is lacking for a resolution of this problem is a rigid mathematical analysis of possible transport by a diffusing carrier of a substance which combines with and leaves the carrier many times during transit. More precise data of oxyhemoglobin gradients along the diffusion path are also needed.

CARBON DIOXIDE

Carbon dioxide is much more soluble in water than is oxygen, and hence transported more rapidly in aqueous solutions by passive diffusion. However, this transport can be enhanced by bicarbonate ions. Figure 1 shows the equilibrium relationships between dissolved CO_2 and bicarbonate ions. For example, at pH 6.8, the bicarbonate ions contain five-sixths of the total CO_2 in an equilibrium solution. Thus bicarbonate ions may play a role in CO_2 transport quite similar to the role of oxyhemoglobin in facilitating oxygen transport. The reversible exchange between dissolved CO_2 and bicarbonate ions is very slow but it is greatly accelerated by carbonic anhydrase. Figure 2 part I shows the reaction by the two postulated paths. Figure 2 part II shows a possible mode of action of the enzyme. Human red cells probably have the highest known carbonic anhydrase activity. It makes more of the bicarbonate ions—under many physiological conditions all of them—available for CO_2 transport.

Figure 3 shows the facilitation of $^{14}CO_2$ transport by bicarbonate ions: with carbonic anhydrase, without carbonic anhydrase, and with carbonic anhydrase inhibited by acetazolamide. These data were obtained in aqueous solutions with 0.025 M bicarbonate and also with 1 mg/ml bovine red cell carbonic anhydrase, conditions approximating those inside the human red cell. From Fig. 3 it can be seen that at pH 7 the transport increase due to carbonic anhydrase is approximately fivefold, while at pH 7.5 it is about ninefold.

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In summary, it may be stated that bicarbonate ions, when their interchange with dissolved CO_2 is catalyzed by carbonic anhydrase, play a role in red cell CO_2 capacity and transport similar to that of oxyhemoglobin in oxygen capacity and transport. The facilitated transport mechanism here is clearly bicarbonate ion diffusion.

The red cells present another interesting possibility in CO_2 transport. The plasma must be traversed by CO_2 entering and leaving the red cells. Plasma contains bicarbonate ions; at pH 7.4 the equilibrium concentration is 20 times that of dissolved CO_2, but it contains no carbonic anhydrase. This does not exclude possible enzyme action on plasma bicarbonate ions if there is enzyme activity on the outside of the cell membranes.

We made two studies of possible enzyme action in red cell membranes. Washed red cell ghosts (3) were found to enhance $^{14}CO_2$ transport, and the effect was inhibited by acetazolamide. Hence they contain the enzyme, but this is not conclusive evidence of its presence on the outer surface of intact cells. Whole blood was then tested for facilitated transport, but the data indicate facilitation only in the cells (5). The last result does not necessarily negate the first as our test blood was unstirred. Blood in capillaries is rather well stirred and exposes more plasma to contact with red cell membranes.

Perhaps the best evidence for carbonic anhydrase activity is shown in Fig. 4 (1). This is an experiment performed by Francis P. Chinard and myself. We injected $H_2{}^{18}O$ into the pulmonary artery of anesthetized dogs. The ordinates of Fig. 4 give the ^{18}O percentage in the exhaled CO_2 as measured continuously with a mass spectrometer. The data show maximum $^{12}C^{16}O^{18}O$ concentration in the first samples followed by rapid exponential decrease. This indicated *a*) complete equilibration of ^{18}O among CO_2 bicarbonate and water between the time of injection and the taking of the first sample (in some experiments less than a second) and *b*) availability of all tagged forms of CO_2 for expiration.

$$pH = 6.1 + \log \frac{[HCO_3^-]}{[CO_2]}$$

$$\frac{[HCO_3^-]}{[CO_2]} = 10^{(pH-6.1)}$$

FIG. 1. Simplified representation of ratios of dissolved CO_2 and bicarbonate ions at equilibrium.

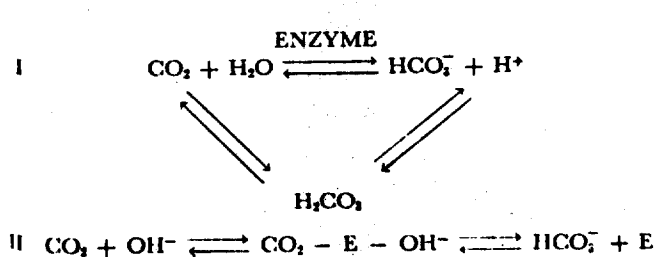

FIG. 2. Carbonic anhydrase and the interconversion of CO_2 and bicarbonate ions. In II, E denotes the carbonic anhydrase.

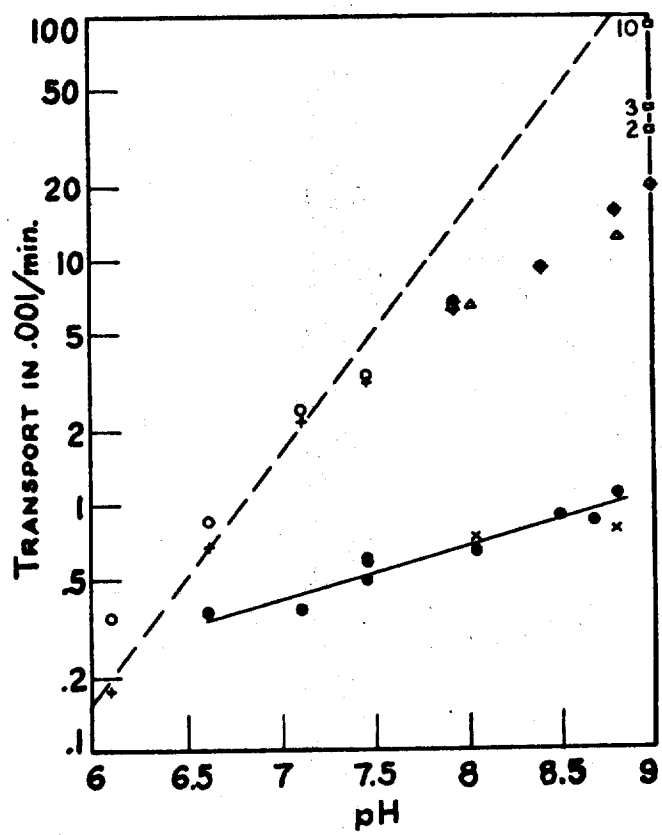

FIG. 3. Carbon dioxide transport through solutions containing 0.025 $NaHCO_3$. Symbols: ○ = total transport with carbonic anhydrase at 1 mg/ml; + = same values minus CO_2 diffusion transport; △ = total transport with carbonic anhydrase at 1 mg/ml and no CO_2 in the lower chamber; □ = increased concentration of carbonic anhydrase (numbers represent concentration of carbonic anhydrase in milligrams per milliliter); ⊗ = buffer solution only; × = 1 mg of carbonic anhydrase/ml plus acetazolamide at 0.01 mg/ml at pH 8 or at 10 mg/ml at pH 8.8. (Reproduced by courtesy of *Science* 155: 44, 1967. Copyright 1967.)

There is no hint of a slow component in the disappearance curve such as might be expected if plasma bicarbonate were exchanging oxygen with water in the absence of enzyme. After administration of inhibitor, the concentration of ^{18}O was greatly decreased and in the latter part of the curve, the values were in the order of the instrumental error.

INERT GASES

I am not aware of any evidence that inert gases enter or leave cells by any process other than passive diffusion. This transport is proportional to diffusion coefficients and concentration gradients. For any given difference in gas tension the gradients are in turn proportional to the solubilities of the gases in the tissues through which they pass.

An observation bearing on the action of inert gases on living systems may be of interest. We have measured the partial molar volume (4), the increase in water volume due to addition of 1 mole of gas, for He, O_2, N_2, A, and CO_2. The values are approximately 30 cm^3 for He, 32

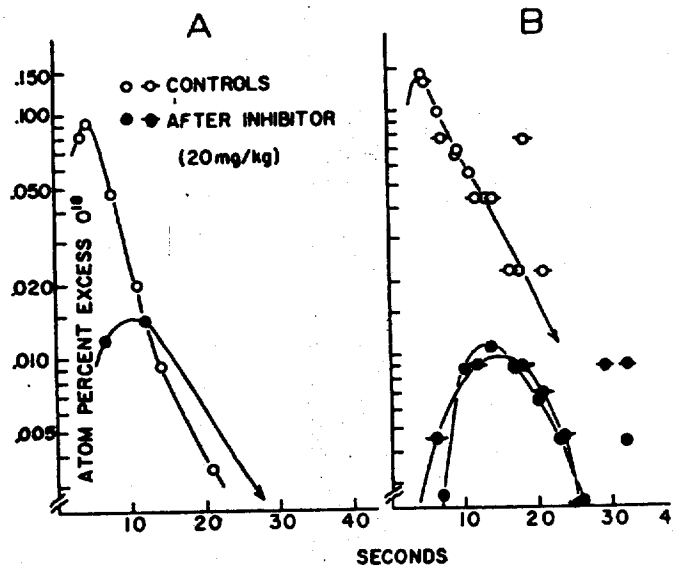

FIG. 4. Atom % excess ^{18}O in expired carbon dioxide as a function of time after injection of $H_2{}^{18}O$. The sequence of the injections is given by the sequence of the symbols. *A* and *B* panels represent experiments in different dogs. The lower curves in panel *B* are duplicate runs in a single animal. (From Chinard, Enns and Nolan (1) reproduced by courtesy of *Am. J. Physiol.*)

cm³ for O_2, N_2, and A, and 35 cm³ for CO_2. They were obtained at gas tensions of one atmosphere and, in some cases, one-half atmosphere. There is no evidence here that any gas has a unique effect on the structure of water. The effect depends only on the amount of inert gas. Hence one may speculate that the effect of inert gases such as nitrogen narcosis may be due entirely to the concentration of gas at the site of action.

SUMMARY

The red cell carries oxygen and carbon dioxide on its circulatory path. The capacity of the cell for oxygen as well as the transport of oxygen into and out of the cell is enhanced by hemoglobin. A similar enhancement of capacity and transport is provided for CO_2 by bicarbonate ions through the action of carbonic anhydrase. There is also some evidence of carbonic anhydrase activity in plasma, possibly from enzyme in the cell membrane.

While inert gases appear to play no significant role in the functioning of red cells at atmospheric pressure, it was pointed out that dissolved gases have nearly equal partial molal volumes. This implies that all inert gases have very nearly the same effect on the physical properties of water.

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E. E. G. Changes During Administration of Carbon Dioxide

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A mixture of 30 per cent carbon dioxide and 70 per cent oxygen is being used in the treatment of certain psychiatric conditions. The physiological basis of this treatment is, however, obscure and theoretical. In an attempt to further elucidate the physiology involved, EEG's were obtained during the course of routine treatments.

Technique

A group of 37 young adults, most of whom had never had a previous treatment, were used as volunteer subjects. Electrodes were placed over the frontal, occipital, and temporal areas of the skull on both sides. Each lead was run to the same bilaterally connected ear lead. In 29 patients, a precordial lead was also used so that an EKG could be obtained simultaneously with the EEG. A control record was obtained with the patient supine; he then voluntarily hyperventilated for three minutes following which the EEG record was obtained for another two minutes or until such time after overbreathing that the record returned to its former pattern. After a five minute rest, and without moving the patient, a mask with an attached bag as a reservoir was applied over the face and nose and 30 per cent CO_2-70 per cent O_2 mixture of about 6 pounds pressure was administered. An EEG was obtained at the same time. The person administering the gas counted aloud the patient's respirations.

Results

A fairly constant EEG pattern was obtained during a treatment. This consisted of a short period of alpha, then loss of this alpha with a general decrease in the amplitude, followed by a fairly sudden appearance of high voltage, slow, predominantly 4 to 6 per second regular waves whose definition and amplitude increased in a crescendo, plateaued and then decreased more rapidly than they appeared. An irregular, low voltage, slow wave pattern was then noted which in some records seemed to differ from the low voltage pattern that immediately preceded the high voltage phase in that the former contained more delta and was more irregular. Finally, the pretreatment pattern reappeared. (Figure 1.) The EEG's which were obtained have been grouped into: (1) results of the total series, (2) results of those in which only 30 breaths of CO_2-O_2 mixture were given, (3) results of those in

From Neurology Section, V.A. Hospital, San Francisco, Calif., and Association for Mental Health, South Bend, Ind.

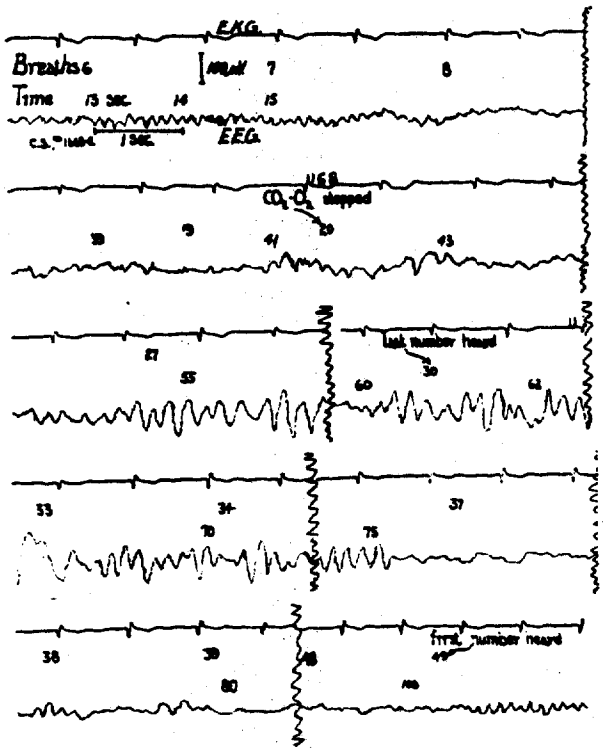

Figure 1. Portions from a record showing the entire sequence of changes: alpha, loss of alpha, high voltage 5/second slow waves ("build-up"), low voltage, irregular slow waves, and reappearance of the alpha. This particular tracing was chosen because of the minimal amount of artifact; however, it is atypical in that the "build-up" begins after the gas was stopped and there is not the usually greater amount of slowing after the "build-up" than before the "build-up."

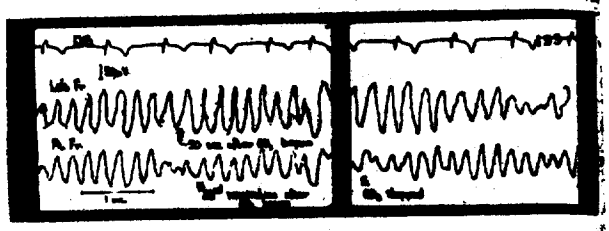

Figure 2. A very clear "build-up."

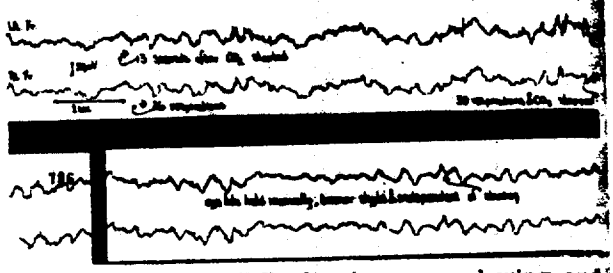

Figure 3. An E.E.G. showing some slowing and increase in voltage but not regular enough to be defined as a "build-up." The record is a continuous one except for about 0.4 second at the black line in the lower half of the figure.

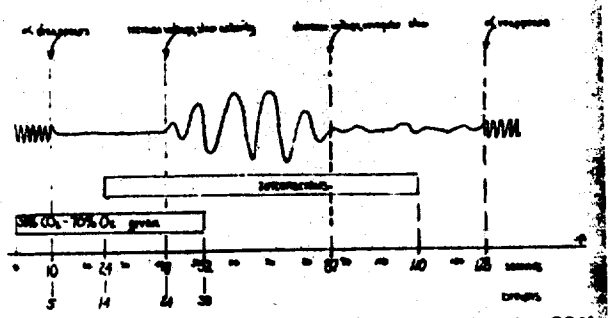

Figure 4. A "typical" E.E.G. response to 30% CO_2-70% O_2 based on the average obtained from our group that received 30 breaths of the gas.

which only 30 breaths of gas were given and there was a "build-up," and (4) those, where regardless of how much gas was given, there was a "build-up." We have used the term "build-up" to describe the appearance of not only high voltage, slow activity, but, rather to define a striking phenomenon during which these high voltage slow waves were well delineated, regular, sinusoidal and with definite limits. (Figures 2 & 3.) The time in seconds and in breaths required for each of the various parts of the EEG sequence have been tabulated (Table 1) and using the averages obtained from these a tracing of a "typical" record has been constructed. (Figure 4.) Because of several minor changes in our routine and the production of artifact during part of some of the records, each of the various phases of the EEG sequence that is tabulated does not represent the total number of EEG's that were obtained; for this reason, each entity in Table 1 has listed with it the number of records upon which the various averages were based. Therefore, where only several, or in some cases only one record is used to form an average, the statistical reliability must be very poor or nil. The various changes in the EEG will usually be discussed on the basis of the average; however, the range of each item is also given in Table 1

1. *Total time the gas was given:* The average time that the CO_2-O_2 mixture was given was about the same in all the groups. It took about 50 to 52 seconds for the average patient to inhale 29 to 30 times. The range of the "no build-up" group was slightly greater than in the other groups; this is probably because these records were from patients who not infrequently exhibited but little change in the EEG during the treatment, so that the administration of the gas

was continued for a longer period of time. On the other hand, there were those in this particular group who became so frightened by the procedure that it had to be terminated earlier than usual. The shorter end of the range in the other groups can be accounted for by patients who exhibited a marked clinical effect—considerable tremor, rigidity, etc.—early in the course of treatment. or who had an early and marked "build-up" in the EEG which caused us to terminate the treatment early.

2. *Changes in alpha:* The time at which the alpha disappeared was, on occasion, difficult to determine because of the frequent loss of alpha when the mask was applied to the face (attention?) or because of the muscle artifact which appeared early in many cases; this artifact did not have to be marked in order to obscure the alpha. However, in those where changes could be seen, the alpha disappeared on the average in 10 to 15 seconds (5 to 7 breaths) after the treatment was begun. The alpha returned earlier if there was no "buld-up," reappearing in approximately 100 seconds after treatment was begun, as compared to 128 to 131 seconds when there was a "build-up."

3. *Changes in consciousness:* This was tested by asking the patient what was the last number that he heard, and then, the first number he heard when he again became aware of the counting. If the patient claimed he heard all the numbers, it was assumed that he did not lose consciousness. This is a common method used when the CO_2-O_2 mixture is given in the clinic; however, it is very unreliable. The answer given by the patient varied according to how soon he was quizzed about it after the treatment was over and it was easily changed by minimal suggestion. One reliable and intelligent patient claimed inability to hear any number between 20 and 70. He was eagerly looking forward to his release from military duty in five days; the statement "you have 50 more days" was used instead of the count "50" and this he heard while by the count method he was regarded as being unconscious. Another male patient who did not remember any count between 50 and 70 did recall hearing the statement "60 beautiful girls just walked into the Lab," which was used in the place of the count "60." However, using the count method as a guide for the loss and regaining of consciousness, the average patient was "unconscious" in 24 to 28 seconds (14 to 16 breaths). "Consciousness" was regained, on the average, within 110 seconds after the treatment was begun.

4. *High voltage, slow activity, and "build-up":* Only one out of 36 patients had no discernible increase in voltage or slowing of his basic rhythm; 29 of the remaining 35 patients had a "build-up." The average duration of the high voltage, slow activity in the group where it was not regular or sinusoidal enough to be classified as a "build-up" by our definition, was 21 seconds and the amplitude was 78 microvolts; this was decidedly less than the group that included a "build-up" where the average duration was 47 seconds and the average amplitude was about 128 microvolts.

5. *Relation of the EEG during treatment to the clinical manifestations:* Of the five apparent grand mal seizures noted in our series, one of the patients represented two of our experimental EEG's; he had a grand mal seizure each time. He had received a course of CO_2-O_2 treatment previously and it was observed that he had a grand mal seizure with almost every treatment; he was purposely chosen for the experimental series. During one of the treatments he had a considerable "build-up" but during the other experimental treatment, the EEG record was so full of artifact that the details could not be made out adequately. The control record before the first treatment was interpreted as borderline because of a small amount of scattered slowing whereas the control record before the second treatment was interpreted as normal. Another patient who also had a grand mal seizure with each treatment and hence was also purposely chosen for our experiment, had a convulsion during the experimental treatment, too. His record was so full of artifact that it could not be decided whether there was a "build-up" or not. Again, the control portion of the EEG was normal. The third patient *may* have had a convulsion; he did not have a "build-up." His convulsion (?) was not a typical tonic seizure, followed by clonic jerking, but there was such rigidity followed

by such tremor that we considered it to be possibly a grand mal seizure. The control portion of the record was mildly abnormal with a scattering of delta activity and poorly defined spiky activity. Of all those who had a convulsion, there was nothing in the readable parts of the treatment record that distinguished them from the other treatment records, although admittedly, the great amount of artifact that was obtained may have obscured some pertinent features.

6. *Relation between the pre-treatment (control) EEG's and the EEG's during treatment:* There were three abnormal pretreatment (control) EEG's in addition to another three which, though they had normal prehyperventilation records, had considerable nonfocal high voltage slow activity during hyperventilation which was sustained for more than a minute after the overbreathing stopped. There was no constant relationship between the pre-CO_2-O_2 EEG and any changes noted in the EEG during treatment.

7. *Changes in pulse rate during treatment:* The pulse rate was counted in 29 patients immediately before the treatment was begun, immediately after the treatment was terminated, and again when the "build-up" was at its height. There was no constant change in the pulse rate; it was the same before and after treatment in 2, it increased after treatment in 12, and it decreased after treatment in 15. Of interest was the presence of extrasystoles in five individuals during and immediately following treatment. There was no constant relationship between the direction of change of the rate of the pulse and the presence of extrasystoles.

Discussion

During 30 per cent CO_2-70 per cent O_2 treatment there is a fairly constant sequence of change in cerebral metabolism as measured by alterations in the pattern of the EEG. The averages we have used to compile our "typical" EEG response are based on figures with a considerable range. This is probably to be expected for Meduna[1] notes that the response may vary from treatment to treatment and from patient to patient during the course of a series of treatments. The loss of alpha and the appearance of a low voltage pattern is the EEG response to increased CO_2[2,3] and decrease O_2[4,5] tension. The most outstanding change in our "typical" EEG was the "build-up." Whether, when seen, this is a definite entity or merely a marked amount of the same slowing noted in several other records is not certain. However, it was so striking that we have considered it, at least tentatively, as a particular phenomenon. Meduna quotes Gibbs as describing 3 to 4 per second high voltage activity in the EEG during the "second phase (state of narcosis)" of a 30 per cent CO_2-70 per cent O_2 treatment; this began 6 to 12 respirations after the treatment was started, and continued for another 6 to 12 respirations. If this is the same change we have described as high voltage, slow activity and/or "build-up" it occurred later in our group. We did not compute the duration of high voltage, slow activity in terms of respirations but rather in seconds; in terms of seconds the duration of this phenomenon in our series was about the same as the time of onset of this change and hence the number of respirations are probably about the same; i.e., average about 23 to 24 breaths The difference in time of the onset and duration of the slow activity between Meduna's and our group may be due to some differences in technique and may not represent anything more important than that. There is, however, another discrepancy and that is between the frequency of the slow activity noted by Gibbs—3 to 4 per second—and that most frequently seen in our series—4 to 6 per second.

The presence of extrasystoles in five patients suggests an increased cardiac irritability. This adds further confirmation to Meduna's[6] belief that heart disease is a contraindication to treatment.

Summary

1. EEG's of 37 persons given 30 per cent CO_2-70 per cent O_2 inhalations in a manner similar to that given in the treatment of certain psychiatric conditions was obtained in an attempt to further elucidate the physiological basis of this particular type of treatment.

2. A fairly characteristic pattern of change in the EEG was seen and has been discussed.

TABLE 1

			Total	30 breaths	Total "build-up"	30 breaths "build-up"	Without "build-up"
Total time CO_2-O_2 given	Average	(seconds)	52	52	50	52	46
	Range	(")	93-26	71-36	71-33	71-36	80-26
	No. entries	(")	38	18	27	15	8
	Average	(breaths)	29	30	29	30	26
	Range	(")	50-15	30-30	40-20	30-30	50-15
	No. entries	(")	37	18	27	15	8
Time & disappears	Average	(seconds)	12	10	11	10	14
	Range	(")	20-3	16-6	16-3	16-6	19-9
	No. entries	(")	21	9	16	8	4
	Average	(breaths)	6	5	5	5	7
	Range	(")	8-2	8-5	8-2	8-5	8-5
	No. entries	(")	21	9	5	8	4
Time consciousness lost	Average	(seconds)	28	24	28	24	24
	Range	(")	67-9	44-12	67-9	44-9	24
	No. entries	(")	24	14	22	13	1
	Average	(breaths)	16	14	16	14	17
	Range	(")	50-5	24-6	50-5	24-6	17
	No. entries	(")	24	14	22	13	1
Time consciousness regained	Average	(seconds)	126	110	105	110	115
	Range	(")	146-56	146-78	146-56	146-78	115
	No. entries	(")	21	12	20	12	1
Time onset increase voltage slow activity	Average	(seconds)	42	45	41	43	42
	Range	(")	81-0	81-0	81-24	81-24	80-0
	No. entries	(")	36	16	27	14	8
	Average	(breaths)	24	23	24	24	23
	Range	(")	58-0	36-0	58-13	34-13	36-0
	No. entries	(")	34	17	26	14	7
Duration of increase voltage, slow activity	Average	(seconds)	41	46	47	51	21
	Range	(")	93-0	88-0	93-10	88-19	64-0
	No. entries	(")	35	17	27	14	8
Amplitude of increase voltage, slow activity	Average	(MV)	120	116	134	125	78
	Range	(")	270-0	210-0	240-60	210-60	270-0
	No. entries	(")	36	17	27	15	8
Time & reappears	Average	(seconds)	128	128	128	131	100
	Range	(")	290-60	183-62	285-73	183-95	141-60
	No. entries	(")	34	16	25	13	7

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PRELIMINARY REPORT ON THE TERATOGENIC ACTION OF CARBON DIOXIDE
[Nota Preliminare sull'Azione Teratogenica dell'Ossido di Carbonio]

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Department of Siena -- Meeting of March 21, 1955

Prompted by a repeated clinical observation noted by Giuntini and Corneli of an infant with multiple congenital deformations born of a mother supposedly poisoned by carbon dioxide fumes during pregnancy, we undertook an experimental study with the object of testing the teratogenic action of carbon dioxide in mammals.

The experiments were performed on guinea pigs in the first and second months of gestation. Using the methodology described below, the animals were made to breathe in an atmosphere containing carbon dioxide.

A) A first group of 40 selected subjects, of a mean weight of 250 g, was placed in a wooden box, specially constructed for the experiment, hermetically sealed and furnished with a special opening through which the carbon dioxide, obtained by dehydrating formic acid through concentrated and heated sulfuric acid, was introduced with the aid of a special apparatus.

$$\underset{46.02\ g}{HCOOH} \qquad \underset{28\ g}{CO + H_2O}$$

As a result of this reaction, a gram molecule of formic acid discharges a gram molecule of carbon dioxide. Thus, from 46.02 g of formic acid, 28 g of carbon dioxide are discharged and, at 0° and 760 mm pressure, make up 22.4 liters. The amount of formic acid used for this first series of experiments was 2.5 g.

Taking into consideration the fact that the box is 0.144 m^3, we verified a concentration of carbon dioxide, expressed in a ratio of 1:18, of 0.48%.

B) A second group of 40 guinea pigs at the same stage of gestation was subjected to a milder intoxication of carbon dioxide, reducing the amount of formic acid by one-half (1.25 g), obtaining a concentration of carbon dioxide equal to 0.42%, or 1:236 expressed in volume. The time of exposure to the gas was 10 minutes per day for 20 days for the first group, starting from the 20th day of gestation, and one hour per day for one month for the second group, starting on the same day of pregnancy.

The results are reported in the following table.

	27 miscarriages with 56 subjects apparently normal	
1st group	23 born on time, of which 18 were apparently normal	
40 guinea pigs with acute carbon dioxide poisoning	5 with flaccid paralysis of the posterior limbs, to a more or less evident degree	
Controls -- (10 guinea pigs) 27 normal births		
	22 normal births (46 well-formed feti)	
2nd group		
40 guinea pigs with chronic carbon dioxide intoxication	18 on time, with 39 microsomic births	24 with defects of the paws and 15 with normal limbs
Controls -- 10 guinea pigs with 23 normal births		

The conclusions which may be drawn from these first experiments on experimental oxycarbonism in mammals are the following:

1) The carbon dioxide demonstrated a clear teratogenic effect on the offspring of guinea pigs subjected in pregnancy to repeated intoxications with this gas.

2) The most distinct results were obtained in the pregnant guinea pigs subjected to chronic intoxication with a concentration of 0.42% carbon dioxide in the atmosphere.

3) For the most part, the deformations were found to be flaws in the posterior extremities, and in some cases evident paralysis of the posterior region.

4) No actual formative defect was noted in the skeleton, such as embriopathies, for we deliberately performed the experiments during a rather advanced stage of pregnancy, that is, when organogenesis could already be considered completely finished.

5) The experimental alterations reported can thus be attributed to the fetal period (fetopathies), consisting of deformations in the limbs (twisted paws, etc.), in which a neuro-muscular genetic component seems to have played a definitely predominant role in some subjects.

Giuntini-'55
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NOTA PRELIMINARE SULL'AZIONE TERATOGENICA DELL'OSSIDO DI CARBONIO. — L. GIUNTINI e F. CORNELI.

(Dalla Clinica Ortopedica dell'Università di Siena).

Sezione di Siena — Seduta del 21 marzo 1955.

Prendendo lo spunto da un'osservazione clinica resa nota da Giuntini e Corneli, di un bambino portatore di malformazioni congenite multiple nato da madre ripetutamente intossicata, durante la gravidanza, da esalazioni di ossido di carbonio, abbiamo creduto opportuno iniziare uno studio sperimentale tendente a saggiare l'azione teratogenica dell'ossido di carbonio nei mammiferi.

Gli esperimenti sono stati eseguiti su cavie nel 1° e 2° mese di gestazione facendole respirare in atmosfera contenente ossido di carbonio con le modalità tecniche qui sotto riportate.

A) Un 1° lotto di 40 soggetti selezionati, del peso medio di g 250, è stato posto in una cassetta di legno, appositamente costruita, a tenuta ermetica, munita di apposita apertura dalla quale, per mezzo di speciale apparecchio, veniva introdotto ossido di carbonio ottenuto disidratando l'ac. formico mediante ac. solforico concentrato e riscaldato.

$$\underset{g\ 46{,}02}{HCOOH} \rightarrow \underset{g\ 28}{CO} + H_2O$$

Come risultato della reazione suddetta una grammomolecola di ac. formico svolge una grammomolecola di ossido di carbonio; da g 46,02 di ac. formico si svolgono così g 28 di ossido di carbonio che occupano a 0° e 760 mm di pressione litri 22,4. La quantità di acido formico adoperata per questa prima serie di esperienze è stata di g 2,5.

Tenendo conto che la cassetta è di m^3 0,144, si è verificata una concentrazione di ossido di carbonio espressa in volume di 1:118 uguale a 0,48%.

B) Un 2° lotto di 40 cavie alla stessa epoca di gravidanza ho sottoposto ad una più leggera intossicazione di ossido di carbonio riducendo la quantità di ac. formico alla metà (g 1,25), ottenendo una concentrazione di ossido di carbonio cioè, espresso in volume, di 1:236 uguale a 0,42%. Il tempo di esposizione al gas è stato per il 1° lotto di 10 minuti al giorno, per la durata di giorni 20, a partire dal 20° giorno di gestazione; per il 2° lotto di un'ora al giorno per la durata di un mese, sempre partendo dallo stesso periodo di gravidanza.

I risultati sono riferiti nella seguente tabella.

1° lotto **40 cavie con intossicazione ossicarbonica di tipo acuto.**	**27 parti abortivi con 56 soggetti apparentemente normali.** **23 nati a termine di cui 18 apparentemente normali.** **5 con paralisi a tipo flaccido degli arti posteriori di grado più o meno evidente.**

Controlli **– (10 cavie) 27 nati normali.**

2° lotto **40 cavie con intossicazione ossicarbonica di tipo cronico.**	**22 parti normali (46 feti ben conformati)** **18 a termine con 39 soggetti, microsomici.**	**24 con difetti del piede e 15 con arti normali.**

Controlli **– 10 cavie con 23 soggetti normali.**

Le conclusioni che si possono trarre da queste prime esperienze sull'ossicarbonismo sperimentale nei mammiferi sono le seguenti:

1) L'ossido di carbonio ha dimostrato una evidente azione teratogenica nei riguardi della prole nata da cavie sottoposte in gradidanza a ripetute intossicazioni di questo gas.

2) I risultati più probativi si sono ottenuti nelle cavie gravide sottoposte ad una intossicazione cronica corrispondente ad una concentrazione dello 0,42% di ossido di carbonio nell'atmosfera.

3) Le malformazioni riprodotte sono consistite per lo più in vizii di posizione dell'estremità posteriore e da qualche caso di evidente paralisi del treno posteriore.

4) Non si è notato alcun difetto formativo vero e proprio dello scheletro sul tipo delle embriopatie, perchè siamo intervenuti deliberatamente in un periodo piuttosto tardivo della gravidanza, allorchè cioè l'organogenesi era da ritenere completamente ultimata.

5) Le riferite alterazioni sperimentali sono quindi da attribuirsi al periodo fetale (fetopatie) consistenti cioè in vizii di posizione degli arti (piede torto ecc.) nei quali una componente genetica neuro-muscolare sembra avere avuto in qualche soggetto una parte sicuramente preponderante.

Goddard-'60
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THE BIOLOGICAL ROLE OF CARBON DIOXIDE

DAVID R. GODDARD, PH.D.

CARBON dioxide is found in all environments in which life occurs. Although some of the large organisms may produce sufficient CO_2 in their metabolism that they are not dependent upon external CO_2, and may be independent of external P_{CO_2} so long as it is low (< 0.01 to 0.05 atmospheres), there is little doubt that all cellular and multicellular organisms require CO_2.

Although the first organisms may have evolved in a world of methane and organic compounds,[1] CO_2 has been part of the aquatic and atmospheric life for well in excess of a billion years.[2] It is not surprising, therefore, to find that CO_2 is an active partner in many aspects of metabolism of all organisms.

During the nineteenth century and the early decades of the twentieth century, it was assumed that the only metabolic role of CO_2 was its utilization in the metabolism of green plants in the light, and that other organisms (nongreen plants, bacteria, animals) produced CO_2 in irreversible reactions. This view, which is still part of much teaching, persisted in spite of the ever-increasing evidence in human physiology of the complex relation between CO_2 of tissues and the external environment. When Rockwell[3] and Rockwell and Highberger[4] reported in 1921 and 1927 that certain bacteria, yeasts, and molds required CO_2 for growth and incorporated the carbon into carbon compounds, their results were ignored or denied.

In 1936 Wood and Werkman[5] showed that the conversion of carbohydrates to propionic acid by the propionic bacteria occurred with the incorporation of CO_2. This was confirmed later[6,7] through the use of carbon isotopes. Work done since 1940 has made it clear that the reactions of CO_2 release are reversible, and that carbon dioxide may be liberated or absorbed, depending upon conditions, in a wide variety of organisms. Carbon dioxide incorporation has largely been obscured in nongreen plants by the excess CO_2 production, but isotopic methods have made it possible to show incorporation in spite of excess production, and to demonstrate that in many (probably all) organisms certain biochemical pathways are necessarily coupled to CO_2 utilization. Though the reduction of CO_2 to carbohydrates in photosynthesis depends upon the energy of sunlight, as we shall show below, the actual pathways of CO_2 incorporation occur in nonphotochemical reactions.

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CARBON DIOXIDE AND METABOLISM

Fortunately, the evolution of biological systems has been such that in spite of great morphological diversity the metabolic systems are surprisingly similar for very dissimilar organisms. This is illustrated in the metabolism of CO_2.

In cellular respiration, many oxidative steps do not directly produce CO_2, as biological oxidation occurs primarily by electron transfer over a series of oxidation-reduction catalysts (the respiratory enzymes and coenzymes) or by dehydrogenation. Carbon dioxide is produced primarily in the decarboxylation of α-keto acids, though these decarboxylations are often coupled to oxidative steps. Thus, we find the citric acid cycle, the Embden-Meyerhof fermentation pathway, and the pentose-phosphate pathway operative in such dissimilar organisms as yeasts and bacteria, mammals, and in higher plants.

Succulent plants (Bryophyllum, cacti, etc.), which produce organic acids (primarily malic acid) in the dark from carbohydrates, do so in oxidative metabolism and with CO_2 absorbed from the environment.[8] Until this was discovered it had been impossible to write balanced equations showing the conversion of sugars to malic acid. This problem is resolved by the incorporation of CO_2. The nonphotochemical reduction of CO_2 requires energy, but here the energy is furnished by the oxidation of organic compounds, through the intermediate action of energy stored in adenosine triphosphate and reduced pyridine nucleotides. In most cases, the production of CO_2 is far greater than the amount fixed, but in the succulent plants, in the dark, there is no CO_2 production but there is a net utiliza-

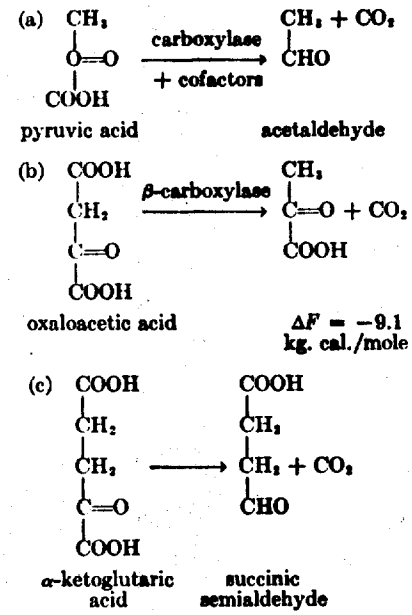
tion of carbohydrate and the energy released in the oxidation of sugars to organic acids is used in the fixation of CO_2.

That the reduction of CO_2 to the level of carbohydrates occurs in photosynthetic reactions without the direct participation of light is clear from the following observations. McAlister[9] found that in green plants undergoing a steady photosynthesis, after a sudden interruption of the light, there is a continued absorption of CO_2 for 90 to 170 seconds. Calvin, *et al.*[10] then demonstrated that if radioactive CO_2 is furnished to plants in the dark, after a prior light period, the CO_2 is reduced to the level of carbohydrates, the amount reduced being equal to that reduced in one minute of photosynthesis; no such reduction occurred in comparable plants lacking the prior light period. It was thus clear that during the light some compounds were produced that could reduce CO_2 to carbohydrates. The energy for CO_2 reduction is furnished by the radiant energy of the light, but the energy is passed on by catalytic substances acting in a cyclical manner. These results made it possible to separate the photochemical aspects of photosynthesis from the enzymatic steps of CO_2 reduction.

Earlier work by Hill[11] had already demonstrated that the photolysis of water, with the liberation of O_2, was independent of CO_2 reduction. The work of Strehler,[12] Frankel,[13] and of Arnon[14] has shown that the reduction of CO_2 occurs in nonphotochemical reactions through reaction with adenosine triphosphate and a hydrogen donor (perhaps reduced pyridine nucleotides). The adenosine triphosphate and the reducing agent are formed during the photochemical reactions. Thus the final stage of CO_2 reduction in photosynthesis is a series of reactions similar to those recognized as part of the carbohydrate metabolism of nonphotosynthetic organisms.

Though we cannot recount here details of the reactions of carbohydrate metabolism, we can give a few of them and so show the reversible entrance of CO_2 into metabolism.

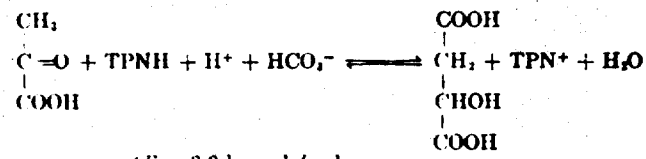
The release of CO_2 may occur in one of several decarboxylation reactions, a few of which are illustrated below:



The actual reactions are far more complex than illustrated. The equilibrium for these reactions lies far on the side of CO_2 release, and, in fact, such reactions were considered irreversible.

Wood, *et al.*[7] were able to demonstrate, with isotopic CO_2, that reaction (b) is reversible, *i.e.*, that isotopic C introduced as CO_2 could be found in the carboxyl group of organic acids. Though the reversal of this particular reaction may not be physiologically of great importance, these experiments changed our whole outlook on the biological activity of CO_2.

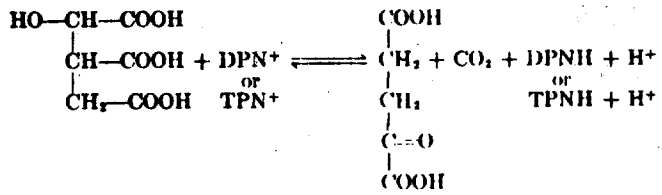
Ochoa[15] found a reaction of considerable interest, the reversible incorporation of CO_2 into malic acid:



ΔF = 3.0 kg. cal./mole
TPNH = reduced triphosphopyridine nucleotide
TPN^+ = oxidized triphosphopyridine nucleotide.

This reaction could be followed on the spectrophotometer, because of high absorption of TPNH at 340 mμ. The reaction is mobile, and may be readily shifted from right to left, or *vice versa*, by varying the concentration of the reactants. Vennesland and co-workers [16] found a similar enzyme in higher plants. The energy for the carboxylation can be thought of as being furnished by the reduced TPNH.

In similar fashion, reversible reactions are known for isocitrate and α-ketoglutaric acids:

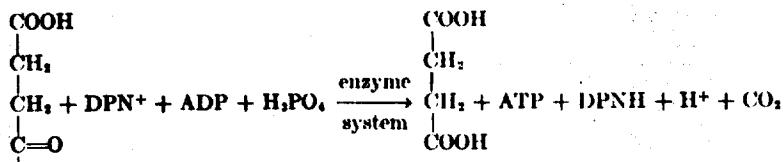


isocitric acid α-ketoglutaric acid

DPN+ and DPNH represent oxidized and reduced diphosphopyridine nucleotides.

Enzymes are known which react either with TPN or DPN, and this reaction is reversible, allowing CO_2 incorporation or release, depending upon the concentration of the reactants.

The decarboxylation of α-ketoglutaric acid is of some interest:



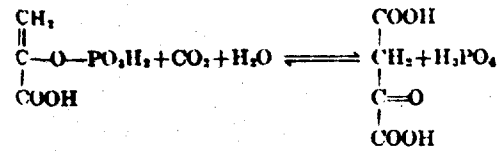
succinic acid

Here, the enzyme system involves at least two enzymes and coenzyme A. The reaction may proceed from left to right or the reverse.

We are interested in these reactions because they are an important part of carbohydrate degradation, but they are all responsive to the P_{CO_2} and may serve, in part, for CO_2 fixation in nonphotosynthetic reactions.

There is some doubt whether any of these reactions is of importance in photosynthesis, since they all require an appreciable P_{CO_2} while photosynthesis occurs at high velocity at $P_{CO_2} = 3 \times 10^{-4}$ atmosphere and at maximum velocity at 1 to 2×10^{-3} atmosphere.

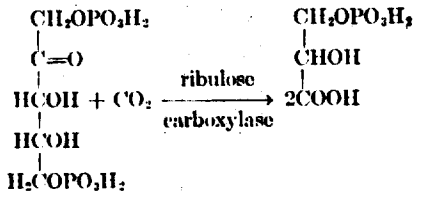
The hydrolysis of a phosphate bond may yield energy that can be used for the addition of CO_2 as a carboxyl group:



phosphoenol pyruvate oxaloacetic acid

This reaction is catalyzed by phosphoenol pyruvic carboxylase.

Perhaps the most startling result was the discovery that in photosynthesis a 5-carbon phosphate sugar gave rise to two molecules of phosphoglyceric acid:



D-ribulose-1,5-diphosphate 3-phosphoglyceric acid

This enzyme, ribulose carboxylase, appears to be limited to the chloroplasts, and is presumably limited to photosynthetic activity. The phosphoglyceric acid may then undergo reduction to triosephosphates through the energy stored in ATP and reduced nucleotides. The trioses not only can be converted to sugars and starches but, through a series of cyclical

TABLE 1

CARBON ON THE EARTH *

	As CO_2	As C	g./cm.² †
	10^{20} g.		
Atmosphere	0.0233	0.00635	0.124
Fresh water	0.009	0.0024	0.047
Seas	1.29	0.352	6.9
Biospere	0.145	0.04	0.784
Rocks			
As carbonates	674.0	184.0	3,600.0
Other forms of C	246.0	67.0	1,313.0
Fossil fuels	0.27	0.73	14.3
Total	921.74	250.93	

* Data from Rubey.[18]

† g./cm.² earth's surface, calculated on basis of earth's surface = 5.1×10^{18} cm.²

reactions, regenerate ribulose diphosphate to keep the process functioning. If one follows the reactions through in detail, it appears that three molecules of ATP and two of reduced TPNH (or DPNH) are required for each molecule of CO_2 reduced to the level of a carbohydrate.

Carbon dioxide has many physiological effects, aside from its role in photosynthesis. We will omit consideration of its role in the mammalian blood system and its effect in the regulation of the frequency and depth of breathing. Loomis[17] has summarized some of the actions of P_{CO_2} as a regulator of embryonic growth. Free CO_2 must act, either by changing the *p*H or participating in chemical reactions, where the equilibrium (or steady state level) is highly sensitive to P_{CO_2}.

The reactions outlined above may not be the critical reactions, but the biochemistry of CO_2 has proceeded far enough for us to recognize that CO_2 is a highly reactive cellular metabolite. We may expect that the understanding of the individual reactions in which CO_2 participates may furnish the understanding of its complex physiological role in the cells and tissues of bacteria, fungi, and higher plants and animals.

INTERNAL ENVIRONMENT AND CARBON DIOXIDE

Carbon dioxide in the atmosphere is at very low level, a P_{CO_2} of about 3.2×10^{-4} atmosphere (0.23 mm.). The leaves of higher plants may function at this low CO_2 pressure. Many microorganisms may be in diffusion equilibrium with environmental CO_2, but because of ecological environments (whether soils, waters, or the tissues of higher animals or plants) the P_{CO_2} is usually from 10 to 200 times that of the atmosphere. We recognize that P_{CO_2} of blood and mammalian tissues is far from diffusion equilibrium with the atmosphere, but it is not so generally recognized that this is also true of plant stems, roots, fruits, and most metazoans. Diffusion equilibrium is apparently the exception in the biological world.

Therefore a clear distinction is required between the internal and external environments. This may be illustrated by the problem of mammalian tissue cultures.[17] If tissue cultures are in mass and in a closed system, the metabolic activity will raise the P_{CO_2}. However, if mammalian tissues are exposed to air, CO_2 will escape from the tissue, which was in prior equilibrium with blood, and the *p*H may rise and the tissue die. At least as early as 1937 this problem was faced and, on theoretical grounds, the proposal was made and adopted to use 5 per cent CO_2 in air when a gaseous stream was passed over a tissue culture in a bicarbonate-containing medium.

ENVIRONMENTAL CARBON DIOXIDE

Carbon is the sixth most abundant cosmic element, though it is less abundant in the outer crust of the earth than the inner. The figures in table 1 are from Rubey[18] and are to be considered as estimates. The total carbon of the lithosphere, hydrosphere and atmosphere is approximately $\times 51 \times 10^{20}$ g. (or 251×10^{14} metric tons). This corresponds to 921×10^{20} g. of CO_2. The CO_2 of the atmosphere is about 1/40,000 of the total, and the total CO_2 of the seas (CO_2, HCO_3^-, and $CO_3^=$) is 55 times the amount of the atmosphere. It is clear that the greatest mass of the earth's carbon is locked in the carbonate of rocks.

The Atmosphere. Though we normally consider the CO_2 content of the atmosphere as constant, and usually assign it a value of 0.03 per cent, considerable variations from this value occur. Though the P_{CO_2} decreases

with altitude, the percentage composition is not markedly affected by altitude[19] and a value of 0.029 ± 0.002 per cent was indicated by data from the rocket Explorer II at 72,000 feet.[20] However, Buch[21] found that over the North Atlantic the CO_2 in air in equilibrium with the sea was 0.0329 per cent, while in arctic the average was 0.0308 per cent, with values as low as 0.015 per cent being recorded. In general, a decrease in the CO_2 content of sea water and in air is found from tropical to northern seas.

Industrial and biological activity can cause considerable variation in atmospheric CO_2. This is most marked on days when the wind velocity is below one to two miles per hour. For example, Chapman, Gleeson and Loomis[22] have followed the diurnal variation of atmospheric CO_2 in an Iowa corn field at one and 152 m. above the ground. On a calm day (wind one to two miles per hour) the peak CO_2 was found at 4:00 A.M. at 1.0 m. to be 0.0477 per cent. By 7:00 A.M. this had fallen to 0.03 per cent, and the minimum of 0.022 per cent occurred at 1:00 P.M. with a rise to 0.03 per cent just after sunset. At 152 m., the changes were smaller, but even at this elevation the photosynthesis lowered the CO_2 level during daylight hours. An Iowa corn field may remove 200 pounds of CO_2 per acre per day (maximum value of 400 pounds). This corresponds to the total CO_2 in a column of air 333 m. Easily detectable decreases during sunny hours were determined even when the wind was eight miles per hour. The increase during the night was probably due largely to plant respiration and in part to respiration of soil organisms.

Fresh Water. Lakes and rivers constitute a small part of the earth's surface, but they are an important habitat for many organisms.

TABLE 2

THE APPARENT DISSOCIATION CONSTANT FOR CARBONIC ACID

Temperature (Centigrade)	K_a	pK_a
0	2.65×10^{-7}	6.58
5	3.04×10^{-7}	6.52
10	3.43×10^{-7}	6.46
15	3.80×10^{-7}	6.42
20	4.15×10^{-7}	6.38
25	4.45×10^{-7}	6.35

TABLE 3

THE PROPORTIONS OF CO_2, HCO_3^-, AND $CO_3^=$ IN WATER AT VARIOUS *pH* VALUES AND 15 C.

pH	CO_2	Total Free HCO_3^-	$CO_3^=$
4	0.996	0.004	1.25×10^{-9}
5	0.962	0.038	1.20×10^{-7}
6	0.725	0.275	0.91×10^{-5}
7	0.208	0.792	2.60×10^{-4}
8	0.025	0.972	3.20×10^{-4}
9	0.003	0.996	0.031
10	2×10^{-4}	0.757	0.243

Hutchinson[23] has given an excellent account of the chemical and physical properties of fresh waters. The composition of lake water is not a constant with depth, and there are also marked seasonal changes.

Carbon dioxide in fresh waters will exist as dissolved CO_2, H_2CO_3, HCO_3^-, and $CO_3^=$. We can consider the following reactions as occuring:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$
$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
$$HCO_3^- \rightleftharpoons H^+ + CO_3^=$$

The dissociation of carbonic may be readily formulated:

$$K_{H_2CO_3} = \frac{(H^+)(HCO_3^-)}{(CO_2 \text{ total})}$$

The difficulty arises because the concentration of H_2CO_3 is not known with precision. It has been reported as 1.105 to 0.56 per cent of the free CO_2. Berg and Patterson[24] have given the value of $K_{H_2CO_3}$ as $1.32 \pm 0.5 \times 10^{-4}$ at 25 C.

The problem is avoided by formulating the equation as follows:

$$K_a = \frac{(H^+)(HCO_3^-)}{(CO_2 \text{ total})}$$

where CO_2 is total analytically determined CO_2. The equation is valid for very dilute solutions, and K_a may be considered the apparent dissociation constant. Its value from Horned and Davis[25] is given in table 2. The constant is not independent of the salt content of the waters; in lakes and rivers the divalent ions predominate, and the constant can be

TABLE 4

CARBON DIOXIDE CONTENT OF SELECTED SOILS

	Percentage CO_2 in Air Spaces	
	At 6-inch depth	At 18-inch depth
Arable land (manured)	0.34	0.5
Arable land (not manured)	0.34	0.45
Grassland	1.46	1.64
Water-logged soil	3.9 to 9.1	

corrected for dissolved salts as follows:

$$pK_a' = pK_a - 0.53\sqrt[3]{m}$$

where m = molarity of the solution.

In fresh waters where the pH is usually below 8.0 we are rarely concerned with the second dissociation constant of CO_2. Table 3, from Hutchinson,[23] is of some interest in showing the distribution of CO_2 in various forms for fresh waters.

The situation in lakes is complex, and the interested reader is referred to Hutchinson.[23] The CO_2 content will depend in part upon equilibrium with the atmosphere, the withdrawal of CO_2 in photosynthesis, the addition by respiration, and the addition of acids or alkali from soil waters. The effect of temperature on CO_2 solubility cannot be ignored. Many lakes will be stratified, with high CO_2 content in lower levels.

Soil Carbon Dioxide. Russell and Appleyard[26] have studied the CO_2 content of agricultural soils. Table 4 has been constructed from their values. Though the composition of the gases of soils is highly variable, and will depend upon water content, total organic matter, and temperature, it is to be recognized that soil organisms and the roots of plants live in environments where the CO_2 content is 10 to 100 times that of the atmosphere.

The Seas. The surface waters of the open oceans in temperate latitudes is constant. The sodium chloride content will be about 3.5 g./l., expressed as salinity (S) per cent 35. The pH will vary from approximately 8.1 to 8.3. The total CO_2 (CO_2, HCO_3^-.$CO_3^=$) is approximately 46 ml./l.

The composition of sea water is shown in table 5. The total CO_2 content and free CO_2 of sea water, in relation to pH, are shown in table 6. Melting arctic ice and large rivers will dilute sea water, and, as the salinity is decreased, the constants affecting the dissociation of CO_2 are also changed.

The dissociation constants of carbonic acid change with the salt content, temperature, and pressure. Buch[27] has constructed tables giving the values of K_a' and K_{a2}' at different temperatures and salinity; these tables have been reproduced by Harvey.[28] The effect of salt on the constants is shown below:

	Water	Sea Water (S per cent 35)
	Temperature 15 C.	
pK_{a1}'	6.43	6.04
pK_{a2}'	10.43	9.07

Since surface sea waters have a pH of 8.0 to 8.3, the bicarbonate/carbonate system will be dominant.

The CO_2 content of surface waters is modified by biological activity. The phytoplankton absorb free CO_2 in photosynthesis, and this results in a decreased P_{CO_2} and a rise in pH. The CO_2 is partially replaced by the conversion of bicarbonate to carbonate:

$$2\,NaHCO_3 \rightleftharpoons Na_2CO_3 + H_2CO_3$$
$$H_2CO_3 \rightleftharpoons H_2O + CO_2$$

TABLE 5

COMPOSITION OF SEA WATER AT 15 C., S per cent 35, pH 8.15

	g./kg.	g./l.	Molarity
Total salts	35.1	36	0.558
Sodium	10.77	11.1	0.483
Magnesium	1.3	1.33	0.0547
Calcium	0.409	0.42	0.0105
Potassium	0.388	0.39	0.00997
Strontium	0.01	0.01	0.00011
Chloride	19.37	19.8	0.558
Sulfate	2.71	2.76	0.0287
Bromide	0.065	0.066	0.00083
Boric acid			
H_3BO_3	—	0.021	0.00033
$H_2BO_3^-$	—	0.0048	0.00008
Total CO_2	—	46.1 ml./l.	0.00209
CO_2	—	0.29 ml./l.	0.0000128
HCO_3^-	—	—	0.00185
$CO_3^=$	—	—	0.00023
O_2	—	5.8 ml./l.	0.00026

TABLE 6

CARBON DIOXIDE OF SEA WATER AT 15 C., S PER CENT 35

pH	7.4	7.8	8.0	8.1	8.2	8.3	8.4	8.5
Total CO_2								
moles/l. × 10^5	238	235	216	211	206	199	191	183
ml./l.	53.3	52.6	48.4	47.3	46.1	44.6	42.8	41
P_{CO_2}								
atmosphere × 10^5	230	85	50	39	29	22	15	12
CO_2								
moles/l. × 10^5	8.94	3.31	1.95	1.57	1.13	0.86	0.58	0.47
ml./l.	2.0	0.74	0.44	0.34	0.25	0.19	0.13	0.1

In northern seas, the rapid growth of diatoms in the spring months results in a photosynthetic level which exceeds the combined respiration of algae, bacteria, and animals, with a resulting increase of *p*H and a fall of P_{CO_2}. In the winter months respiration may exceed photosynthesis, with an increasing P_{CO_2} and a fall of *p*H.

THE SEA AS A REGULATOR OF CARBON DIOXIDE

As early as 1880, Schlösing [29] proposed that the sea was a regulator of atmospheric CO_2. The seas contain 55 times as much CO_2 as does the atmosphere. Krogh [30] and more recently Buch [31] have studied the equilibrium between atmospheric CO_2 and sea water. An excess of atmospheric CO_2 should result in absorption by the sea, with a resulting decrease in *p*H and a dissolution of $CaCO_3$. Rubey [18] has calculated what would happen if the CO_2 content of air was increased fivefold, to 0.015 per cent (after equilibrium with the sea). To raise the level in the atmosphere fivefold would increase the total atmospheric CO_2 from 2.33×10^{18} g. to 11.65×10^{18} g. To increase the CO_2 of the atmosphere by this amount would require the addition of 81×10^{18} g., of which some 71.7×10^{18} g. would have dissolved in the sea. This increased CO_2 in the sea would result in the dissolution of $CaCO_3$, the dissolution of lime rods would have added 62×10^{18} g. of CO_2 and the final *p*H would be 7.8.

There is a belief [28] that the CO_2 content of the atmosphere has increased about 10 per cent since 1900. Such an increase would mean 2.2×10^{17} g. of CO_2 in the atmosphere and, if equilibrium had been reached with the sea, 90 per cent of the CO_2 would be expected to have been absorbed by the sea or, in other words, there would have been a total increase of 2.2×10^{18} g. This corresponds to 8.5×10^{11} metric tons of coal. The 2.2×10^{17} g. of CO_2 in the atmosphere is not out of order with expected industrial production, as Callendar [32] estimated in 1940 an industrial production of 43×10^{15} g. of CO_2 per year and for sixty years this is 2.68×10^{17} g. in comparison with a suggested atmospheric increase of 2.2×10^{17} g. The industrial production of CO_2 is inadequate to cause an increase of 10 per cent CO_2 in the atmosphere if nine-tenths of the CO_2 has entered the seas. Either the increase has not in fact occurred, or equilibrium with the seas has not been established. One would also expect an increase in atmospheric CO_2 to result in an increase in photosynthesis and an increased locking up of CO_2 in the biosphere.

GEOLOGICAL ORIGIN

Urey [1] and Kuiper [33] have proposed that the initial earth had a reducing atmosphere of methane, ammonia and hydrogen. Carbon dioxide is not stable in a hydrogen atmosphere. The gravitational field of the earth cannot permanently hold a hydrogen atmosphere and hydrogen would escape except as it is bound in water, methane, metal hydrides and ammonia. The CO_2 of the atmosphere may have had its origin from volcanos and hot springs.[2] Though the rate of production is low (3 to 7 × 10^{12} g. per year), only about 0.04 to 0.07 per cent of the current photosynthetic rate, the current

TABLE 7 [1]

RATE OF PHOTOSYNTHESIS ON LAND

	Area, 10^8 cm.^{2}	Areal Fixation, mg./cm.^{2} year		Total Fixation for Whole Area, 10^{15} g./year	
		C	CO_2	C	CO_2
Forest	44	20	73.3	8.8	32.3
Cultivated	27	16	58.6	4.3	15.8
Grassland	31	6	22	1.9	7
Desert	24	0.5	1.8	0.1	0.2
Total	126	12	44	15.1	55

[1] From Hutchinson.[2]

atmosphere CO_2 could be produced in 60,000 years and that of the seas and atmosphere in a few million years. These are geologically very short times. On the other hand, life may have had its origin in an anaerobic world, with little or no CO_2 and the CO_2 may be of biological origin. Though one considers CO_2 to be produced by living organisms by the use of O_2 in respiration, chemical mechanisms for the degradation of organic compounds without utilization of oxygen are possible.

We do not know which gas occurred first, O_2 or CO_2. Oxygen could have been first produced by the photochemical decomposition of water at high altitudes:

$$H_2O + h\nu \longrightarrow H_2 + O$$
$$O + O \longrightarrow O_2.$$

The hydrogen would then be lost to the earth. Kuiper [33] has calculated that the rate of this process could produce our atmosphere in 5×10^8 years. This is a very low rate compared with present-day photosynthesis, since the estimate of photosynthetic rates indicates an O_2 production of 55×10^{15} g. per year,[2, 34] or the atmospheric O_2 of 12×10^{21} g. could be produced in 22,000 years, and even a rate of one-fifth of this would form our O_2 in 100,000 years.

Thus the primitive O_2 and CO_2 may have had a nonbiological origin, but once photosynthetic life was firmly established the activities of organisms must have become dominant in the biological evolution of O_2, water, and CO_2.

THE CIRCULATION OF CARBON DIOXIDE

Generations of students have been taught that the respiration of animals balances the photosynthesis of plants. Such statements are usually in nonquantitative terms, and frequently ignore the role of plant respiration. Several workers have made efforts to estimate the quantity of photosynthesis. Table 7, from Hutchinson,[2] makes a respiratory correction for plants, and arrives at an over-all rate for land plants of $73 \pm 18 \times 10^{15}$ g. CO_2 per year.

The respiratory component is made up of the respiration of higher plants, approximately 20 per cent, of higher animals, and of soil organisms (bacteria, fungi, protozoa and soil invertebrates). The human contribution can be approximated; if we assume a population of 2.7×10^9 and a daily calorie intake of 2500 kg. cal., the yearly CO_2 production is $1.0 \pm 0.2 \times 10^{15}$ g. CO_2. Thus human respiration may account for 1.4 per cent of land photosynthesis.

In a mature forest, the respiration of material on and within the soil will nearly balance the net photosynthesis (total photosynthesis minus respiration of the higher plants). The total CO_2 added by respiration of soil organisms may be 80 per cent of total photosynthesis under these conditions, whereas for agricultural land it may be only half of this. What percentage of the respiration is to be ascribed to bacteria, fungi, invertebrates, and higher plant roots is unknown.

TABLE 8 *

TOTAL METABOLISM OF THE EARTH

	CO_2 Fixed	O_2 Produced
Ocean	$462 \pm 303 \times 10^{15}$ g./yr.	$336 \pm 218 \times 10^{15}$ g./yr.
Land	$73 \pm 18 \times 10^{15}$ g./yr.	$53 \pm 15 \times 10^{15}$ g./yr.
Whole earth	$535 \pm 311 \times 10^{15}$ g./yr.	$389 \pm 231 \times 10^{15}$ g./yr.
Unit area	105 ± 63 mg./cm.^{2}/yr.	76 ± 45 mg./cm.^{2}/yr.

* From Hutchinson.[2]

Though the photosynthesis of certain areas of the seas may rival or exceed that of agricultural land,[2] an evaluation of the total photosynthesis is difficult. Riley[34] has given estimates, which are used as the basis for the information in table 8. Though there is uncertainty concerning the absolute magnitude, it is probably safe to assume that photosynthesis of land plants constitutes about one-fifth of the total, the remaining four-fifths occurring in the open seas. The respiration of marine animals probably comes far closer to a balance of marine photosynthesis than does that of land animals balance photosynthesis of land organisms. Though the productivity of the seas may be limited by light or temperature, during part of the year, the rapid growth of diatoms depletes the surface waters of nitrates, phosphates, and silicates. It is doubtful if CO_2 ever limits the growth of marine life. The rapid circulation of marine C through living organisms is limited to surface waters (0 to 400 m.) and the slow mixing in the seas means that the C of the ocean deeps moves through the cycle of life at a very low rate.

Conclusions

All life appears to be dependent upon CO_2, indirectly through the role of CO_2 in photosynthesis and directly as CO_2 is an essential part of the organism's own biosynthetic pathways and a determinant in development and differentiation. Carbon dioxide is part of the internal environment of all organisms and many small organisms which are unable to satisfy their needs by endogenously produced CO_2 are dependent upon external CO_2. Larger organisms are rarely in diffusion equilibrium with their environments for CO_2.

The CO_2 contents of the environments are varied. Though the CO_2 content of air is considered a constant, it may change by 100 per cent within a day over agricultural land. The CO_2 content of soils and waters, though far from constant, is frequently strikingly above that of the air. In micro-environments the activity of organisms plays an important role in the determination of CO_2 levels.

Though the quantity of CO_2 appears large, the atmospheric CO_2 passes through living organisms once in ten years. The quantities in the seas and carbonate rocks appear to be tremendous, but in terms of biological activity over a period of one to two billion years these sums are not large.

The origin of life and its evolution cannot be understood without considering the geochemistry of the early earth and the recognition that the physiological functions found in advanced organisms are in part derived from mechanisms established by evolution in more primitive organisms. Nowhere is this more clearly established than in the relation of organisms to CO_2.

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Significance of Carbon Dioxide in the Metabolism of Animals

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Detailed study of the metabolic significance of carbon dioxide was begun comparatively recently.

But even in the second half of the 19th century, physiologists had begun to consider carbon dioxide not simply as a final product of metabolism, but as the most important metabolite, which is capable of very significantly influencing the function of a number of organ systems in man and animals (1). Interest in this problem has arisen in connection with the development of aviation, space research, and underwater, underground and other studies, in which possible changes in carbon dioxide concentration are characteristic in inhaled air. It is necessary to emphasize that up to the present time, many researchers have studied precisely these questions.

What concerns biochemists in their very important contribution in the development of this problem is the evidence that the carbon in carbon dioxide is utilized in the tissues of heterotrophic organisms in the process of biosynthesis of fats, carbohydrates, nucleic acids, proteins, and other organic compounds, with the help of CO_2 fixation reactions (carboxylation) (2-11). For a long time it was considered (8-11) that this process in heterotrophic microorganisms was first discovered in 1935 by Wood and Werkman. However, the presence of this process had already been proven in 1910 by our countryman, A. F. Lebedev (2,3). Interest in these processes arose especially after they were discovered in the tissues of higher animals (5,6). Up to this time, carboxylation has been thoroughly studied in many laboratories all over the world, and it is now known that in animal tissues, more than ten basic reactions occur, in which the carbon in carbon dioxide is transformed into the carbon of organic compounds (11).

At the present time, two basic problems are being intensively studied: 1) the physiological significance of carbon dioxide which takes part in the respiratory cycle, and 2) the mechanisms and physiological significance of carbon dioxide fixation reactions in tissues.

It must be emphasized that, in recent years, the study of metabolic properties involved in changes in concentration and proportions of different forms of carbon dioxide in tissues has increased significantly. In this respect, there is a huge number of studies which, even in the second half of the 19th century (12-15) and up to the present time (16-20) did elucidate and are now making clear the properties of physiological functions and metabolism in animals and man in the so-called acidose and alkalose states. It was shown (16-32) that the reason for observed shifts in metabolism in acidosis and alkalosis was not only a change in H^+ concentration, but also, to a lesser degree, change in the concentration and values of the proportions of various forms of carbon dioxide. Nevertheless, considering the mechanisms of carbon dioxide influence on metabolism, investigators have overlooked the important role of carboxylation processes, whose intensity of occurrence depends to a definite degree on a number of factors, and first of all, on the concentration of carbon dioxide in the tissues.

It must also be noted that the biological role of carbon dioxide is not limited to its participation in carboxylation processes, in the formation of a bicarbonate buffer system, or in regulating the activity of the respiratory center. Thus, it was indicated relatively recently that the activity of a number of enzymes which have no direct relation to CO_2 fixation, may be substantially altered, depending on carbon dioxide concentration in the media (ATP-ase, aconitate-hydrolase, glucose-6-phosphate phosphohydrolase).

In our laboratory, it was shown that carboxylation lies at the basis of the most important biosynthetic processes in animal tissues and that affecting the intensity of its occurrence may be an important means of influencing the intensity of the latter processes' occurrence (10, 11, 33). The discovery of this pattern is not only important from the theoretical point of view, but it has great significance in the solution of many problems in medical and veterinary practice, animal husbandry, and microbiology.

Forms of Carbon Dioxide and the Regulation of its Content in the Organism

Forms of Carbon Dioxide

Carbon dioxide in the animal organism may be in the form of soluble carbon dioxide gas (pCO_2), carbonic acid (H_2CO_3), bicarbonates (HCO_3^-), carbonates ($CO_3^=$), or carbamates ($R\text{-}N<^{H}_{COOH}$) (Fig. 1).

pCO_2. The major source of carbon dioxide in tissues is endogenic carbon dioxide gas. Its solubility in water at 37° C (in millimoles) is 0.334 X p (p is the partial pressure of carbon dioxide, mm Hg td). It is emphasized the pCO_2 concentration in the blood plasma of man and animals is about 1.0-1.2 mmole/liter (34-40).

H_2CO_3 is formed in tissues from pCO_2 and bicarbonates, with the participation of carboanhydrase (34). In tissues it is very low, and it constitutes 1/300-1/900 of the total amount of pCO_2 (35-36). Speaking of pCO_2, very often both these forms of carbon dioxide are calculated together.

HCO_3^- is formed from pCO_2 with the participation of carboanhydrase, as well as H_2CO_3 and $CO_3^=$, with corresponding changes in hydrogen ion concentration in the tissues. Blood studies done on man and animals have indicated that the bicarbonates make up about 95% of total carbon dioxide, which is separated from the plasma with the addition of a strong acid in a vacuum, and the remaining forms of carbon dioxide make up only 5% (37, 39). This relationship (under normal conditions) is quite constant, and because of this, Henderson and Hasselbach (39) developed an equation which expresses the interrelationship of such indices as pH and H_2CO_3 and HCO_3^- concentrations in the blood of man:

$$pH = 6.10 + \log \frac{HCO_3^-}{H_2CO_3} .$$

According to the data of many investigators, becarbonate content in the blood plasma of man and animals is about 18-25 mmole/liter, but in a number of pathological states, it may oscillate between 10 and 50 mmole/liter (21, 37-41). Approximately the same amount of bicarbonate is contained in the intercellular and cerebrospinal fluids, where it is found in direct relation to its content in the blood (39, 42). The amount of bicarbonate in intracellular fluids is also

determined by its extracellular concentration, but it is usually lower than the latter by approximately 25-30% (43). Bicarbonate content in saliva, pancreatic juice, and bile is very high (60-130 mmole/ liter (44-46).

CO_3^-, as data in the literature confirm, is formed from bicarbonate with the participation of carboanhydrase:

$$2\ HCO_3^- \longrightarrow H_2CO_3 + CO_3^- .$$

This form of carbon dioxide has substantial significance in the formation of bone tissue. Thus, it is indicated that carbonate ions make up 6.0% of the mineral components of animal bones (48). Carbonates play an especially important role in the process of shell formation in birds' eggs: Ca^{++} from the blood enters the oviduct glands and is transformed into the crystalline calcium carbonate of the shell. For some time, ammonia has also been considered to play an important role in this process (49):

$$HCO_3^- + Ca^{++} + NH_3 \longrightarrow CaCO_3 + NH_4^+ .$$

$R\text{-}N\langle^{H}_{COOH}$, carbamates or carbaminic acids, are formed due to the interaction of pCO_2 with free non-polarized amino groups of proteins (50-52). This reaction is exothermic. Carbamates are rather strong acids (pK = 5.8), which are stable in an alkaline medium and which break down in an acidic medium. Their calcium and barium salts are soluble in water. In human blood, about 5% of pCO_2 is in the form of carbamates (37).

It is shown that 10-30% of carbon dioxide participating in the respiratory cycle is developed in the surrounding medium, thanks to the formation of carbamates (37, 50-52).

Regulation of Carbon Dioxide Content in the Animal Organism

From what has been presented, it is evident that the basic forms of carbon dioxide in animal organisms are bicarbonates and soluble carbon dioxide gas. Not only the concentrations of other forms of carbon dioxide but that of hydrogen ions depends on the content of these two forms and the proportions between them.

Let us look at the mechanisms for regulating carbon dioxide content in tissues, as well as a number of physiological and pathophysiological states in animals and man, which characterize alterations in the concentration and proportions of the different forms of carbon dioxide in the organism.

Sources and Mechanisms of Eliminating Carbon Dioxide from the Organism

pCO_2. As was already pointed out, the major source of carbon dioxide in the organism is endogenic carbon dioxide gas. Thus, in adult man, an average of 600 g of carbon dioxide is formed in 24 hours (37). Its neutralization and elimination from the organism are very complicated processes which are represented at the present time in the following manner.

On the one hand, H_2CO_3 is transformed in tissues into HCO_3^- depending on the degree of carbon dioxide formation and oxyhemoglobin

transformation into reduced hemoglobin. HCO_3^- is transformed in the lungs into CO_2 and H_2O, thanks to the carboanhydrase of the erythrocytes, after which CO_2 is excreted into the surrounding medium. This may be represented as:

in tissues: $$Hb\text{-}O_2 + CO_2 + H_2O \xrightarrow{\text{tissue carboanhydrase}} Hb\text{-}H + HCO_3^- + \underline{O_2};$$

in the lungs: $$Hb\text{-}H + HCO_3^- + O_2 \xrightarrow[\text{carboanhydrase}]{\text{erythrocyte}} Hb\text{-}O_2 + \underset{CO_2 \quad H_2O}{H_2CO_3}.$$

It is considered that this mechanism provides for the elimination of about 2/3 of expirated carbon dioxide gas (37). On the other hand, the hemoglobin of the erythrocytes (37) and plasma albumen (52), reacting with tissue carbon dioxide, form carbamates. In the lungs, the carbamates are el minated and the albumen is transferred to the free state. Robinson indicates that almost one third of all expirated carbon dioxide is eliminated by precisely the same method (37). It must be noted that the intensity of lung ventilation, and thus pCO_2 elimination into the surrounding medium, is regulated by the respiratory center, whose activity occurs in direct relation to pCO_2 and H^+ content in the blood (1). Approximately 5% of the total amount of expirated carbon dioxide penetrates into the surrounding medium through the skin (53).

$\underline{HCO_3^-}$. The source of HCO_3^- may be various forms of carbon dioxide (Fig. 1). Bicarbonate concentrations in tissues are determined mainly by the proportion of alkali (Na, K, Ca, Mg) and acid (Cl, S, P) equivalents in them. Therefore it is necessary to talk above all about the mechanism of HCO_3^- content regulation in the tissues. In short, let us consider the transformation of acids and bases in the organism, which may be formed within the cells or may be eliminated from the gastrointestinal tract. Getting into the organism, these compounds are subjected primarily to strong dilution in different biological liquids. In addition, a significant portion of them undergo metabolic transformation. Thus, organic acids are, in the majority, oxidized in CO_2 and H_2O. Therefore significant shifts in carbon dioxide content in the organism will take place during changes in the intensity with which organic acids or their bases enter the tissues. It is an entirely different matter with mineral acids or bases. The most important homeostatic mechanism here is the buffer system: bicarbonate, protein, phosphate, and in erythrocytes, hemoglobin. It is necessary to emphasize that the strongest and most important of these is the bicarbonate buffer system (37).

Ion exchange between extracellular liquids and mineral components of the skeleton also has great significance in regulating active reactions in the blood. Therefore in a number of acidose states, disruption of mineral exchange is frequently and nonrandomly observed, involving the skeleton (osteomalaria, osteoporosis, fragility of egg shells).

Thus, when a definite amount of acids enter the organism, a significant portion of the tissue bicarbonate which reacts with them is transformed into carbon dioxide, a surplus of which is eliminated through the lungs. On the other hand, the bases entering the organism, reacting with carbon dioxide, form bicarbonate, increasing their total content in the tissues. This is the great importance of a bicarbonate buffer, which results in protecting the organism from

sharp pH oscillations in biological liquids, due to changes in their concentrations and proportions of bicarbonate and carbon dioxide.

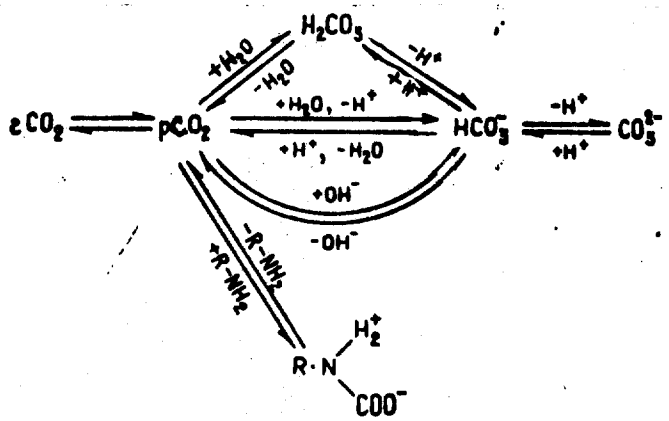


Fig. 1. Forms of carbon dioxide in animal organisms.

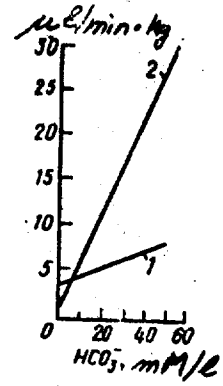


Fig. 2. Formation of bile (1) and pancreatic juice (2) in dogs vs. bicarbonate content in the blood.

The great importance in maintaining acid-alkaline homeostasis and, more exactly, in eliminating excess HCO_3^- and H^+ from the organism, is that of the kidneys (37). It is accomplished in the following manner. Bicarbonates in the blood freely penetrate into the glomerular kidney filtrate, and their content in it is determined by their concentration in the blood. However, if the blood pH is lower than normal, "reabsorption" of the bicarbonates into the blood occurs, with a parallel increase in H^+ excretion in the urine. The nature of this process is that under the influence of carboanhydrase of the kidney tube cells, H_2CO_3 is transformed into HCO_3^- and H^+. The latter is excreted in the glomerular filtrate, from which, meanwhile, the equivalent amount of sodium ions is reabsorbed. Therefore, instead of each hydrogen ion eliminated in the urine, one molecule of $NaHCO_3$ is transformed in the plasma, which is utilized for neutralizing excess H^+ in the blood. The rate of H^+ excretion in urine and corresponding reabsorption of Na^+ into the blood occurs in direct proportion to pCO_2 content in the blood, i.e. the pH of the urine eliminated will be determined by the bicarbonate concentration in the original urine and the intensity of H^+ excretion in the glomerular kidney cells. In acidose states, bicarbonate may be observed to be insufficient in the original urine to neutralize excreted H^+ ions. In such a case, they will be neutralized by primary phosphates or ammonia, which leads to increased elimination of phosphorus and nitrogen with the urine (54-56).

A significant portion of the bicarbonate is freed from the blood in the process of forming saliva, bile, and intestinal and pancreatic juices (45, 46). It is established that the rate of formation of pancreatic juices and bile in dogs is directly proportional to HCO_3^- concentration in the blood (Fig. 2) (46). However, to judge the amount of bicarbonate eliminated through the gastrointestinal

tract is difficult, since a significant portion of it is neutralized by the acids in food and stomach juices, and the salts forming in the meantime are absorbed back into the blood.

Physiological and Pathophysiological States of Animals and Man, which are Characterized by Changes in the Content and Proportions of Different Forms of Carbon Dioxide in the Organism

Van Slyke and other investigators (39), even in the 1920's, having studied the acid-alkali balance in the blood of animals and man in a number of physiological and pathophysiological states, established that there are nine different, inherent states of the organism: normal, metabolic acidosis and alkalosis, respiratory acidosis and alkalosis, respiratory acidosis and alkalosis with partial kidney compensation, and compensated acidosis and alkalosis. The last two deserve special attention, since they are characterized by essential differences in carbon dioxide content with a normal H^+ concentration in the tissues. These states are schematically represented in Fig. 3 (39). Let us consider each of them.

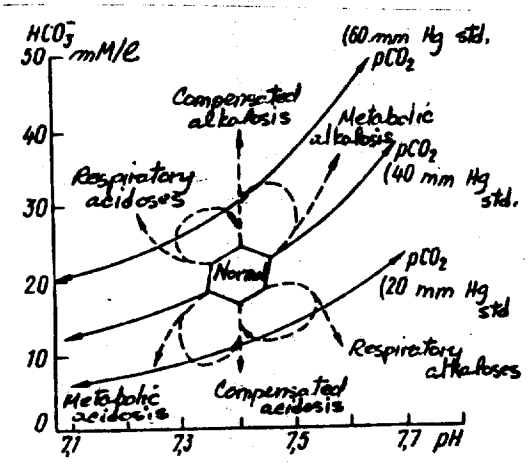


Fig. 3. Normal and pathophysiological states of the animal and human organism, which are characterized by changes in the interrelationships of HCO_3^- content and pCO_2 and H^+ in blood plasma

Normal is characterized by indices such as these: pH = 7.36-7.44; HCO_3^- = 18-25 mmole/liter; pCO_2 = 1.0-1.2 mmole/liter.

Metabolic alkalosis (pH > 7.44, HCO_3^- greater than normal, pCO_2 normal or above) develops when an excess of bicarbonate or sodic or potassic salts of organic acids enters the organism, after frequent washing out of the stomach, during hyperexcretion of HCl into the stomach cavity, or during successive nephritis (kidney alkalosis) (39, 57, 58). Under these conditions, with an increase in HCO_3^- content in the blood, respiration is immediately slowed down, which leads to a parallel increase in the amount of pCO_2 in the tissues. Simultaneously, elimination of bicarbonate with the urine is increased. These processes tend to lower the blood pH value to a normal level, although HCO_3^- and pCO_2 content in the blood may be significantly higher than normal at this point. If blood pH is normalized, the so-called state of compensated alkalosis is developed. If the pH continues to increase (> 7.8), tetanic phenomena develop and death is possible.

Metabolic acidosis (pH < 7.36, HCO_3^- below normal, pCO_2 normal or below) is observed in animals and man after administration of ammonium salts of inorganic acids or acid solutions, in severe forms of diabetes, in complications of childbirth, in a number of postoperative states, in extensive burn cases, in the first days of a neonate, etc. (39, 57, 59-61). Consequently, the state of metabolic acidosis is developed in those cases in which an excess of acid (of alimentary or endogenic origin) is neutralized by bicarbonates of

tissue and blood, which leads to an increase in pCO_2 content in the blood and a decrease in the amount of HCO_3^-. Meanwhile, respiration is quickened, and simultaneously, the reabsorption of bicarbonates from the glomerular kidney filtrate back into the blood is increased. These two processes are directed t ard an increase in pH, i.e. its normalization. If the pH is normalized, the state of compensated acidosis has developed. If the pH continues to decrease (< 7.0), a comatose state occurs and death is possible.

Respiratory alkaloses (pH > 7.44, HCO_3^- normal or below, pCO_2 below normal) develop upon breathing a gas mixture devoid of CO_2, and also in spontaneous hyperventilation of the lungs (37, 39). Elimination of bicarbonates in the urine is increased (respiratory alkalosis with partial kidney compensation), which, simultaneously with hypoventilation of the lungs, creates conditions for normalization of the blood pH and a transition to the state of compensatory acidosis.

Respiratory acidosis (pH < 7.36, HCO_3^- normal or above, pCO_2 above normal) arises during suppression of the respiratory center's activity by morphine, by breathing gas mixtures containing a large percentage of carbon dioxide, and also in severe forms of pneumonia and emphysema, or spontaneous checking of breathing (37, 39, 57, 62, and 63). Elimination of bicarbonates in the urine is inhibited (respiratory acidosis with partial kidney compensation), which, simultaneously with hyperventilation of the lungs, creates conditions for blood pH normalization and a transition to the state of respiratory alkalosis.

Compensated alkalosis (pH normal, HCO_3^- and pCO_2 above normal) is observed in the compensation of metabolic alkaloses and respiratory acidoses. But it appears most frequently in chronic intake into the organism with food of an excess of alkaline equivalents relative to acidic equivalents, in long periods of staying in an atmosphere with somewhat increased (over normal) CO_2 content, and frequently in emphysema of the lungs and chronic bronchial pneumonia (37, 39, 57). The transition from this state to normal is accomplished by kidney activity (which increases elimination of bicarbonate in the urine) and the respiratory center's activity (hyperventilation of the lungs).

Compensated acidosis (pH normal, HCO_3^- and pCO_2 below normal) is developed in compensation of metabolic acidosis and respiratory alkalosis. But it occurs most frequently with chronic intake into the organism, in food, of an excess of acidic, relative to alkaline, equivalents, while remaining in an atmosphere with a CO_2 concentration below 3%, in slight and moderate forms of diabetes, fever, and burn illnesses, after operations or being wounded, in highly-productive animals, etc. (37, 39, 40, 57). The transition from this state to normal is accomplished with action by the kidneys (which inhibit bicarbonate elimination with the urine) and the respiratory center (hyperventilation of the lungs). Ultraviolet irradiation has a favorable effect on this process (58).

It is necessary to note that, in the case of a deficiency of potassium in food, intracellular acidosis is developed, although the pH of the blood and intracellular fluids may already be somewhat above normal (64). There is also information that the cellular membranes are more permeable to pCO_2 and H_2CO_3 than to HCO_3^- and H^+ (65). This property of the cell membranes serves to preserve to a distinct degree the constancy of the intracellular pH value.

We have briefly considered the basic forms of carbon dioxide

which is contained in animal tissues and the mechanisms of regulating its content, and we have noted a number of physiological and pathophysiological states of the animal and human organism, corresponding to substantial changes in the content and proportions of the various forms of CO_2 and in the hydrogen ions.

The wide distribution of the indicated states was a major reason why, even at the end of the 19th century (12-15) and at the present time (16-20), numerous studies by Russian and foreign scientists were and are being devoted to the features of metabolism in tissues in different sorts of acidoses and alkaloses.

However, the approaches to the study of this problem and the explanation of the results obtained are based, in the majority of cases, only on the roles of H^+ concentration changes in metabolism. They exclude almost completely the possible metabolic significance of changes in content and proportions of the different forms of carbon dioxide in these states.

In the last few years, interest in explaining the metabolic significance of carbon dioxide has risen remarkably (10, 11, 21-33, 66-80), especially since it was shown in our laboratory (10, 11, 33, 66-80) that carbon dioxide fixation lies at the basis of the most important biosynthetic processes, not only in autotrophs, but in all heterotrophic organisms, and that, in changing the amount of carbon dioxide in tissues or media, it is possible to directly affect the intensity of its fixation processes and consequently to affect the intensity of biosynthesis of proteins, nucleic acids, lipids, carbohydrates, and other organic tissue components.

Basic Reactions of Carboxylation and their Physiological Significance in Animal Tissues

Even in 1910, Lebedev (2), studying chemical synthesis in *Bacillus hydrogenes* under conditions of autotrophic and heterotrophic nutrition, showed that in both the first and second cases, the indicated microorganisms assimilated carbon dioxide in the process of growth. Furthermore, this process (carbon dioxide fixation, carboxylation) was discovered in different types of heterotrophic microorganisms (3, 4), and, in 1940, in the tissues of animals (5, 6), i.e. it became clear that carbon dioxide fixation is present in all living organisms. This served as a stimulus for the development in many of the world's laboratories of studies directed toward mechanisms of carbon dioxide fixation and the factors which regulate their processes.

At the present time, a number of large review articles on these questions has been published (7, 9). They are revealed most completely in a monograph (10) and other review publications (11, 33) from our laboratory.

Summing up the accumulated data, it is possible to conclude, at the present time, that in animal tissues more than ten basic carboxylation reactions take place (Fig. 4), which may be subdivided into three groups: 1) those occurring due to the energy of reduced forms of pyrimidine nucleotides, 2) those due to ATP energy, and 3) those not requiring any outside energy.

It is established that carbon dioxide fixation preceded carbon dioxide activation, which is brought about by means of its interaction with ATP (Fig. 5). In some cases (81), carbonyl adenilate is formed, and in others (82), carbonyl phosphate. Linen *et al.* (83) consider

that carboxylation of biotin enzyme, which then delivers CO_2 to the corresponding acceptor, can also be considered a process of carbon dioxide activation.

Carboxylation Reactions which Occur due to the Energy of Reduced Formations of Pyrimidine Nucleotides

To this group belong "malic" reactions, and the carboxylation of α-ketoglutarate and serine.

"Malic" reactions. The "malic" enzyme was first discovered by Ochoa and coworkers in the liver of doves (6). It catalyzes the synthesis of malate at the expense of pyruvate, CO_2, and hydrogen in $NADPH_2$, and Mg^{++} and Mn^{++} are required it. They consider that, in the normal state, this enzyme (together with malate dehydrogenase) is "answerable", in exactly the same degree as pyruvate carboxylase, for the synthesis of oxalacetate in tissues (84). However, in malnutrition, diabetes, or after the action of adrenocorticoides, i.e. under conditions of gluconeogenesis stimulation, this reaction

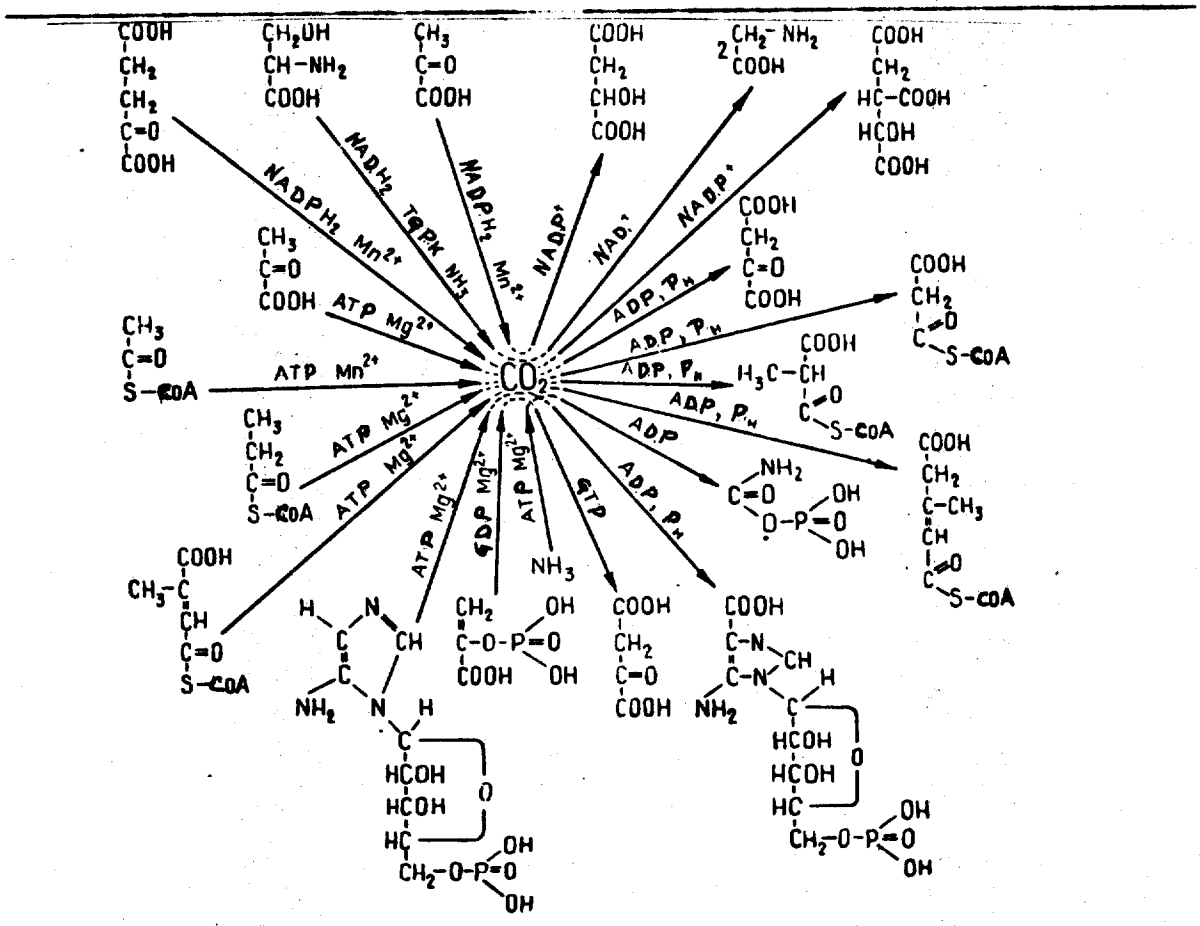


Fig. 4. Basic carboxylation reactions in animal tissues

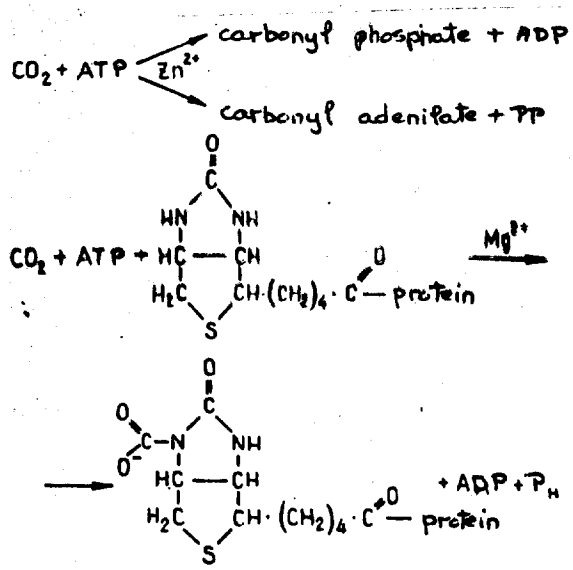


Fig. 5. Carbon dioxide activation

tends more toward the decarboxylation of malate in pyruvate, i.e. it emerges as one of the mechanisms of providing cells with hydrogen from $NADPH_2$ (85-90).

Carboxylation reaction of α-ketoglutarate. In 1948, Ochoa and his coworkers (85) first indicated the possibility of synthesizing isocitrate at the expense of α-ketoglutarate, CO_2, and the hydrogen of $NADPH_2$ by enzyme preparations obtained from the heart of pigs and doves. This reaction is reversible, and Mn^{++} is necessary for it to proceed (85, 91, 92).

The physiological significance of this reaction is mainly in that it is an important link in the metabolism of citrate from the cell, which is very important in metabolism (10). This reaction, similar to the "malic" reaction, plays a role in providing cells with a reduced form of $NADPH_2$ (93) and in the intracellular transport of hydrogen and acetyl-CoA (92).

Carboxylation reaction of serine. The presence of this reaction had already been proposed in 1955-1962 (94, 95). However, it was studied *in detail only* in 1969 (96). This reaction occurs in the kidneys, liver, and a number of other tissues in animals and birds. Carboxylation of serine takes place in the presence of ammonia, bicarbonate, $NADPH_2$, and tetrahydrofolic acid, and is stimulated by pyridoxyl phosphate. As a result, two molecules of glycine are formed.

The physiological significance of the reaction lies in the fact that it emerges not only as a mechanism of CO_2 fixation and mutual transformation of serine and glycine, but also as one of the means of ammonia fixation in animal tissues.

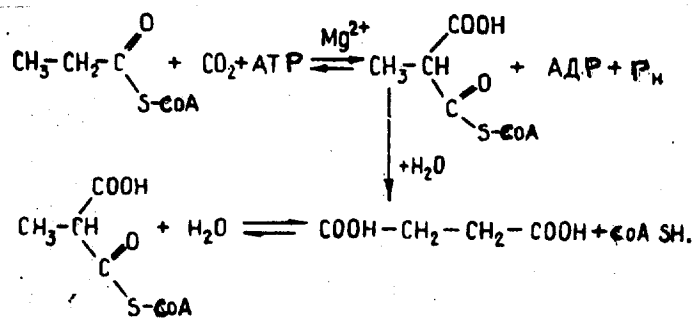
It is assumed that the complex which forms in this reaction (tetrahydrofoliate, a single-carbon fragment) as an intermediate product is the donor of a methyl group in the formation of thymine.

Carboxylation Reaction Occurring due to ATP Energy

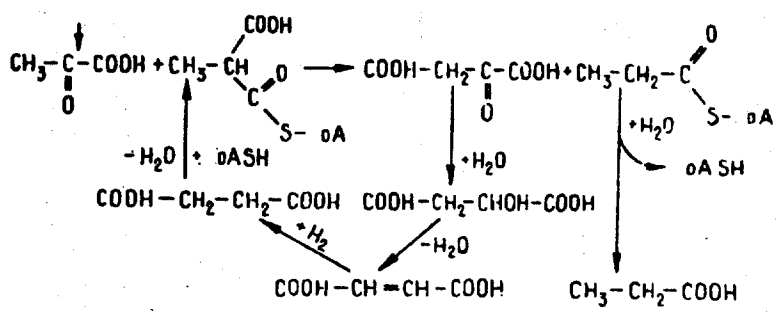
In this group belong the carboxylation reactions of propionyl CoA, β-methyl crotonyl CoA, acetyl CoA, 5-amino-imide-azol ribo-

nucleotide, and the formation of carbamyl phosphate.

The propionyl-CoA-carboxylase reaction. In 1955, Fleyvin et al. (99), simultaneously with Katz and Chaykovii (100), showed that in animal tissues, propionate in the presence of CoA-SH, Mg^{++}, and ATP is transformed into β-methyl-malonyl-CoA. Soon afterward, the reversibility of this reaction was shown (10), as well as the fact that β-methyl-malonyl-CoA, with the participation of methyl-malonyl-CoA-isomerase is reversibly transformed into succinate (102), i.e. the mechanism of the reversible transformations of propionate into succinate was known:



This reaction was discovered in microorganisms (103, 104). Therefore, for some time it was considered that the propiono-acidic fermentation of carbon takes place by such a method. However, it is now known that this process takes place due to the process called transcarboxylation (105), which is catalyzed by methyl-malonyl-oxalacetate transcarboxylase:



The coenzyme of propionyl-CoA-carboxylase and transcarboxylase is biotin. Propionyl-CoA-carboxylase is not strongly specific and may carboxylyze acetyl-, butyryl-, and isobutyryl-CoA (106).

This reaction has especially great significance in metabolism in ruminants, in the rumens of which about 25% of the carbon is fermented to propionic acid (107).

The -methyl-crotonyl-CoA-carboxylase reaction. This reaction was discovered by Rilling and Coon (108) in the liver of long-horned cattle and chickens. Its physiological significance is mainly that it is one of the links in metabolism of leucine and isovaleric acid.

The acetyl-CoA-carboxylase reaction. This is the initial reaction of lipid formation. In it, acetyl-CoA, in the presence of CO_2, ATP, and Mg^{++} or Mn^{++}, is transformed into malonyl-CoA (109). The coenzyme of acetyl-CoA-carboxylase is biotin (110). The enzyme is not

strongly specific and can carboxylyze propionyl-, butyryl-, caprylyl-, and capronyl-CoA (111). Citrate (112) and carnitine (113) are allosteric stimuli, and acyl-CoA-producing, high-molecular-weight fatty acids are allosteric inhibito s of the enzyme (114).

The physiological importance of this reaction is that it is the initial and thus the limiting reaction in the process of fatty acid biosynthesis (115).

The pyruvate-carboxylase reaction. A proposal of the existence of this reaction had already been set forth by Wood and Werkman in 1938 (116). But only in 1960 did Utter and Keech succeed in isolating, from the liver, kidneys, and other tissues, the enzyme which catalyzes the transformation of pyruvate into oxalacetate (in the presence of CO_2, ATP, Mg^{++}, and acetyl-CoA) (117-118). Acetyl-CoA is the allosteric stimulus of the enzyme. The activity of the latter is also increased during an increase in the size of the ratio ATP/ADP in the cell, as well as after hormonal stimulation of gluconeogenesis (119-121).

The major physiological significance of this reaction is that it is the main means of providing cells with C-4-dicarbonic acids.

The carbamyl-phosphate-synthetase reaction. The synthesis of carbamyl-phosphate, which is utilized in the biosynthesis of citrulline, takes place in the presence of NH_3, CO_2, ATP, and Mn^{++} (or Co^{++} or Zn^{++}), as well as in the biosynthesis of acetyl glutamate (122-126). Acetyl glutamate has a catalytic value in this reaction, forming an auxiliary compound with CO_2. The process of carbamyl phosphate synthesis is gradual and begins with the formation of carbonyl phosphate, which, subsequently reacting with acetyl glutamate, ATP, and NH_3, is transformed into carbamyl phosphate.

Synthesis of carbamyl phosphate, which is utilized for the synthesis of nitrogen bases of pyrimidine nucleotides, is accomplished by another carbamyl phosphate synthestase. The reaction proceeds due to NH_3, CO_2, ATP, and in the presence of Mn^{++} and glutamate (126, 127).

The physiological significance of these reactions is their participation in processes of biosynthesis of carbamides and pyrimidine nucleotides, and consequently nucleic acids.

The 5-amino-imide-azol-ribonucleotide carboxylase reaction. In this reaction, 5-amino-imide-azol-nucleotide, in carboxylation, transformed into 5-amino-imide-azol carbonic acid. The enzyme which catalyzes the reaction has been isolated from the liver of doves (128). It is highly specific and is activated by Mg^{++}. The reaction proceeds through ATP energy, although it was originally thought that it did not require any outside energy (8, 129).

The reaction's physiological significance is that it is one of the links in the chain of reactions in the biosynthesis of pyrine nucelotides and therefore of nucleic acids.

Carboxylation Reactions which Proceed without Requiring Outside Energy

In heterotrophic microorganisms, several reactions belong to this group (7-11), but only one appears in animal tissues, the phospho-enol-pyruvate-carboxykinase reaction.

The phospho-enol-pyruvate-carboxykinase reaction. Its existence had been proposed long ago (130), but only in 1953 did Utter and Kurahashi (131) succeed in isolating, from the liver of birds, the enzyme which catalyzed the synthesis of oxalacetate through phospho-

enol-pyruvate. The reactions proceed in the presence of CO_2, guanosine diphosphate (GDP), and Mg^{++}. GDP may be replaced by uridine diphosphate (UDP) (132). Originally the enzyme was called oxalacetate carboxylase (130), and then phospho-enol-pyrivate-carboxykinase (133). The activity of the enzyme depends directly on the value of the ratio of GTP/GDP (134).

The physiological significance of the reaction is that it represents the limiting link in the chain of reactions in gluconeogenesis, and any change in its intensity will determine corresponding changes in this process (84, 120). It is also one of the mechanisms for providing cells with C-4-dicarbonic acids. The latter are especially important to muscle tissues, since pyruvate carboxylase is not present in them (135).

In summing up, it may be concluded that CO_2 is an extremely important metabolite. It is difficult to name any other substance which would have as many different transformation reactions as CO_2. If we consider that a number of these reactions is catalyzed by not one but several enzyme systems, it becomes clear that in this respect CO_2 is a unique metabolite in life.

The uniqueness of the physiological significance of carbon dioxide is determined mainly by the fact that it participates in reactions of biosynthesis for the most important cell components: lipids, carbohydrates, a number of amino acids (asparaginic, glutaminic, glycine, serine, citrulline, arginine), the nitrogen bases of nucleotides, and nucleic acids.

If we consider that 4 out of the 9 intermediate members of the tricarbon cycle (oxalacetate, malate, isonitrate, and succinate) form a means of carboxylating the corresponding substrates (pyruvate, α-ketoglutarate, propionate), then it becomes completely clear that its functioning also depends on the processes of carbon dioxide formation (Fig. 6).

Taking into account the participation of carbon dioxide gas in all the basic biosynthetic and oxidation processes in the cell, we came to the conclusion in our laboratory that all these pro-

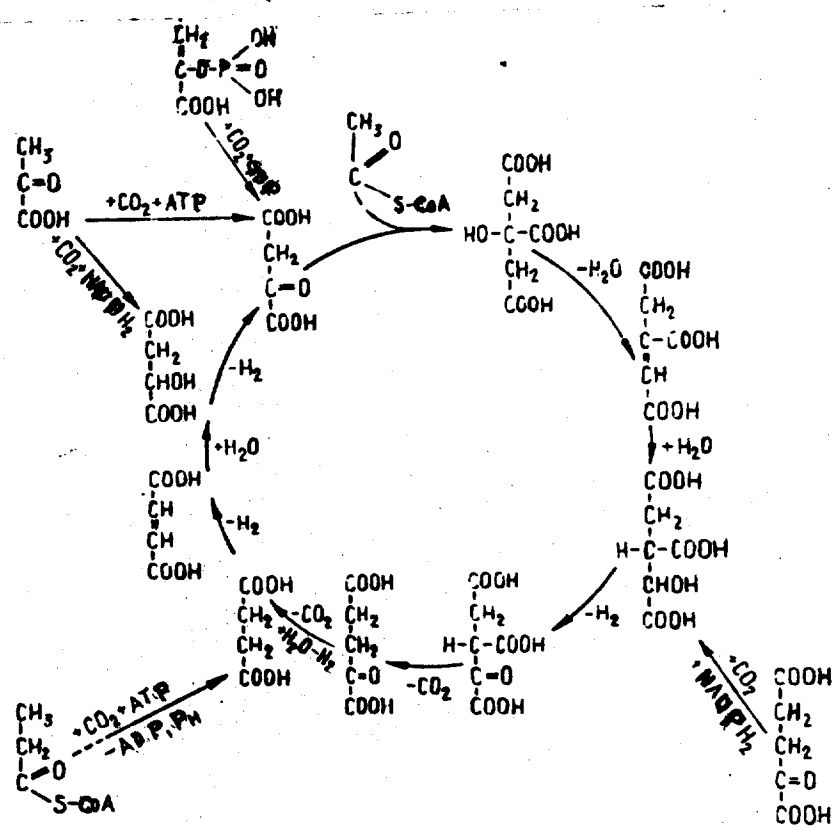


Fig. 6. Carbon dioxide and the tricarbon cycle

cesses, i.e. the biosynthesis of many amino acids (and this means proteins, as well), lipids, and carbohydrates of pyrinic and pyrimidine bases (and this means the corresponding nucleotides and nucleic acids, also). The functioning of the tricarbon cycle must also be found in continuous dependence on the processes of CO_2 fixation.

On this basis, the idea was proposed that in developing means of acting on carboxylation intensity, it may purposefully influence all the indicated biosynthetic processes in animal tissues and in microbial cells (10, 11, 73).

This also served as the base for the wide interest in studies in the area of biosynthesis and biological properties of protein at the Institute of Biochemistry, Academy of Sciences USSR, which were directed toward investigating means of effectively changing the intensity of CO_2 fixation processes and correspondingly, the biosynthetic processes in the animal organism.

Theoretically (7-11), it is possible to influence the intensity of processes of CO_2 fixation in the organism by various means, such as:

1) changing the concentration of the substrates underlying carboxylation;

2) varying the concentration and proportions of different forms of carbon dioxide in the tissues;

3) changing the amount of energy (ATP, $NADPH_2$, $NAPH_2$) provided and the corresponding coenzymes of carboxylase (biotin, nicotinamide, vitamin B_6, tetrahydrofolic acid);

4) changing concentrations of magnesium, manganese, and zinc ions (enzyme activators), as well as of heavy metal ions (carboxylase inhibitors) and of other cofactors (acetyl glutamate, glutamate, citrate, carnitine, acetyl-CoA, and others), which are allosteric regulators of carboxylase activity;

5) evoking changes in the hormonal status in the organism.

In our laboratory, the possibility of acting on the intensity of CO_2 fixation processes and the indicated biosynthetic processes were studied, in changing carbon dioxide content in the tissues as a substrate of carboxylase enzymes, as well as changing the amount of bivalent metals which are activators of these enzymes.

We present below the results of the studies, which confirm the possibility of altering the intensity of biosynthetic processes in animal tissues by acting on the processes of carboxylation.

Stimulation of Carboxylation Reactions - Means of Activating Biosynthetic Processes in the Tissues

In experiments done on rat liver sections (66), it was shown that during their incubation with $NaHC^{14}O_3$, a yield of Mg^{++} in the incubation medium caused an increase of 27.7% in the intensity of radioactive carbon fixation in protein, Zn^{++} caused a 26% increase and Mn^{++} a 12% increase (in the last case, $p > 0.05$). CO_2 fixation in lipids in the liver sections increased only under the influence of Mg^{++} (21.6%).

In further experiments on lactating rabbits, which were fed a salt mixture called carboxyline for 10 days (the dosage calculated at 0.174 mg per g live weight; composition: sodium bicarbonate, 20 parts by weight, magnesium, manganese, and zinc sulfates, 4.0, 0.1, and 0.1 parts by weight, respectively), a significant increase

in C^{14} fixation intensity (in comparison to the control) was shown, due to $NaHC^{14}O_3$ in proteins and carbons of blood and milk serum, as well as in liver and muscle glycogen (69). In feeding carboxyline at 1 mg per g live weight, its stimulating effect was even greater.

Under the influence of the 10-day carboxyline feeding, the intensity of C^{14} inclusion similarly increases, due to $NaHC^{14}O_3$ in the organic components (protein, glycogen) of the liver, muscles, and blood of male rabbits (68), as well as in the protein of rooster livers (67).

Transformation of the carbon in $NaHC^{14}O_3$ into organic acid-soluble compounds of homogenates in the liver of roosters, which received 0.42 mg/g live weight of carboxyline for 120-150 days, also occurs by far more intensely than in liver homogenates from the control chickens (70).

Thus, these results completely verify the proposal that, by increasing the carbon dioxide content and providing a definite concentration of ions (Mg^{++}, Mn^{++}, and Zn^{++}) to animal tissues, CO_2 fixation processes may be stimulated. This was also noted by Adams and Haynes (136) in the mitochondria of rat livers, which showed that an increase in HCO_3^- concentration in the incubation medium from 6 to 12 mmole/liter almost doubled carbon dioxide fixation.

At the same time, it was established in our laboratory that under conditions of stimulating CO_2 fixation processes, the functioning of the tricarbon cycle increases in tissues. This is very important, since it is known that the tricarbon cycle is not just the basic biological oxidation of organic matter. The intensity of biosynthesis of fats, carbon, protein, and nucleotides of animal tissues occur in direct proportion to its fixation intensity (10, 137-144). In particular, in experiments on rat and chicken liver sections, it has been observed that partial replacement of phosphate in a Krebs-Ringer medium with bicarbonate increases oxidation of lysine-1-C^{14} and acetate-2-C^{14} to $C^{14}O_2$, although the pH of the media remained constant (7.05), while this effect of bicarbonate increases with its addition with Mg^{++}, Mn^{++}, or Zn^{++} (66, 67).

In experiments on chickens, which were fed 120-150 days on carboxyline, it was also shown that oxygen absorption and acetate-1-C^{14} oxidation to $C^{14}O_2$ in liver sections from chickens in the experimental groups proceed more intensely, by 24.0 and 77.5% respectively, than in liver sections from chickens in the control groups (70).

The results of these studies are fully verified by Longmore (22), who established that with an increase in amount of HCO_3^- in the incubation medium from 10 to 40 mmole/liter (pH 7.40), the intensity of glycerine-1,3-C^{14} and pyruvate-2-C^{14} oxidation to $C^{14}O_2$ in liver sections from normal rats increases 20-60%. Trohmen and Orlen (145) also observed that feeding sodium bicarbonate to rats with alloxanic diabetes sharply stimulates acetate-1-C^{14} oxidation to $C^{14})_2$, and the content of intermediate products of the tricarbon cycle in tissues in this situation increases to an almost normal level. It may be suggested that an increase in the fixation process of the tricarbon cycle under conditions of carbon dioxide fixation stimulation in tissues is determined by the fact that four out of nine of its components are formed by carboxylation of the corresponding substrates (Fig. 6).

Concerning the participation of carbon dioxide in the regulation of biological oxidation processes in the cell, in particular in tri-

carbon cycle reactions, a huge amount of data from direct and indirect observations have been accumulated (18, 19, 23, 146-153).

Speaking of the important role of carbon dioxide in the realization of tricarbon cycle fixation processes in animal tissues, we must turn our attention to the activity of a number of enzymes which have no direct relation to CO_2 fixation, but which under constant pH in the media may substantially be altered in relation to their HCO_3^- content. Thus, Hastings et al. (154) indicated that ATP-ase activity in the liver mitochondria of rats may increase almost 3-4 times with an increase in HCO_3^- concentration in the medium from 0 to 60 mmole/liter (pH 7.5). Backer (155) observed a similar change in ATP-ase activity, and McDaniel and Longmore (24) discovered that HCO_3^- (with normal pH) inhibits aconitate-hydrolase activity in ox livers. A similar thing is observed for glucose-6-phosphate-phosphohydrolase in rat livers (156). It is possible to explain this by saying that carbon dioxide may enter into a number of enzyme reactions as a regulator, fulfilling the role of an allosteric effector.

Numerous data obtained in our laboratory confirm that carbon dioxide, in taking part in many reactions of carboxylation which involve the synthesis and utilization of a number of the previous proteins, lipids, carbon, nucleic acids, etc. (Fig. 4) show substantial regulatory action in the basic biosynthetic processes in the organism. Thus, in in vivo experiments on rat liver sections, it was shown that introduction of carboxyline in increasing dosages gradually increases C^{14} inclusion, due to acetate-2-C^{14} and lysine-1-C^{14} in proteins and lipids (68). Of all the components of carboxyline, sodium bicarbonate has the greatest effect, and Mg, Mn, and Zn ions increase its action. Similar results were obtained in experiments on chicken liver sections (67).

In subsequent experiments on rats which were fed daily for 10 days 0.604 mg of carboxyline per g liver weight, then were given acetate-2-C^{14} intravenously and were killed after 1 or 3.5 hours, a significant increase was observed by including tracers in proteins and glycogen in liver and muscles (80).

In in vivo experiments (71, 76, 80) on roosters, which were fed carboxyline for 10 days, a significant stimulation of C^{14} inclusion was observed, due to acetate-2-C^{14} in proteins, lipids, and glycogen of the liver, as well as in blood serum protein. In this case, the effect of carboxyline was more distinct in adult birds in comparison to young ones.

An increase in exchangeability of proteins, lipids, and free nucleotides under the influence of carboxyline feeding was also observed in in vitro experiments on rooster liver sections (67, 70, 79).

Thus, it may be concluded that in stimulating processes of carbon dioxide fixation in tissues, basic biosynthetic processes may be activated in animal organisms, i.e. the biosynthesis of proteins, carbon, lipids, nucleotides, etc.

The correctness of this conclusion is also confirmed by the results of experiments on liver homogenates from rats, rabbits, and chickens, in which the processes of carbon dioxide fixation were depressed by avidin (68, 78, 79). In this situation, tracer inclusion in proteins and lipids was depressed, in parallel with carbon dioxide fixation inhibition, due to acetate-1-C^{14} and glycine-1-C^{14}.

The results from a number of other investigators correspond exactly to these data, showing that under conditions of increasing carbon dioxide content in the tissues, the biosynthesis of lipids and

glycogen is stimulated (21, 22, 25-30, 157, 158).

The established pattern, that of the dependence of basic biosynthetic processes on the intensity of carbon dioxide fixation and, correspondingly, on its content in tissues, has an exceptionally important conceptual and practical significance, especially in view of a great number of factors which are free to influence the content and proportion of different forms of carbon dioxide in the tissues of animals and man and on which we have dwelt extensively above.

Proceeding from this, in the Department of Biosynthesis and Biological Properties of Protein in the Institute of Biochemistry, Academy of Sciences USSR, it was established, in experiments on animals with regenerating tissues, that stimulation of carboxylation in the tissues, evoking an increase in intensity of biosynthetic processes, speeds up tissue regeneration. Thus in experiments on partially hepatectomized rabbits (70-80% of the liver removed), it is shown that under the influence of carboxyline feeding for 10 days up until the operation, biosynthesis increases for protein, lipids, and glycogen in the regenerating liver, recovery of the rabbit's original weight is accelerated, and the content of lean matter is increased (73, 77).

In experiments on rabbits with experimental burn diseases, it was found (75) that feeding them carboxyline for 7 days accelerates the recovery of the original body weight, intensifies the functioning of the tricarbon cycle, and increases the intensity of C^{14} inclusion from acetate in muscle tissue and proteins of the liver, kidneys, spleen, and blood serum, as well as liver lipids.

It was also shown that in experimental post-hemorrhagic anemia, under the influence of carboxyline in the blood serum of anemic rabbits, recovery of the number of erythrocytes, hemoglobin, and proteins in blood serum is accelerated. In the liver, kidneys, and spleen, the fixation processes of the tricarbon cycle and protein synthesis are intensified, the reactivity of bone marrow is increased in erythropoiesis, and the acid-alkaline balance is normalized (74).

The results of Morton's experiments (159) agree fully with these data. He showed that the intensity of reticulocyte formation in the bone marrow of rats <u>in vitro</u> is in direct proportion to CO_2 content in a gaseous medium, reaching a maximum at 30%. The data from a number of investigators (160-166), the list of which might be made much longer, also confirm the dependence of the intensity of growth in many kinds of heterotrophic organisms and yeasts on the amount of carbon dioxide in the medium.

The close relationship of biosynthetic processes on CO_2 fixation and the potential for increasing the intensity of biosynthesis of the basic organic components of animal tissues by stimulating carboxylation reactions in them, as well as the fact that the conditions used at the present time for feeding farm animals (feeding large amounts of silage, pulp, grains, and concentrates) often lead to a decrease in carbon dioxide content in tissues (compensated and metabolic acidoses), served as a basis for work on a method of increasing the productivity of farm animals and birds, conducted at the Institute of Biochemistry, USSR Academy of Sciences and the Ukrainian Agricultural Academy (167-185).

The effectiveness of the proposed method was confirmed in a number of scientific research establishments (186-195), and it is now widely used on state and collective farms in the Ukrainian SSR (196-203).

Conclusion

1. Carbon dioxide is contained in animal tissues in the form of soluble carbon dioxide gas, carbonic acid, bicarbonate, carbonates, and carbamates. About 94-95% of the total amount of carbon dioxide in the blood of animals occurs as bicarbonates. The carbon dioxide content in other biological liquids and tissues is in direct proportion to its content in the blood.

2. In tissues of animals and man, the contents of, and proportions between, different forms of carbon dioxide and hydrogen ions may vary, and thus they determine 9 states of the organism: normal, metabol c acidosis and alkalosis, respiratory acidosis and alkalosis, respiratory acidosis and alkalosis with partial compensation, and compensated acidosis and alkalosis.

3. More than 10 carbon dioxide fixation reactions occur in animal tissues, in which the carbon of carbon dioxide is transformed into the carbon of organic compounds. These reactions are divided into three groups: those proceeding due to ATP energy, those due to the energy of reducing forms of pyrimidine nucleotides, and those not requiring outside energy.

4. The intensity of processes of carbon dioxide fixation in tissues is determined by the following factors: 1) the concentration of substrates underlying carboxylation; 2) the concentrations and proportions of different forms of carbon dioxide in tissues; 3) the degree of energy and corresponding coenzymes of carboxylase provided (biotin, nicotinamide, vitamin B_6, tetrahydrofoliate); 4) the presence in definite concentrations of activators (ions of magnesium, manganese, and zinc) and inhibitors of carboxylase (ions of heavy metals, etc.), as well as a number of cofactors (acetyl glutamate, glutamate, citrate, carnitine, acyl-CoA, etc.), which are allosteric regulators of carboxylase activity; 5) corresponding hormonal status in the organism.

5. Carbon dioxide, being utilized in carboxylation reactions in animal tissues, takes part in the biosynthesis of fatty acids, lipids, many replaceable amino acids, and therefore proteins, in the synthesis de novo of carbon, in the formation of pyrine and pyrimidine bases, and thus nucleotides and nucleic acids, as well as in the reactions of the tricarbon cycle.

6. There is a dependence of the intensity of biosynthesis of the organic compounds listed above on the processes of carbon dioxide fixation, which is expressed in the fact that stimulation or depression of the latter increases or decreases respectively the process of the former.

7. Increasing, within definite limits, the content of carbon dioxide and ions of magnesium, manganese, and zinc in tissues may noticeably increase the intensity of biosynthetic processes and stimulate tricarbon cycle reactions in the organism. This confirms that carbon dioxide in the animal organism is not only a regulator of processes of respiration and the major, central buffer system in animals and man, but it is also a regulator of basic, biosynthetic processes in the organism.

8. The indicated regulatory role of carbon dioxide in the biosynthetic processes of the organism may be effectively utilized for practical purposes. Increasing the biosynthetic processes by stimulating processes of carbon dioxide fixation, one can noticeably increase the productivity of farm animals and birds, as well as signi-

ficantly accelerate the process of tissue regeneration, in particular, in hepatectomy, burns, and post-hemorrhagic anemias.

9. Besides the participation in the processes of carboxylation and regulation of reactions of the medium, carbon dioxide has the property of causing specific changes in the activity of several enzymes (ATP-ase, aconitate-hyd lase, and glucose-6-phosphate-phosphohyd lase), which have no direct relation to carbon dioxide fixation processes.

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ЗНАЧЕНИЕ УГЛЕКИСЛОТЫ В ОБМЕНЕ ВЕЩЕСТВ У ЖИВОТНЫХ

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Детально изучать метаболическое значение углекислоты в организме животных начали сравнительно недавно.

Уже со второй половины XIX века физиологи стали считать углекислоту не просто конечным продуктом обмена, а важнейшим метаболитом, который способен весьма существенно влиять на функции ряда систем органов человека и животных [1]. Особенно возрос интерес к этой проблеме в связи с развитием авиации, исследованием космоса, подводными, подземными и другими работами, для которых характерно возможное изменение концентрации углекислоты во вдыхаемом воздухе. Надо подчеркнуть, что и до сих пор очень многие исследователи изучают именно эти вопросы.

Что касается биохимиков, то, пожалуй, самым важным их вкладом в развитие этой проблемы является доказательство того, что углерод углекислоты используется в тканях гетеротрофных организмов в процессе биосинтеза жиров, углеводов, нуклеиновых кислот, белков и других органических соединений с помощью реакций фиксации CO_2 (карбоксилирования) [2—11]. Длительное время считалось [8—11], что этот процесс у гетеротрофных микроорганизмов впервые был открыт в 1935 г. Вудом и Веркманом [4]. Однако наличие его у них было доказано еще в 1910 г. нашим соотечественником А. Ф. Лебедевым [2, 3]. Особенно возрос интерес к этим процессам после того, как они были обнаружены в тканях высших животных [5, 6]. С этого времени карбоксилирование усиленно изучали во многих лабораториях мира, и теперь известно, что в тканях животных протекает более 10 основных реакций, в которых углерод углекислоты превращается в углерод органических соединений [11].

В настоящее время интенсивно изучаются две основные проблемы: 1) физиологическое значение углекислоты, участвующей в респираторном цикле; 2) механизмы и физиологическое значение реакций фиксации углекислоты в тканях.

Следует подчеркнуть, что в последнее время значительно расширилось изучение особенностей обмена веществ при изменении концентрации и соотношения различных форм углекислоты в тканях. Имеется в виду огромное количество исследований, которые уже со второй половины XIX века [12—15] и до настоящего времени [16—20] посвящались и посвящаются особенностям физиологических функций и обмена веществ у животных и людей при так называемых ацидозных и алкалозных состояниях. При этом было показано [16—32], что причиной наблюдаемых сдвигов в обмене веществ при ацидозах и алкалозах являются не только изменение концентрации H^+, но и не в меньшей мере — изменение концентрации и величины соотношения различ

ных форм углекислоты. Тем не менее, обсуждая механизмы влияния углекислоты на обмен веществ, исследователи упускали из вида важную роль процессов карбоксилирования, интенсивность протекания которых в определенной степени зависит от ряда факторов, в первую очередь — от концентрации углекислоты в тканях.

Следует также отметить, что биологическая роль углекислоты не ограничивается участием ее в процессах карбоксилирования, в образовании бикарбонатной буферной системы и в регуляции деятельности дыхательного центра. Так, сравнительно недавно показано, что активность ряда ферментов, которые не имеют прямого отношения к фиксации CO_2, может существенно изменяться в зависимости от концентрации углекислоты в средах (АТФ-аза, аконитат-гидролаза, глюкозо-6-фосфат-фосфогидролаза).

В нашей лаборатории было показано, что карбоксилирование лежит в основе важнейших биосинтетических процессов в тканях животных и что, воздействуя на интенсивность его протекания, можно существенным образом влиять на интенсивность протекания последних [10, 11, 33]. Открытие этой закономерности важно не только с теоретической точки зрения, но и имеет очень большое значение для решения многих проблем медицинской, животноводческой, ветеринарной и микробиологической практики.

ФОРМЫ УГЛЕКИСЛОТЫ И РЕГУЛЯЦИЯ ЕЕ СОДЕРЖАНИЯ В ОРГАНИЗМЕ

Формы углекислоты

Углекислота в организме животных может содержаться в форме растворенного углекислого газа (pCO_2), угольной кислоты (H_2CO_3), бикарбонатов (HCO_3^-), карбонатов (CO_3^{--}) и карбаматов ($R-N\begin{smallmatrix}H\\COOH\end{smallmatrix}$) (рис. 1).

pCO_2. Главным источником ее в тканях является эндогенный углекислый газ. Растворимость его в воде при 37° С (в миллимолях со-

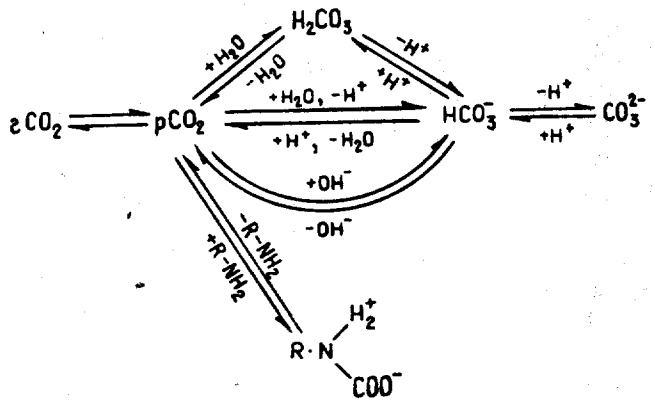


Рис. 1. Формы углекислоты в организме животных.

ставляет $0{,}334 \times p$ (p — парциальное давление углекислоты, *мм* рт. ст.). Подсчитано, что концентрация pCO_2 в плазме крови человека и животных составляет около 1,0—1,2 *ммоль/л* [34—40].

H_2CO_3 образуется в тканях за счет pCO_2 и бикарбонатов с участием карбоангидразы [34]. В тканях ее очень мало и составляет она $^1/_{300}$ — $^1/_{900}$ часть общего количества pCO_2 [35—36]. Говоря о pCO_2, очень часто подразумевают обе эти формы углекислоты вместе.

HCO_3^- образуется за счет pCO_2 с участием карбоангидразы, а также H_2CO_3 или CO_3^{--} при соответствующих изменениях концентрации ионов водорода в тканях. Исследования крови людей и животных показали, что на долю бикарбонатов приходится около 95% общего количества углекислоты, которая выделяется из плазмы при добавлении к ней сильной кислоты в вакууме, и лишь около 5% — на долю остальных ее форм [37, 39]. Это соотношение (при нормальных условиях) довольно постоянное, и исходя из него Хандерсон и Хассельбах [39] составили уравнение, которое выражает взаимозависимость таких показателей, как pH, концентрация H_2CO_3 и HCO_3^- в крови человека:

$$pH = 6{,}10 + \lg \frac{HCO_3^-}{H_2CO_3}.$$

По данным многих исследователей, содержание бикарбонатов в плазме крови людей и животных составляет около 18—25 *ммоль/л*, а при ряде патологических состояний может колебаться от 10 до 50 *ммоль/л* [21, 37—41]. Примерно такое же количество бикарбонатов содержится в межклеточной и цереброспинальной жидкости, причем оно находится в прямой зависимости от содержания их в крови [39, 42]. Количество бикарбонатов во внутриклеточной жидкости также определяется внеклеточной их концентрацией, но обычно оно ниже последней примерно на 25—30% [43]. Очень высоко содержание бикарбонатов в слюне, соке поджелудочной железы и в желчи (60—130 *ммоль/л*) [44—46].

CO_3^{--} как свидетельствуют данные литературы [47], образуется за счет бикарбонатов при участии карбоангидразы:

$$2\,HCO_3^- \longrightarrow H_2CO_3 + CO_3^{--}.$$

Эта форма углекислоты имеет существенное значение для формирования костной ткани. Так, показано, что карбонатные ионы составляют около 6,0% минеральных компонентов кости животных [48]. Особенно важную роль играют карбонаты в процессе образования скорлупы яиц у птиц: Ca^{2+} из крови поступает в железы яйцеводов и превращается в кристаллический карбонат кальция скорлупы. В последнее время важную роль в этом процессе отводят также аммиаку [49]:

$$HCO_3^- + Ca^{2+} + NH_3 \longrightarrow CaCO_3 + NH_4^+.$$

$R-N\langle^{H}_{COOH}$ — карбаматы, или карбаминовые кислоты, образуются при взаимодействии pCO_2 со свободными неполяризованными аминогруппами белков [50—52]. Эта реакция экзотермическая. Карбаматы — довольно сильные кислоты (pK — 5,8), устойчивы в щелочной среде и расщепляются в кислой. Их кальциевые и бариевые соли растворимы в воде. В крови человека в виде карбаматов находится примерно 5% pCO_2 [37].

Показано, что 10—30% углекислоты, участвующей в респираторном цикле, выводится в окружающую среду благодаря образованию карбаматов [37, 50—52].

Регуляция содержания углекислоты в организме животных

Из изложенного видно, что основными формами углекислоты в организме животных являются бикарбонаты и растворенный углекислый газ. От их содержания и величины соотношения между ними в основном зависит не только концентрация других форм углекислоты, но и ионов водорода.

Рассмотрим механизмы регуляции содержания углекислоты в тканях, а также ряд физиологических и патофизиологических состояний животных и человека, характеризующихся изменениями в концентрации и соотношении различных форм углекислоты в организме.

Источники и механизмы выделения углекислоты из организма

pCO_2. Как уже указывалось, основным источником его в организме является эндогенный углекислый газ. Так, у взрослого человека за сутки образуется в среднем 600 *г* углекислоты [37]. Нейтрализация и выделение ее из организма — очень сложные процессы, которые в настоящее время представляются следующим образом.

С одной стороны, в тканях по мере образования углекислоты и превращения оксигемоглобина в восстановленный гемоглобин происходит превращение H_2CO_3 в HCO^-_3, а в легких благодаря карбоангидразе эритроцитов HCO_3^- превращается в CO_2 и H_2O, после чего CO_2 выделяется в окружающую среду. Это можно представить так:

$$\text{в тканях: } Hb-O_2+CO_2+H_2O \xrightarrow[\text{тканей}]{\text{карбоангидраза}} Hb-H+HCO_3^-+\underline{O_2};$$

$$\text{в легких: } Hb-H+HCO_3^-+O_2 \xrightarrow[\text{эритроцитов}]{\text{карбоангидраза}} Hb-O_2+\underset{CO_2 \quad H_2O}{H_2CO_3}.$$

Считают, что этот механизм обеспечивает выделение около $^2/_3$ выдыхаемого углекислого газа [37]. С другой стороны, гемоглобин эритроцитов [37] и белки плазмы [52], реагируя с углекислотой тканей, образуют карбаматы. В легких карбаматы расщепляются и белки переходят в свободное состояние. Робинсон указывает, что почти треть всей выдыхаемой углекислоты выделяется именно таким путем [37]. Следует отметить, что интенсивность вентиляции легких, а значит, и выделения pCO_2 в окружающую среду регулируется дыхательным центром, деятельность которого находится в прямой зависимости от содержания pCO_2 и H^+ в крови [1]. Примерно 0,5% общего количества выделяемой углекислоты проникает в окружающую среду через кожу [53].

HCO^-_3. Источником его могут быть различные формы углекислоты (рис. 1). Концентрация бикарбонатов в тканях определяется в основном величиной соотношения в них щелочных (Na, K, Ca, Mg) и кислотных (Cl, S, P) эквивалентов. Поэтому прежде чем говорить о механизмах регуляции содержания HCO_3^- в тканях, вкратце рассмотрим превращение кислот и оснований в организме, которые могут образовываться внутри клеток или всасываться из пищеварительного тракта. Попадая в организм, эти соединения прежде всего подвергаются сильному разведению в различных биологических жидкостях. Кроме того, значительная часть их подвергается различным метаболическим превращениям. Так, органические кислоты в большинстве своем окисляются в CO_2 и H_2O. Поэтому существенные сдвиги в содержании углекислоты в организме будут иметь место при изменении интенсивности поступления органических кислот или образования их в тканях. Совсем другое дело с минеральными кислотами или основаниями. Важнейшим гомеостатическим механизмом здесь выступают буферные системы: бикарбонатная, белковая, фосфатная, а в эритроцитах — гемоглобин. Необходимо подчеркнуть, что наиболее сильной и важной из них является бикарбонатная буферная система [37].

Важное значение в регуляции активной реакции крови имеет также обмен ионов между внеклеточными жидкостями и минеральными

компонентами скелета. Поэтому не случайно при ряде ацидозных состояний часто наблюдаются нарушения минерального обмена, касающегося скелета (остеомаляцию, остеопороз, слабость скорлупы яиц).

Таким образом, при поступлении в организм определенного количества кислот значительная часть бикарбонатов тканей, реагируя с ними, превращаются в угольную кислоту, избыток которой выделяется через легкие. И наоборот — поступающие в организм основания, реагируя с угольной кислотой, образуют бикарбонаты, повышая их общее содержание в тканях. В этом состоит большое значение бикарбонатного буфера, которое заключается в предохранении организма от резких колебаний pH биологических жидкостей за счет изменения в них концентрации и соотношения биокарбонатов и угольной кислоты.

Важное значение в поддержании кислотно-щелочного гомеостаза, а точнее — в выделении из организма избытка HCO_3^- и H^+, принадлежит почкам [37]. Осуществляется это следующим образом. Бикарбонаты крови свободно проникают в почечный гломерулярный фильтрат, и содержание их в нем определяется концентрацией их в крови. Однако если pH крови ниже нормы, происходит «реабсорбция» бикарбонатов в кровь при параллельном усилении экскреции H^+ в мочу. Сущность этого процесса заключается в том, что под влиянием карбоангидразы клеток почечных канальцев H_2CO_3 превращается в HCO_3^- и H^+. Последний экскретируется в гломерулярный фильтрат, из которого при этом реабсорбируется эквивалентное количество ионов натрия. Поэтому вместо каждого выведенного в мочу иона водорода в плазму возвращается одна молекула $NaHCO_3$, используемая для нейтрализации избытка H^+ в крови. Скорость экскреции H^+ в мочу и соответственно реабсорбции Na^+ в кровь находится в прямой зависимости от содержания pCO_2 в крови, т. е. pH выделяемой мочи будет определяться концентрацией бикарбонатов в первичной моче и интенсивностью экскреции H^+ в гломерулярных клетках почек. При ацидозных состояниях может наблюдаться недостаток бикарбонатов в первичной моче для нейтрализации экскретируемых ионов H^+. В таком случае они будут нейтрализоваться первичными фосфатами или аммиаком, что приводит к усиленному выведению фосфора и азота с мочой [54—56].

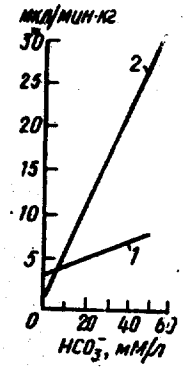


Рис. 2. Зависимость образования желчи (1) и панкреатического сока (2) у собак от содержания бикарбонатов в крови.

Значительная часть бикарбонатов высвобождается из крови в процессе образования слюны, желчи, кишечного и поджелудочного сока [45, 46]. Доказано, что скорость образования поджелудочного сока и желчи у собак прямо пропорциональна концентрации HCO_3^- в крови (рис. 2) [46]. Однако судить о количестве бикарбонатов, выделяемых через желудочно-кишечный тракт, трудно, поскольку значительная часть их нейтрализуется кислотами пищи и желудочного сока, а образующиеся при этом соли всасываются обратно в кровь.

Физиологические и патофизиологические состояния животных и человека, характеризующиеся изменениями в содержании и соотношении различных форм углекислоты в организме

Ван-Слайк и другие исследователи [39] уже в 20-х годах, изучив кислотно-щелочной баланс крови животных и людей при ряде физиологических и патофизиологических состояний, установили, что организму присущи девять различных состояний: норма, метаболиче-

ские ацидоз и алкалоз, респираторные ацидоз и алкалоз, респираторные ацидоз и алкалоз с частичной почечной компенсацией, компенсированные ацидоз и алкалоз. Последние два заслуживают особого внимания, поскольку они характеризуются существенными различиями в содержании углекислоты при нормальной концентрации H^+ в тканях. Схематически эти состояния представлены на рис. 3 [39]. Рассмотрим каждое из них.

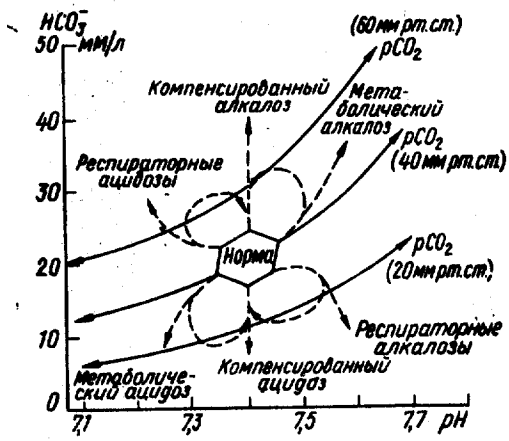


Рис. 3. Нормальные и патофизиологические состояния организма животных и людей, характеризующиеся изменениями взаимоотношения содержания HCO_3; pCO_2 и H^+ в плазме крови.

Норма характеризуется такими показателями: pH = = 7,36—7,44; HCO_3^- = 18—25 *ммоль/л*; pCO_2 = 1,0—1,2 *ммоль/л*.

Метаболический алкалоз (pH>7,44; HCO_3^- больше нормы; pCO_2 — норма или больше нормы) развивается при поступлении в организм избытка бикарбонатов, натриевых или калиевых солей органических кислот, после частых промываний желудка, при гиперсекреции HCl в полость желудка, при ряде нефритов (почечный алкалоз) [39, 57, 58]. В этих условиях с увеличением содержания HCO_3^- в крови сразу же замедляется дыхание, что приводит к параллельному увеличению количества pCO_2 в тканях. Одновременно с этим усиливается выделение бикарбонатов с мочой. Эти процессы направлены в сторону снижения величины pH крови до нормального уровня, хоть содержание HCO_3^- и pCO_2 в ней при этом может быть значительно выше нормы. Если pH крови нормализуется, развивается так называемое состояние компенсированного алкалоза. Если же оно продолжает возрастать [>7, 8], то развиваются тетанические явления и возможна смерть.

Метаболический ацидоз (pH <7,36; HCO_3^- меньше нормы; pCO_2 — норма или меньше нормы) наблюдается у животных и людей после введения им аммонийных солей неорганических кислот или растворов кислот, при тяжелых формах диабета, при осложненных родах, при ряде нефритов (почечный ацидоз), после тяжелых травм, при ряде послеоперационных состояний, при обширных ожогах, в первые дни у новорожденных и др. [39, 57, 59—61]. Следовательно, состояние метаболического ацидоза развивается в тех случаях, когда избыток кислот (алиментарного или эндогенного происхождения) нейтрализуется бикарбонатами тканей и крови, что приводит к увеличению содержания pCO_2 в крови и снижению количества HCO_3^-. При этом учащается дыхание и одновременно усиливается реабсорбция бикарбонатов из гломерулярного фильтрата почек обратно в кровь. Эти два процесса направлены на повышение pH, т. е. нормализацию его. Если pH нормализуется, развивается состояние компенсированного ацидоза. Если же оно продолжает снижаться (<7,0), то развивается коматозное состояние и возможна смерть.

Респираторные алкалозы (pH>7,44; HCO_3^- — норма или меньше нормы; pCO_2 — меньше нормы) развиваются при вдыхании газовой смеси, лишенной CO_2, а также при самопроизвольной гипер-

вентиляции легких [37, 39]. При этом усиливается выделение бикарбонатов с мочой (респираторный алкалоз с частичной почечной компенсацией), что одновременно с гиповентиляцией легких создает условия для нормализации pH крови и перехода в состояние компенсированного ацидоза.

Респираторные ацидозы (pH <7,36; HCO_3^- — норма или больше нормы; pCO_2 — больше нормы) возникает при подавлении деятельности дыхательного центра морфием, при вдыхании газовой смеси, содержащей большой процент углекислоты, а также при тяжелых формах пневмоний и эмфизем, самопроизвольной задержке дыхания [37, 39, 57, 62, 63]. При этом задерживается выделение бикарбонатов с мочой (респираторный ацидоз с частичной почечной компенсацией), что одновременно с гипервентиляцией легких создает условия для нормализации pH крови и перехода в состояние компенсированного алкалоза.

Компенсированный алкалоз (pH — норма; HCO_3^- и pCO_2 — выше нормы) наблюдается при компенсации метаболического алкалоза и респираторного ацидоза. Но чаще всего он появляется при хроническом поступлении в организм с пищей избытка щелочных эквивалентов по сравнению с кислотными, при длительном пребывании в атмосфере с несколько повышенным (по сравнению с нормой) содержанием CO_2, часто при эмфиземе легких и хронических бронхопневмониях [37, 39, 57]. Переход из этого состояния в норму осуществляется благодаря деятельности почек (усиливающих выделение бикарбонатов с мочой) и дыхательного центра (гипервентиляция легких).

Компенсированный ацидоз (pH — норма; HCO^-_3 и pCO_2 — ниже нормы) развивается при компенсации метаболического ацидоза и респираторного алкалоза. Но чаще всего он наступает при хроническом поступлении в организм с пищей избытка кислотных эквивалентов по сравнению со щелочными, при пребывании в атмосфере с концентрацией CO_2 ниже 0,3%, при легких и средних формах диабета, лихорадке, ожоговой болезни, после ранений и операций, у высокопродуктивных животных и т. д. [37, 39, 40, 57]. Переход от этого состояния к норме осуществляется благодаря деятельности почек (задерживающих выделение бикарбонатов с мочой) и дыхательного центра (гиповентиляция легких). Благоприятное влияние на этот процесс оказывает ультрафиолетовое облучение [57].

Необходимо отметить, что в случае недостатка в пище калия развивается внутриклеточный ацидоз, хотя pH крови и межклеточной жидкости может быть даже несколько выше нормы [64]. Имеются также сведения о том, что клеточные мембраны более проницаемы для pCO_2 и H_2CO_3, чем для HCO_3^- и H^+ [65]. Это свойство клеточных мембран в определенной степени способствует сохранению постоянства величины внутриклеточного pH.

Мы вкратце рассмотрели основные формы углекислоты, содержащейся в животных тканях, механизмы регуляции ее содержания, а также отметили ряд физиологических и патофизиологических состояний организма человека и животных, сопровождающиеся существенными изменениями содержания и величины соотношения различных ее форм и ионов водорода.

Широкое распространение указанных состояний стало главной причиной того, что еще с конца XIX века [12—15] и по настоящее время [16—20] многочисленные исследования отечественных и зарубежных ученых посвящались и посвящаются изучению особенностей обмена веществ в тканях при различного рода ацидозах и алкалозах.

Однако подходы к изучению этой проблемы и объяснение полученных результатов основываются в большинстве случаев только на

роли изменения концентрации H^+ в обмене веществ и почти совершенно исключается возможное метаболическое значение изменения содержания и соотношения различных форм углекислоты при этих состояниях.

В последние годы интерес к выяснению метаболического значения углекислоты значительно возрос [10, 11, 21—33, 66—80], особенно после того, как в нашей лаборатории [10, 11, 33, 66—80] было показано, что фиксация углекислоты лежит в основе важнейших биосинтетических процессов не только у автотрофов, но и у всех гетеротрофных организмов и что, изменяя количество углекислоты в тканях или средах, можно целенаправленно воздействовать на интенсивность процессов ее фиксации и соответственно на интенсивность биосинтеза белков, нуклеиновых кислот, липидов, углеводов и других органических компонентов тканей.

ОСНОВНЫЕ РЕАКЦИИ КАРБОКСИЛИРОВАНИЯ И ИХ ФИЗИОЛОГИЧЕСКОЕ ЗНАЧЕНИЕ В ТКАНЯХ ЖИВОТНЫХ

Еще в 1910 г. Лебедев [2], исследуя хемосинтез у *Bacillus hydrogenes* в условиях автотрофного и гетеротрофного питания, показал, что как в первом, так и во втором случае указанные микроорганизмы в процессе роста ассимилируют углекислоту. В дальнейшем этот процесс (фиксация CO_2, карбоксилирование) был обнаружен у других видов гетеротрофных микроорганизмов [3, 4], а в 1940 г.— и в тканях животных [5, 6], т. е. стало ясно, что фиксация углекислоты присуща всем живым организмам. Это послужило стимулом к развертыванию во многих лабораториях мира исследований, направленных на изучение механизмов фиксации углекислоты и факторов, регулирующих эти процессы.

В настоящее время по этим вопросам опубликован ряд больших обзорных статей [7, 9]. Наиболее полно они освещены в монографии [10] и других обзорных изданиях [11, 33] нашей лаборатории.

Суммируя накопленные данные, можно заключить, что в тканях животных в настоящее время обнаружено свыше 10 основных реакций карбоксилирования (рис. 4), которые можно подразделить на три группы: 1) протекающие за счет энергии восстановленных форм пиримидиновых нуклеотидов; 2) за счет энергии АТФ; 3) не требующие энергии извне.

Установлено, что фиксации углекислоты предшествует ее активация, осуществляющаяся путем взаимодействия ее с АТФ (рис. 5). При этом в одних случаях [81] образуется карбониладенилат, а в других [82] — карбонилфосфат. Линен и др. [83] считают, что карбоксилирование биотинового фермента, который затем передает CO_2 на соответствующий акцептор, также можно рассматривать как процесс активации углекислоты

Реакции карбоксилирования, протекающие за счет энергии восстановленных форм пиримидиновых нуклеотидов

К этой группе относятся реакции «малатная», карбоксилирования α-кетоглутарата и серина.

«Малатная» реакция. Фермент «малик» впервые был обнаружен Очоа и сотр. в печени голубя [6]. Он катализирует синтез малата за счет пирувата, CO_2, водорода $НАДФН_2$ и нуждается в Mg^{2+} или Mn^{2+}. Считают, что в норме этот фермент (вместе с малатдегидрогеназой) в одинаковой мере с пируваткарбоксилазой «ответственны» за синтез оксалацетата в тканях [84]. Однако при голодании, диабете, после воздействия адренокортикоидами, т. е. в условиях стиму-

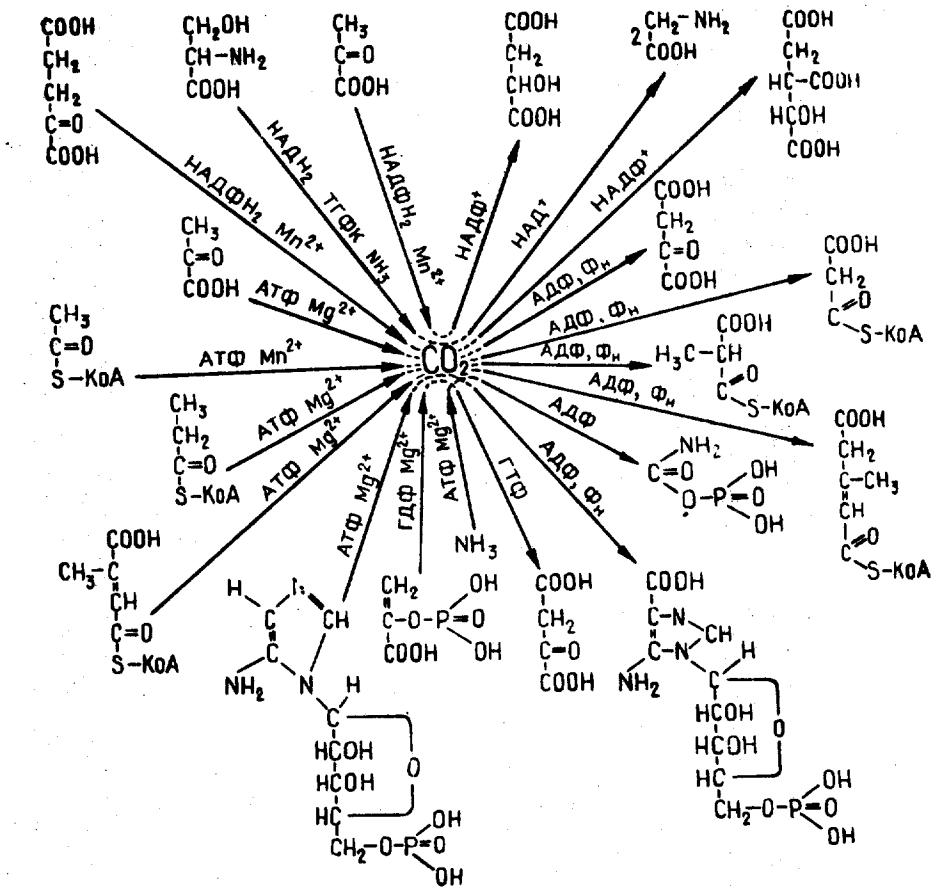


Рис. 4. Основные реакции карбоксилирования в тканях животных.

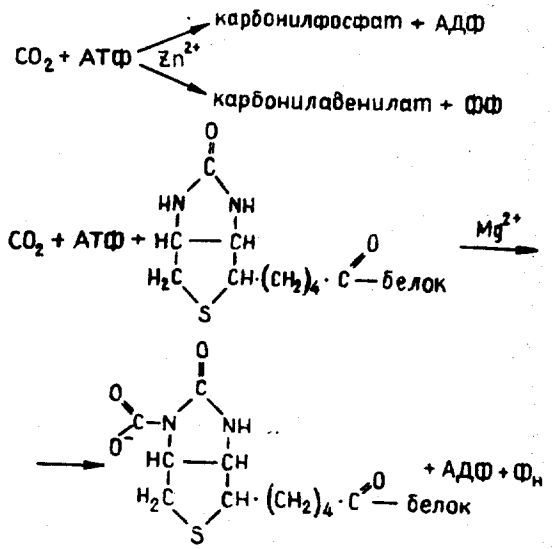


Рис. 5. Активация углекислоты.

ляции глюконеогенеза, эта реакция больше направлена в сторону декарбоксилирования малата в пируват, т. е. выступает как один из механизмов обеспечения клетки водородом НАДФH_2 [85—90].

Реакция карбоксилирования α-кетоглутарата. В 1948 г. Очоа и сотр. [85] впервые показали возможность синтеза изоцитрата за счет α-кетоглутарата, CO_2 и водорода НАДФH_2 ферментными препаратами, полученными из сердца свиньи и голубя. Реакция эта обратима и для ее протекания необходим Mn^{2+} [85, 91, 92].

Физиологическое значение этой реакции определяется прежде всего тем, что она является важным звеном в метаболизме цитрата в клетке, имеющего большое значение в обмене веществ [10]. Эта реакция, подобно «малатной», играет роль в обеспечении клетки восстановленной формой НАДФH_2 [93] и во внутриклеточном транспорте водорода и ацетил-КоА [92].

Реакция карбоксилирования серина. Наличие этой реакции предполагалось уже в 1955—1962 гг. [94, 95]. Однако детально она была изучена лишь в 1969 г. [96]. Эта реакция обнаружена в почках, печени и ряде других тканей животных и птиц. Карбоксилирование серина в ней происходит в присутствии аммония, бикарбоната, НАДH_2, тетрагидрофолиевой кислоты и стимулируется пиридоксальфосфатом. В результате образуется две молекулы глицина.

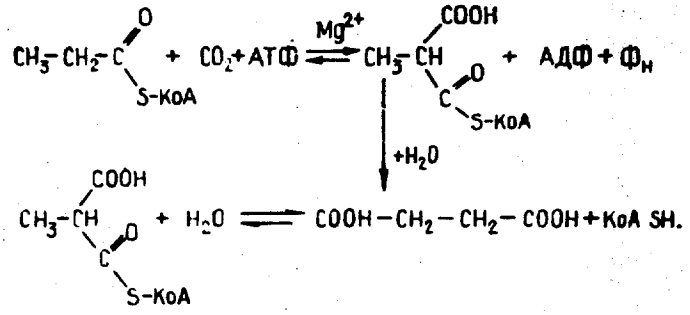
Физиологическое значение этой реакции определяется тем, что она выступает не только как механизм фиксации CO_2 и взаимопревращения серина и глицина, но и является одним из путей фиксации аммиака в тканях животных.

Допускают, что образующийся в этой реакции комплекс (тетрагидрофолат — одноуглеродистый фрагмент) как промежуточный продукт является донором метильной группы при образовании тимина [97, 98].

Реакции карбоксилирования, протекающие за счет энергии АТФ

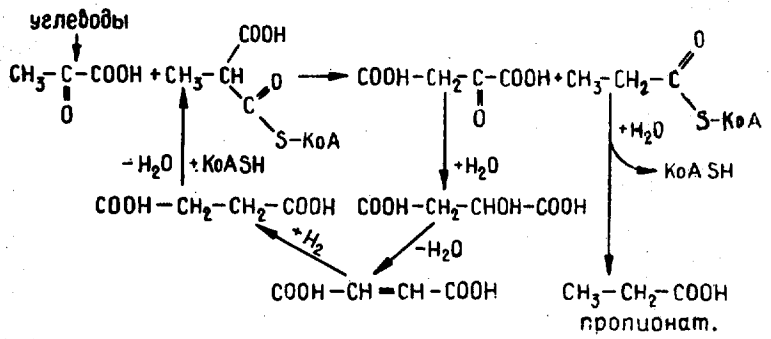
К этой группе относятся реакции карбоксилирования пропионил-КоА, β-метилкротонил-КоА, ацетил-КоА, 5-аминоимидазолрибонуклеотида и образования карбамилфосфата.

Пропионил-КоА-карбоксилазная реакция. В 1955 г. Флейвин и др. [99] одновременно с Катцом и Чайковым [100] показали, что в животных тканях пропионат в присутствии КоА—SH, Mg^{2+} и АТФ превращается в β-метилмалонил-КоА. Вскоре была доказана обратимость этой реакции [101], а также то, что β-метилмалонил-КоА с участием метилмалонил-КоА-изомеразы обратимо превращается в сукцинат [102], т. е. стал известен механизм обратимого превращения пропионата в сукцинат:



Эта реакция была обнаружена и у микроорганизмов [103, 104]. Поэтому некоторое время считалось, что таким путем осуществляется про-

пионово-кислое брожение углеводов. Однако теперь известно, что этот процесс осуществляется благодаря так называемому процессу транскарбоксилирования [105], который катализируется метилмалонилоксалацетаттранскарбоксилазой:



Коферментом пропионил-КоА-карбоксилазы и транскарбоксилазы является биотин. Пропионил-КоА-карбоксилаза не строго специфична и может карбоксилировать ацетил-, бутирил- и изобутирил-КоА [106].

Особо важное значение имеет эта реакция в обмене веществ у жвачных, в преджелудках которых около 25% углеводов взбраживается до пропионовой кислоты [107].

β-Метилкротонил-КоА-карбоксилазная реакция. Эта реакция была обнаружена Райлином и Куном [108] в печени крупного рогатого скота и кур. Физиологическое ее значение определяется прежде всего тем, что она является одним из звеньев метаболизма лейцина и изовалериановой кислоты.

Ацетил-КоА-карбоксилазная реакция. Это начальная реакция липогенеза. В ней ацетил-КоА в присутствии CO_2, АТФ и Mg^{2+} или Mn^{2+} превращается в малонил-КоА [109]. Коферментом ацетил-КоА-карбоксилазы является биотин [110]. Фермент не строго специфичен и может карбоксилировать пропионил-, бутирил-, каприлил- и капронил-КоА [111]. Цитрат [112] и карнитин [113] являются аллостерическими стимуляторами, а ацил-КоА-производные высокомолекулярных жирных кислот — аллостерическими ингибиторами фермента [114].

Физиологическое значение этой реакции определяется тем, что она является начальной и притом лимитирующей в процессе биосинтеза жирных кислот [115].

Пируваткарбоксилазная реакция. Предположение о существовании этой реакции высказывалось Вудом и Веркманом еще в 1938 г. [116]. Но лишь в 1960 г. Уттеру и Кичу из печени, почек и других тканей удалось выделить фермент, который катализирует превращение пирувата (в присутствии CO_2, АТФ, Mg^{2+}, ацетил-КоА) в оксалацетат [117, 118]. Ацетил-КоА является аллостерическим стимулятором фермента. Активность последнего усиливается также при возрастании величины соотношения АТФ/АДФ в клетке, а также после гормональной стимуляции глюконеогенеза [119—121].

Основное физиологическое значение этой реакции заключается в том, что она является главным путем обеспечения клетки С-4-дикарбоновыми кислотами.

Карбамилфосфатсинтетазная реакция. Синтез карбамилфосфата, который используется при биосинтезе цитруллина, осуществляется в присутствии NH_3, CO_2, АТФ и Mn^{2+} (или Co^{2+}, или

Zn^{2+}), а также ацетилглутамата [122—125]. Ацетилглутамат имеет каталитическое значение в этой реакции, образуя с CO_2 временное соединение. Процесс синтеза карбамилфосфата ступенчатый и начинается с образования карбонилфосфата, который, последовательно реагируя с ацетилглутаматом, АТФ и NH_3, превращается в карбамилфосфат.

Синтез карбамилфосфата, который используется для синтеза азотистых оснований пиримидиновых нуклеотидов, осуществляется другой карбамилфосфатсинтетазой. Реакция осуществляется за счет NH_3, CO_2, АТФ и в присутствии Mn^{2+} и глутамата [126, 127].

Физиологическое значение этих реакций заключается в их участии в процессах биосинтеза мочевины, пиримидиновых нуклеотидов и соответственно нуклеиновых кислот.

5-Аминоимидазолрибонуклеотидкарбоксилазная реакция. В этой реакции 5-аминоимидазолрибонуклеотид, карбоксилируясь, превращается в 5-аминоимидазолкарбоновую кислоту. Фермент, катализирующий ее, выделен из печени голубя [128]. Он высокоспецифичен и активируется Mg^{2+}. Реакция протекает за счет энергии АТФ, хотя вначале считали, что она не нуждается в энергии извне [8, 129].

Физиологическое значение ее в том, что она является одним из звеньев в цепи реакций биосинтеза пуриновых нуклеотидов и соответственно нуклеиновых кислот.

Реакции карбоксилирования, протекающие без потребления энергии извне

У гетеротрофных микроорганизмов к этой группе относятся несколько реакций [7—11], а в тканях животных обнаружена только одна — фосфоэнолпируваткарбоксикиназная.

Фосфоэнолпируваткарбоксикиназная реакция. Существование ее предполагалось уже давно [130], но лишь в 1953 г. Уттеру и Курагаши [131] удалось выделить из печени птиц фермент, который катализировал синтез оксалацетата за счет фосфоэнолпирувата. Реакция протекает в присутствии CO_2, ГДФ, Mg^{2+}. ГДФ может быть замещен УДФ [132]. Вначале фермент был назван оксалацетаткарбоксилазой [130], а затем фосфоэнолпируваткарбоксикиназой [133]. Активность фермента находится в прямой зависимости от величины соотношения ГТФ/ГДФ [134].

Физиологическое значение реакции состоит в том, что она представляет собой лимитирующее звено в цепи реакций глюконеогенеза и любое изменение ее интенсивности будет определять соответствующие изменения в этом процессе [84, 120]. Она является также одним из механизмов обеспечения клетки С-4-дикарбоновыми кислотами. Последнее особенно важно для мышечной ткани, поскольку пируваткарбоксилазы в ней не обнаружено [135].

Суммируя изложенное, можно заключить, что CO_2 является чрезвычайно важным метаболитом. Трудно назвать какое-либо другое вещество, которое имело бы столько различных реакций превращения, как CO_2. Если же учесть, что ряд этих реакций катализируется не одной, а несколькими ферментными системами, то становится ясным, что в этом отношении CO_2 является в живой природе уникальным метаболитом.

Уникальность физиологического значения углекислоты определяется прежде всего тем, что она участвует в реакциях биосинтеза важнейших компонентов клетки: липидов, углеводов, ряда аминокислот (аспарагиновой, глутаминовой, глицина, серина, цитруллина, аргинина), азотистых оснований нуклеотидов и нуклеиновых кислот.

Если учесть и то, что четыре из девяти промежуточных членов трикарбонового цикла (оксалацетат, малат, изоцитрат и сукцинат) образуются путем карбоксилирования соответствующих субстратов (пирувата, α-кетоглутарата, пропионата), то становится ясным, что функционирование его также зависит от процессов фиксации углекислоты (рис. 6).

Принимая во внимание участие углекислого газа во всех основных биосинтетических и окислительных процессах в клетке, мы в нашей лаборатории пришли к выводу, что все эти процессы, т. е. биосинтез многих аминокислот (а значит, и белков), липидов и углеводов, пуриновых

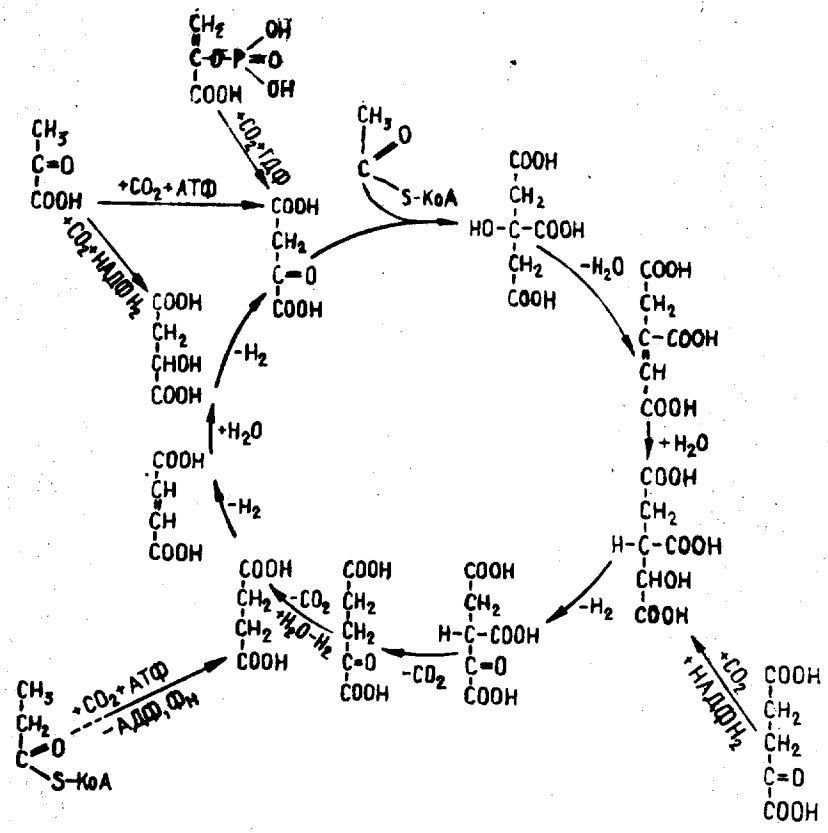


Рис. 6. Углекислота и трикарбоновый цикл.

и пиримидиновых оснований (а значит, и соответствующих нуклеотидов и нуклеиновых кислот), а также функционирование трикарбонового цикла должны находиться в неразрывной зависимости от процессов фиксации CO_2.

На основании этого была высказана мысль о том, что, найдя пути воздействия на интенсивность карбоксилирования, можно будет целенаправленно влиять на все указанные биосинтетические процессы в тканях животных и в микробиальных клетках [10, 11, 33].

Это и послужило основой для широкого развертывания исследований в отделе биосинтеза и биологических свойств белка Института биохимии АН УССР, направленных на изыскание путей эффективного изменения интенсивности процессов фиксации CO_2 и соответственно биосинтетических процессов в организме животных.

Теоретически [7—11] пытаться влиять на интенсивность процессов фиксации CO_2 в организме можно по-разному, а именно: 1) путем изменения концентраций субстратов, подлежащих карбоксилированию;

2) варьируя концентрацию и величину соотношения различных форм углекислоты в тканях;

3) изменяя степень обеспеченности энергией (АТФ, НАДФH_2, НАДH_2) и соответствующими коферментами карбоксилаз (биотином, никотинамидом, витамином B_6, тетрагидрофолиевой кислотой);

4) путем изменения концентраций ионов магния, марганца и цинка (активаторов ферментов), а также ионов тяжелых металлов (ингибиторов карбоксилаз) и других кофакторов (ацетилглутамата, глутамата, цитрата, карнитина, ацетил-КоА и др.), являющихся аллостерическими регуляторами активности карбоксилаз;

5) вызывая изменения гормонального статуса в организме.

В нашей лаборатории изучали возможность воздействия на интенсивность процессов фиксации CO_2 и указанных биосинтетических процессов путем изменения содержания углекислоты в тканях как субстрата ферментов карбоксилаз и количества двухвалентных металлов, являющихся активаторами этих ферментов.

Ниже приводим результаты исследований, свидетельствующие о возможности изменения интенсивности биосинтетических процессов в тканях животных путем воздействия на процессы карбоксилирования.

СТИМУЛЯЦИЯ ПРОЦЕССОВ КАРБОКСИЛИРОВАНИЯ — СПОСОБ АКТИВАЦИИ БИОСИНТЕТИЧЕСКИХ ПРОЦЕССОВ В ТКАНЯХ

В опытах на срезах печени крыс [66] было показано, что при инкубации их с $NaHC^{14}O_3$ добавление в инкубационную среду Mg^{2+} вызывает повышение интенсивности фиксации радиоактивного углерода в белки на 27,7%, Zn^{2+} — на 26% и Mn^{2+} — на 12% (в последнем случае $p>0{,}05$). Фиксация CO_2 в липиды срезов печени усиливалась только под влиянием Mg^{2+} (на 21,6%).

В дальнейших опытах с лактирующими крольчихами, которым в течение 10 дней скармливали из расчета 0,174 *мг* на 1 *г* живого веса солевую смесь, названную карбоксилином (состав: бикарбонат натрия — 20 весовых частей, сульфаты магния, марганца и цинка — 4,0, 0,1 и 0,1 весовых частей соответственно), показано достоверное по сравнению с контролем увеличение интенсивности фиксации C^{14} за счет $NaHC^{14}O_3$ в белки и углеводы сыворотки крови и молока, а также в гликоген печени и мышц [69]. При скармливании карбоксилина из расчета 1 *мг* на 1 *г* живого веса стимулирующий эффект его еще больше.

Под влиянием 10-дневного скармливания карбоксилина аналогично увеличивается интенсивность включения C^{14} за счет $NaHC^{14}O_3$ в органические компоненты (белки, гликоген) печени, мышц и крови кроликов-самцов [68], а также в белки печени петухов [67].

Превращение углерода $NaHC^{14}O_3$ в органические кислоторастворимые соединения гомогенатов печени петухов, получавших 120—150 дней карбоксилин из расчета 0,42 *мг* на 1 *г* живого веса, также протекает намного интенсивнее, чем в гомогенатах печени контрольных кур [70].

Таким образом, эти результаты полностью подтверждают высказанное предположение о том, что, повышая содержание углекислоты и обеспечивая определенную концентрацию ионов (Mg^{2+}, Mn^{2+} и Zn^{2+}) в тканях животных, можно стимулировать процессы фиксации CO_2. Это отмечено также и Адамом и Гейнесом [136] на митохондриях печени крыс, показавших, что увеличение в инкубационной среде концентрации HCO_3^- с 6 до 12 *ммоль/л* почти вдвое усиливает фиксацию углекислоты.

Одновременно с этим в нашей лаборатории было установлено, что в условиях стимуляции процессов фиксации CO_2 усиливается функционирование трикарбонового цикла в тканях. Это имеет очень важное

значение, так как известно, что трикарбоновый цикл является не только основой биологического окисления органических веществ; интенсивность биосинтеза жиров, углеводов, белков и нуклеотидов тканей животных находится в прямой зависимости от интенсивности его функционирования [10, 137—144]. В частности, в опытах на срезах печени крыс и кур наблюдали, что частичная замена фосфата в среде Кребса — Рингера бикарбонатом усиливает окисление лизина-1-C^{14} и ацетата-2-C^{14} до $C^{14}O_2$, хотя pH сред оставалось постоянным (7,05), причем этот эффект бикарбоната усиливался при добавлении его совместно с Mg^{2+} Mn^{2+} или Zn^{2+} [66, 67].

В опытах на курах, которым 120—150 дней скармливали карбоксилин, также показано, что поглощение кислорода и окисление ацетата-1-C^{14} до $C^{14}O_2$ в срезах печени кур опытных групп происходит интенсивнее на 24,0 и 77,5% соответственно, чем в срезах печени кур контрольных групп [70].

Результаты этих исследований полностью подтверждены Лангморе [22], который установил, что при увеличении количества HCO_3^- в инкубационной среде с 10 до 40 *ммоль/л* (pH 7,40) интенсивность окисления глицерина-1,3-C^{14} и пирувата-2-C^{14} до $C^{14}O_2$ в срезах печени нормальных крыс повышается на 20—60%. Фрогман и Ортен [145] также наблюдали, что скармливание крысам с аллоксановым диабетом бикарбоната натрия резко стимулирует окисление ацетата-1-C^{14} до $C^{14}O_2$, а содержание промежуточных продуктов трикарбонового цикла в тканях при этом повышается почти до уровня нормы. Можно полагать, что усиление функционирования трикарбонового цикла в условиях стимуляции фиксации углекислоты в тканях обусловливается тем, что четыре из девяти его компонентов образуются путем карбоксилирования соответствующих субстратов (рис. 6).

Об участии углекислоты в регуляции процессов биологического окисления в клетке, в частности в реакциях трикарбонового цикла, накоплено огромное количество данных прямых и косвенных наблюдений [18, 19, 23, 146—153].

Говоря о важной роли углекислоты в регуляции функционирования трикарбонового цикла в тканях животных, следует обратить внимание еще и на то, что активность ряда ферментов, которые не имеют прямого отношения к фиксации CO_2, при постоянном pH сред может существенно изменяться в зависимости от содержания HCO_3^- в них. Так, Хастингсом и др. [154] было показано, что АТФ-азная активность митохондрий печени крыс может повышаться почти в три-четыре раза при увеличении концентрации HCO_3^- в среде с 0 до 60 *ммоль/л* (pH 7,5). Подобное изменение активности АТФ-азы наблюдал Рекер [155], а Макдениж и Лангморе [24] обнаружили, что HCO_3^- (при нормальном pH) ингибирует аконитат-гидролазную активность в печени быка. Подобное отмечено и для глюкозо-6-фосфат-фосфогидролазы печени крыс [156]. Возможно, это объясняется тем, что углекислота может выступать в ряде ферментных реакций в качестве регулятора, выполняя роль аллостерического эффектора.

Многочисленные данные, полученные в нашей лаборатории, свидетельствуют о том, что углекислота, участвуя во многих реакциях карбоксилирования, касающихся синтеза и использования ряда предшественников белков, липидов, углеводов, нуклеиновых кислот и т. д. (рис. 4), оказывает существенное регуляторное воздействие на основные биосинтетические процессы в организме. Так, в опытах in vitro на срезах печени крыс было показано, что внесение в инкубационные среды (pH 7,05) карбоксилина в возрастающих дозах ступенчато усиливает включение C^{14} за счет ацетата-2-C^{14} и лизина-1-C^{14} в белки и липиды [68], причем из всех компонентов карбоксилина наибольшим эффектом обладает бикарбонат натрия, а ионы Mg, Mn и Zn усиливают его действие. По-

добные результаты получены и в опытах на срезах печени кур [67].

В последующих опытах на кроликах, которым ежедневно в течение 10 дней скармливали 0,604 *мг* карбоксилина на 1 *г* живого веса, а затем вводили внутривенно ацетат-2-C^{14} и забивали через 1 и 3,5 *ч*, наблюдалось достоверное увеличение интенсивности включения метки в белки и гликоген печени и мышц [80].

В опытах in vivo [71, 76, 80] на петухах, которым 10 дней скармливали карбоксилин, наблюдалась значительная стимуляция включения C^{14} за счет ацетата-2-C^{14} в белки, липиды и гликоген печени, а также в белки сыворотки крови. При этом эффект карбоксилина был более выражен у взрослой птицы по сравнению с молодой.

Усиление обмениваемости белков, липидов и свободных нуклеотидов под влиянием скармливания карбоксилина наблюдалось также и в опытах in vitro на срезах печени петухов [67, 70, 79].

Таким образом, можно заключить, что, стимулируя процессы фиксации углекислоты в тканях, можно активировать основные биосинтетические процессы в организме животных, т. е. биосинтез белков, углеводов, липидов, нуклеотидов и т. д.

О правильности этого вывода свидетельствуют также результаты опытов на гомогенатах печени крыс, кроликов и кур, в которых процессы фиксации углекислоты угнетались авидином [68, 78, 79]. При этом параллельно с торможением фиксации углекислоты угнеталось включение метки в белки и липиды за счет ацетата-1-C^{14} и глицина-1-C^{14}.

Этим данным полностью соответствуют результаты ряда других исследователей, показавших, что в условиях повышения содержания углекислоты в тканях стимулируется биосинтез липидов и гликогена [21, 22, 25—30, 157, 158].

Установленная закономерность — зависимость основных биосинтетических процессов от интенсивности фиксации углекислоты и, соответственно, от содержания ее в тканях,— имеет исключительно важное не только познавательное, но и практическое значение, особенно если иметь в виду огромное число факторов, способных влиять на величину содержания и соотношения различных форм углекислоты в тканях животных и человека, на чем мы подробнее останавливались выше.

Исходя из этого, в отделе биосинтеза и биологических свойств белка Института биохимии АН УССР в опытах на животных с регенерирующими тканями установили, что стимуляция карбоксилирования в организме, вызывая повышение интенсивности биосинтетических процессов, ускоряет регенерацию тканей. Так, в опытах на частично гепатэктомированных (удаление 70—80% печени) кроликах показано, что под влиянием скармливания им карбоксилина в течение 10 дней до операции усиливается биосинтез белков, липидов и гликогена в регенерирующей печени, заметно ускоряется восстановление исходного веса ее и увеличивается содержание в ней сухих веществ [73, 77].

В опытах на кроликах с экспериментальной ожоговой болезнью, выяснилось [75], что скармливание им карбоксилина на протяжении 7 дней ускоряет восстановление исходного веса тела, интенсифицирует функционирование трикарбонового цикла, повышает интенсивность включения C^{14} из ацетата в ткань мышц и белки печени, почек, селезенки и сыворотки крови, а также в липиды печени.

Было также показано, что при экспериментальной постгеморрагической анемии под влиянием карбоксилина в сыворотке крови анемичных кроликов ускоряется восстановление количества эритроцитов, гемоглобина и белков сыворотки крови; в печени, почках и селезенке интенсифицируются функционирование трикарбонового цикла и синтез белков; повышается реактивность костного мозга в сторону эритропоэза; нормализуется кислотно-щелочной баланс [74].

Полностью согласуются с этими данными результаты опытов Мортона [159], который показал, что интенсивность образования ретикулоцитов в костном мозге крыс in vitro находится в прямой зависимости от содержания CO_2 в газовой среде, достигая максимума при 30%. О зависимости интенсивности роста многих видов гетеротрофных микроорганизмов и дрожжей от количества углекислоты в среде также свидетельствуют данные ряда исследований [160—166], список которых можно было бы намного продолжить.

Тесная зависимость биосинтетических процессов от фиксации CO_2 и возможность повышения интенсивности биосинтеза основных органических компонентов тканей животных путем стимуляции реакций карбоксилирования у них, а также то, что сложившиеся в настоящее время условия кормления сельскохозяйственных животных (скармливание в больших количествах силоса, жома, барды, концентратов) часто приводят к снижению содержания углекислоты в тканях (компенсированный и метаболический ацидозы), послужило основой для разработки в Институте биохимии АН УССР и Украинской сельскохозяйственной академии метода повышения продуктивности сельскохозяйственных животных и птицы [167—185].

Эффективность предложенного метода подтверждена в ряде научно-исследовательских учреждений [186—195], и теперь он широко применяется в совхозах и колхозах УССР [196—203].

ЗАКЛЮЧЕНИЕ

1. В тканях животных углекислота содержится в форме растворенного углекислого газа, угольной кислоты, бикарбонатов, карбонатов и карбаматов. На долю бикарбонатов в крови животных приходится около 94—95% общего количества углекислоты. Содержание углекислоты в других биологических жидкостях и тканях находится в прямой зависимости от количества ее в крови.

2. В тканях животных и человека величины содержания и соотношения между различными формами углекислоты и ионов водорода могут варьировать и, отсюда, обусловливать девять состояний организма: норму, метаболические ацидоз и алкалоз, респираторные ацидоз и алкалоз, респираторные ацидоз и алкалоз с частичной компенсацией, компенсированные ацидоз и алкалоз.

3. В тканях животных обнаружено свыше 10 реакций фиксации CO_2, в которых углерод углекислоты превращается в углерод органических соединений. Эти реакции подразделяют на три группы: протекающие за счет энергии АТФ, за счет энергии восстановленных форм пиримидиновых нуклеотидов, не требующие энергии извне.

4. Интенсивность процессов фиксации углекислоты в тканях определяется следующими факторами: 1) концентрацией субстратов, подлежащих карбоксилированию; 2) величинами концентрации и соотношения различных форм углекислоты в тканях; 3) степенью обеспеченности энергией и соответствующими коферментами карбоксилаз (биотином, никотинамидом, витамином B_6, тетрагидрофолатом); 4) наличием в определенных концентрациях активаторов (ионов магния, марганца и цинка) и ингибиторов карбоксилаз (ионов тяжелых металлов и др.), а также ряда кофакторов (ацетилглутамата, глутамата, цитрата, карнитина, ацил-КоА и др.), являющихся аллостерическими регуляторами активности карбоксилаз; 5) соответствующим гормональным статусом в организме.

5. Углекислота, используясь в реакциях карбоксилирования в тканях животных, принимает участие в биосинтезе жирных кислот, липидов, многих заменимых аминокислот, а значит, и белков, в синтезе углеводов de novo, в образовании пуриновых и пиримидиновых осно-

ваний, а значит, нуклеотидов и нуклеиновых кислот, а также в реакциях трикарбонового цикла.

6. Существует зависимость интенсивности биосинтеза указанных выше органических соединений от процессов фиксации углекислоты, выражающаяся в том, что стимуляция или угнетение последних соответственно усиливает или ингибирует протекание первого.

7. Увеличивая в определенных пределах содержание углекислоты и ионов магния, марганца и цинка в тканях, можно заметно повышать интенсивность биосинтетических процессов и стимулировать реакции трикарбонового цикла в организме. Это свидетельствует о том, что углекислота в организме животных является не только регулятором процессов дыхания и основой главной буферной системы у животных и человека, но и регулятором основных биосинтетических процессов в организме.

8. Указанную регуляторную роль углекислоты в биосинтетических процессах организма можно эффективно использовать в практических целях. Усиливая биосинтетические процессы путем стимуляции процессов фиксации углекислоты, можно заметно повышать продуктивность сельскохозяйственных животных и птицы, а также значительно ускорять процессы регенерации тканей, в частности, при гепатэктомии, ожогах и постгеморрагических анемиях.

9. Помимо участия в процессах карбоксилирования и регуляции реакции среды углекислота обладает свойством вызывать специфические изменения в активности некоторых ферментов (АТФ-азы, аконитат-гидролазы и глюкозо-6-фосфат-фосфогидролазы), которые не имеют прямого отношения к процессам фиксации CO_2.

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A simplified procedure for the measurement of $^{14}CO_2$ in blood

The collection and determination of $^{14}CO_2$ in blood is an important method in the study of the oxidation of ^{14}C-labeled substances *in vivo*. This often requires analysis of large series of arterio-venous differences, and none of the available methods[1,2] are well suited for large series of samples. The simplified procedure described here has the advantage that it does not involve any pipetting, and all the materials used are discardable.

METHOD

Blood was drawn into graduated, heparinized all-glass syringes, which were sealed and chilled in an ice-bath until analyzed.

The liberation and collection of $^{14}CO_2$ was performed in the ordinary screw-cap glass vials used for liquid scintillation counting (24 mm neck diameter, Packard Instrument Company, Inc., Downers Grove). A small plastic cap (for centrifuge tubes, Labora, Stockholm) was placed upside down in the counting vial, forming a cup to hold 0.5 ml concentrated sulphuric or lactic acid. The blood (about 5 ml) was added directly from the syringe through a needle into the vial at the side of the plastic cup causing this to float in the blood. A wad of glass wool was glued to the inside of the screw-cap and soaked with 0.1 ml ethanolamine. Care was taken to make this wad so small that it did not touch the margins of the vial when the cap was screwed on. The vial was then gently tilted and rotated slowly by hand until the plastic cap sank and the blood was thoroughly mixed with the acid. The vials were left standing at room temperature for 24 h. The screw-cap was then transferred to another counting vial containing 4 ml ethylene glycol monomethyl ether, and this vial was left standing upside down for about 10 min to dissolve the glue. After addition of 10 ml scintillation fluid (5 g/l 2,5-diphenyloxazole and 0.3 g/l 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene) the vial was cooled and counted in a Tri Carb liquid scintillation counter with a Model 3003 spectrometer and external standardization. Counting efficiency for ^{14}C was about 80%.

For standardization a $NaH^{14}CO_3$-solution was made through the liberation of $^{14}CO_2$ from labeled barium carbonate (New England Nuclear Corporation, Boston) by the method of Friedberg and Estes[2]. The $^{14}CO_2$ was collected in water containing a slight excess of NaOH. This solution was prepared fresh each day, since it was observed that old solutions of labeled sodium bicarbonate contained radioactivity that was not liberated as $^{14}CO_2$ on acidification. The radioactivity of the standard solution was determined by liquid scintillation counting in the same system as was used for the samples.

The other radioactive substances used were obtained from The Radiochemical Centre, Amersham.

RESULTS

The efficiency of the liberation and collection of $^{14}CO_2$ was tested by addition of a standard solution of $NaH^{14}CO_3$ to 5 ml samples of either water or blood. From 10 samples of water the recovery was 99.2 ± 2.1% (± S.D.) and from 15 samples of blood the recovery was 95.2 ± 3.3%.

The influence of the incubation time was studied and 24 h gave the best results. Most of the $^{14}CO_2$ was liberated and trapped immediately and the recovery was almost 70% after 1 h and then increased slowly to the 95% found after 24 h. Longer incubation times did not give higher recoveries.

The interference from labeled substances that may be present in the analyzed blood was tested and the results are presented in Table I. 0.1 μC of each of the tested substances was added to 5 ml water and carried through the procedure.

TABLE I

INTERFERENCE FROM LABELED SUBSTANCES PRESENT IN THE SAMPLES

Substance added (0.1 μC)	*Number of samples*	*Per cent of radioactivity recovered as $^{14}CO_2$*	*Standard deviation*
[1-^{14}C]Glycerol	5	0.11	0.03
[3-^{14}C]Sodium β-hydroxybutyrate	5	0.10	0.03
[1-^{14}C]Sodium lactate	4	0.15	0.03
[1-^{14}C]Sodium pyruvate	4	0.85	0.17
[1-^{14}C]Palmitic acid (albumin-bound)	5	0.008	0.007

DISCUSSION

The use of ethanolamine for trapping of $^{14}CO_2$ for liquid scintillation counting was introduced by Jeffay and Alvarez[3] and has proved well adaptable to the present technique. The amount of ethanolamine used represents at least a 4-fold excess compared to the CO_2 present in 5 ml blood. The influence of the wad of glass wool on counting efficiency is negligible, and it does not raise the background count rate by more than 1 or 2 counts/min.

Shortly after the mixing of blood and acid the samples solidify to a thick jelly. This apparently has little influence on the recovery of $^{14}CO_2$, but may explain the slightly lower recovery obtained from blood than from water samples. Dilution of the blood with water before the analysis to avoid this thickening did not improve the recovery.

Both lactic and sulphuric acid has been used to liberate the CO_2 from blood without any difference in the results. The data presented in Table I were obtained using sulphuric acid. With easily oxidizable labeled substances present in the blood, lactic acid should be preferred.

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Cardiac Malformations in Rats Induced by Exposure of the Mother to Carbon Dioxide During Pregnancy

By Olga M. Haring, M.D.

EXPERIMENTS of previous investigators proved that many teratogenic agents induced malformations by arresting or retarding growth. In finding an agent to act as "metabolic stimulator" instead of "metabolic inhibitor," it might be expected that the type of induced anomalies would also be different. A combination of different agents in proper time and adequate dosage may yield a wide range of selective malformations.

Methods

In the current investigations, a gas mixture of 6 per cent carbon dioxide with 20 per cent oxygen and 74 per cent nitrogen was used as teratogenic agent. Rats utilized in the study were pregnant young adults of a Sprague Dawley strain purchased in groups of 6 to 12. Pregnancy was calculated from the time observed-copulation occurred (0 hour).

The pregnant rats in groups of 2 were placed in a plastic chamber for a single 24-hour period. Here they were exposed to a gas mixture containing 6 per cent carbon dioxide, 20 per cent oxygen and 74 per cent nitrogen. The earliest day of exposure was the fifth day of pregnancy, the latest day was the twenty-first day. Gas samples from the chamber were analyzed at regular intervals for carbon dioxide and oxygen content. Laboratory pellets and water were placed in the cages in free amounts. As the plastic chamber was transparent, the rats could be observed during experiments. With each group of exposed rats, control animals of the same breed, age, and mating time were kept in separate cages maintained on the same food but in normal atmospheric conditions. After removal from the chamber, the test rats, properly identified, continued under normal atmospheric conditions until delivery.

The majority of rats were permitted to deliver spontaneously. A Caesarean section was performed in a certain number of cases where spontaneous delivery had already started. The Caesarean section was performed under light ether anesthesia. After spontaneous or induced delivery was accomplished, the uteri were inspected, the implantation sites counted and noted, and the mothers weighed. The newborn rats were measured and weighed immediately after birth and before they had suckled. In addition to recording the number of young per litter; the sex, weight and size of each, memoranda were made of the visible malformations, the condition of the young at birth, the method of delivery (spontaneous or Caesarean), and the way they were sacrificed and preserved for serial sectioning. After proper fixation, the chest organs were removed *in toto* and simultaneously embedded in paraffin. This method proved to be the best for maintenance of proper topographic relationship. Hearts and lungs were sectioned serially as a unit, stained with hematoxylin and eosin, and microscopically studied. Projection drawings were made from 2 series.

Results

Mortality, Sex Distribution and Weight of Young

Seventy-one pregnant rats in the test series yielded 530 young. Twenty-one mothers in the control group gave birth to 159 infant rats. The average size of a litter was very similar in both groups: 7.57 in the control group and 7.47 in the treated group (table 1). The size of the control litter was low because the mothers were young.[1] The number of rats stillborn or dying shortly after birth was 41 out of 530 (7.7 per cent) among the test animals and 5 out of 159 (3.1 per cent) in the untreated group. Of the 41 perinatal-death cases in the test group, 27 resulted after spontaneous delivery and 14 after Caesarean delivery. In 4 dams from the test series (on days marked with an asterisk in table 2) the entire litter was resorbed.

Exposure of the mother to 6 per cent carbon dioxide increased the mortality rate among the male fetuses. In the 159 control animals, 80 (50.3 per cent) were males and 79 (49.7 per cent) were females, while in the 530 animals of the test group, 238 (44.9 per

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Table 1

Experimental Data

	Control series	Test series
Number of rats bred	159	530
Number of male offsprings	80 (50.3%)	238 (44.9%)
Number of female offsprings	79 (49.7%)	292 (55.1%)
Average weight of newborn rats in mg.	5,275	6,276 (18.9% higher than control)
Average number of rats recovered per litter	7.6	7.4
Number of rats stillborn or dead immediately after birth	5 (3.1%)	41 (7.7%)
Number of rats with skeletal malformations	1 (0.6%)	58 (10.9%)

Table 2

Mean Weights

Day of mother's exposure to CO_2	Number of litters	Number of newborn	Mean weight of newborn in mgm
5th	5*	31	6,338 ± 6.47
6	3	26	6,292 ± 6.43
7	5*	40	6,277 ± 6.37
8	5	40	6,255 ± 6.31
9	5	45	5,748 ± 6.39
10	4	23	6,480 ± 5.88
11	6	44	6,013 ± 7.45
12	7	29	6,129 ± 6.27
13	4	36	6,237 ± 6.34
14	3	30	5,861 ± 5.83
15	6*	42	6,206 ± 6.27
16	4	31	6,134 ± 6.27
17	3*	23	6,215 ± 6.52
18	3	22	6,158 ± 6.31
19	3	22	6,331 ± 6.49
20	3	27	6,331 ± 6.46
21	2	19	5,862 ± 6.04
Total for test animals	71	530	6,276
Controls	21	159	5,275

*In one dam, the entire litter was resorbed.

cent were males and 292 (55.1 per cent) were females. The difference is significant by statistical analysis ($p < .001$).

The sex distribution, number, and mean weights of the newborn offspring on the different days of exposure and the number of mothers exposed on each day are shown in table 1 and compared with the control group. The average weight of the newborn rat whose mother was exposed to increased carbon dioxide during pregnancy is higher than the average weight of the control group. The differences between the means were all statistically highly significant ($p < .001$). There was no correlation between the age and weight of the mean weight of the offspring. This was equally true of control and treated animals. In every size litter, the average weight of CO_2-treated young was higher than that of the control young in the same litter size. The average weight of the 159 control offspring was 5,275 mg., while the average weight of the 530 treated animals was 6,276 mg. or 18.9 per cent higher.

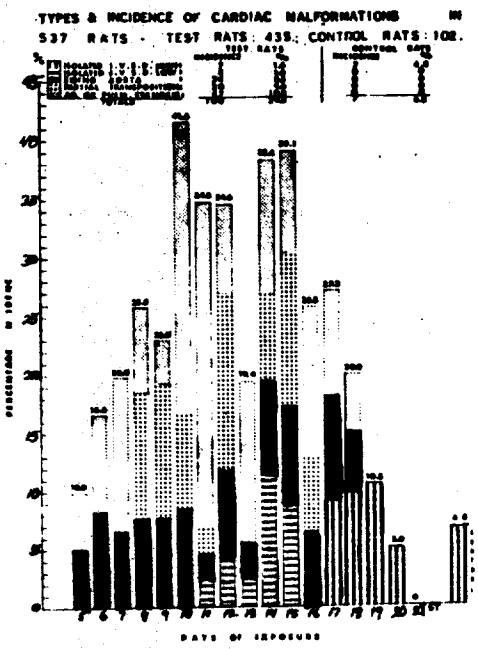

Figure 1

In this figure the total incidence of cardiac malformations is shown in the test animals and in the control animals. Notice that the only type of malformation found among control animals was a defect in the membranous part of the interventricular septum.

The length of the gestation period in both the test and control groups was an average of 22 days, 6 hours. Once parturition started, the entire litter was delivered within 1½ to 2 hours. Only in 2 cases was delivery prolonged in the CO_2-treated animals to about 3 hours. It is not likely, however, that this fact would bear significant influence on the weight of the young.

Test Series; Cardiovascular Defects

Four hundred and thirty-five hearts from the experimental series and 102 from the control series have been sectioned serially and studied microscopically. The incidence of cardiovascular defects was increased in the experimental series as compared with the control group. One hundred and six (24.3 per cent) animals had cardiac anomalies among the treated group and only 7 (6.8 per cent) in the untreated (fig. 1). The 106 young with abnormal hearts among the experimental series can be divided into 5 groups: (1)

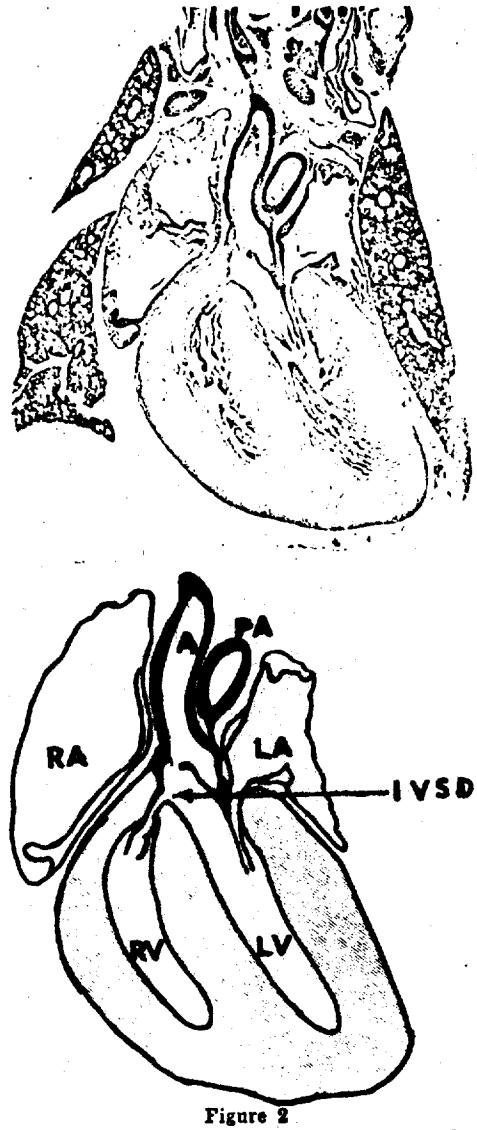

Figure 2

(Top) Frontal section through the heart of a control newborn rat with a high interventricular defect in the membranous part of the septum. (Bottom) Diagram of the heart in figure 2 (top).

high interventricular septal defects (isolated), 7 cases; (2) low interventricular septal defects (isolated), 8 cases; (3) overriding aorta, 24 cases; (4) partial transposition, 24 cases; and (5) pulmonic or aortic stenosis with intact ventricular septum and myocardial hypertrophy, 47 cases.

Group 1: High Interventricular Septal Defects (Isolated)

Seven cases presented this anomaly. The defect was located in the membranous part of the septum only. The days on which this type of defect was found were after exposure on the late days of pregnancy: the seventeenth, eighteenth, nineteenth and twentieth days (fig. 2).

Group 2: Low Interventricular Septal Defects (Isolated)

Single or multiple septal defects situated in the midportion of the muscular interventricular septum were found in 8 cases (fig. 3). They occurred in rats exposed on the eleventh, twelfth, thirteenth, fourteenth and fifteenth days.

Group 3: Riding Aorta

In these 24 specimens, the aorta emerged from both ventricles and the pulmonary artery solely from the right ventricle. The interventricular septal defect was not large; however, it involved not only the membranous septum but also the adjacent muscular portion (septum ventriculorum interaorticum) (fig. 4). The term riding aorta was coined by Rokitansky[2] who first described the anomaly. Spitzer[1] called it Type I Transposition, also Lev[1] includes this lesion among his Transposition Complexes. In the present description, Rokitansky's criteria were applied and only those cases were listed as transposition where one of the large vessels emerged entirely from the wrong ventricle. Five of the 24 cases had a narrowing of the pulmonary outflow channel and hypoplasia of the pulmonary artery. In the other 19, the pulmonary trunk was normal. Riding aorta with pulmonary hypoplasia was found only in rats treated on the seventh, eighth and tenth days. Riding aorta with normal pulmonary artery occurred be-

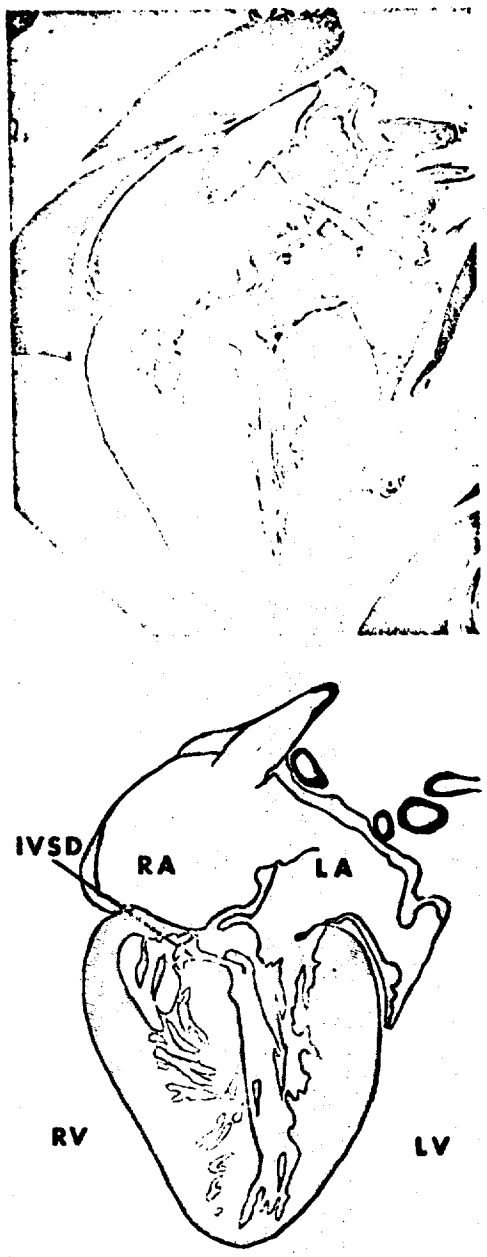

Figure 3

(Top) Frontal section through the heart of test rat with defect in the muscular portion of the interventricular septum. (Bottom) Diagram of the heart in figure 3 (top).

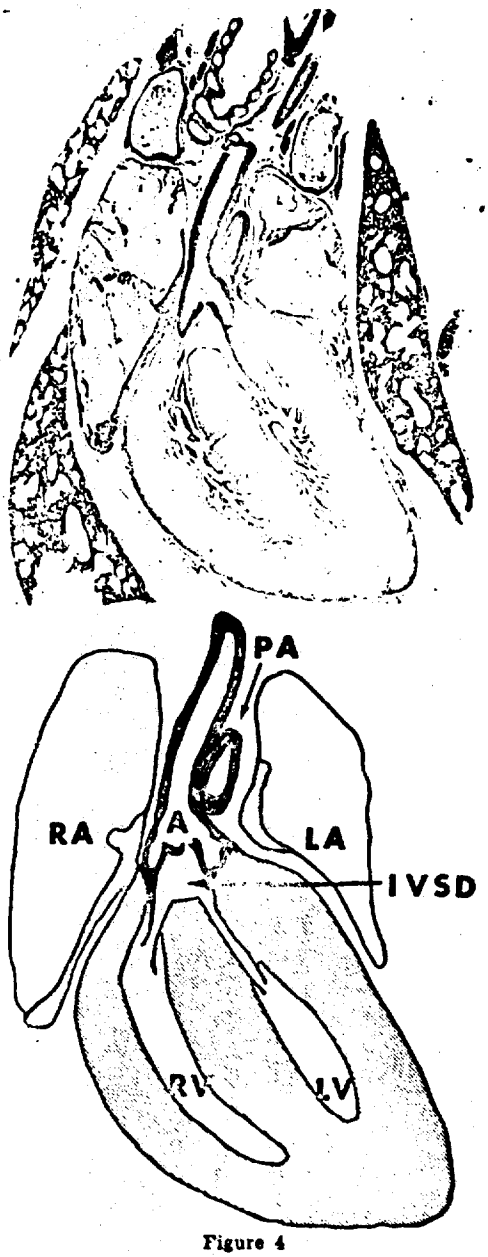

Figure 4

(Top) Frontal section through the heart of test rat with interventricular septal defect, overriding aorta and moderate narrowing of the pulmonary artery. (Bottom) Diagram of the heart in figure 4 (top).

tween the fifth and the eighteenth day and on every day except the eighth and tenth days.

Group 4: Partial Transposition

In 10 specimens, both the aorta and the pulmonary artery arose from the right ventricle. They all had an interventricular septal defect similar to the defect described in the previous group. There were 6 cases in the present series accompanied by pulmonary hypoplasia. In 4 others, the pulmonary artery was of normal caliber (fig. 5). They occurred after treatment on the following 5 days: 2 cases on the eighth day, 3 on the ninth, 2 on the tenth, 2 on the twelfth, and 1 on the fourteenth day.

In this group were also included 10 cases of common trunk in which a single vessel emerged from the right ventricle. This vessel gave off the coronary and systemic arteries and the pulmonary branches. The pulmonary trunk and the ductus arteriosus were absent in 8 out of the 10 cases. In the 2 others, a partial septum separated the mouth of the markedly hypoplastic main pulmonary artery from the common trunk and the ductus was connected with a small left pulmonary artery. In every instance, a large interventricular septal defect was found. Atrial septal defects and common atrioventricular canal were associated 5 times (fig. 6). The 10 cases were found among the offspring treated on the eighth, eleventh, twelfth, fourteenth, fifteenth and sixteenth days.

Group 5: Pulmonic or Aortic Stenosis with Intact Interventricular Septum

This condition with evidence of myocardial hypertrophy, was found in 47 specimens (fig. 7). Twenty-six had a predominant pulmonic stenosis; in the other 21, the aortic stenosis was more marked. Some degree of dextroposition of the aorta or levoposition of the pulmonary artery was noticed in the majority of instances and the stenosis was almost subvalvular. The valves were normal in respect to the number of cusps and their structure. There was also some degree of hypoplasia of the affected vessel and in a certain number of cases besides the narrowing of the outflow

tract, the inflow chamber was also reduced to a slit-like cavity surrounded by markedly hypertrophied walls and thickened endocardium. This type of malformation was not induced when rats were exposed to increased CO_2 after the seventeenth day of pregnancy. The highest incidence occurred on the eleventh day.

Ductus Arteriosus

Out of the 435 in the test series, the ductus was functionally closed in 95 cases (21.8 per cent), somewhat contracted but still open in 115 (26.4 per cent), large and patent in 217 cases (49.9 per cent), and absent 8 times (1.9 per cent).

Control Series

Not a single case with low interventricular septal defect, riding aorta, partial transposition or outflow tract-stenosis was found in the control group. The only type of cardiac anomaly presented was the isolated high interventricular septal defect in the membranous part (in other words, a persistent interventricular foramen). This anomaly was found in 7 of the 102 control-offspring (6.8 per cent) as a small, thin slit-like defect. In 3 of the 7, there was no real communication between the 2 ventricles. The defect presented itself as a pocket-like aneurysm of the membranous septum. The ductus arteriosus was functionally closed in 30 cases (29.4 per cent), open in 55 (53.9 per cent), and widely patent 17 times (16.7 per cent).

Noncardiovascular Defects

Skeletal System

Fifty-eight animals had a marked kyphosis, 25 a moderate hydrocephalus.

Lungs

Microscopic studies of the serially sectioned lungs revealed abnormalities in 376 of the test animals (86.4 per cent). The 3 types of abnormalities found were:

1. Overgrowth of connective tissue around the pulmonary vessels was found in 283 test animals (65.1 per cent), with the highest incidence among those exposed to excessive CO_2 between the fifth and tenth days. Cross-sec-

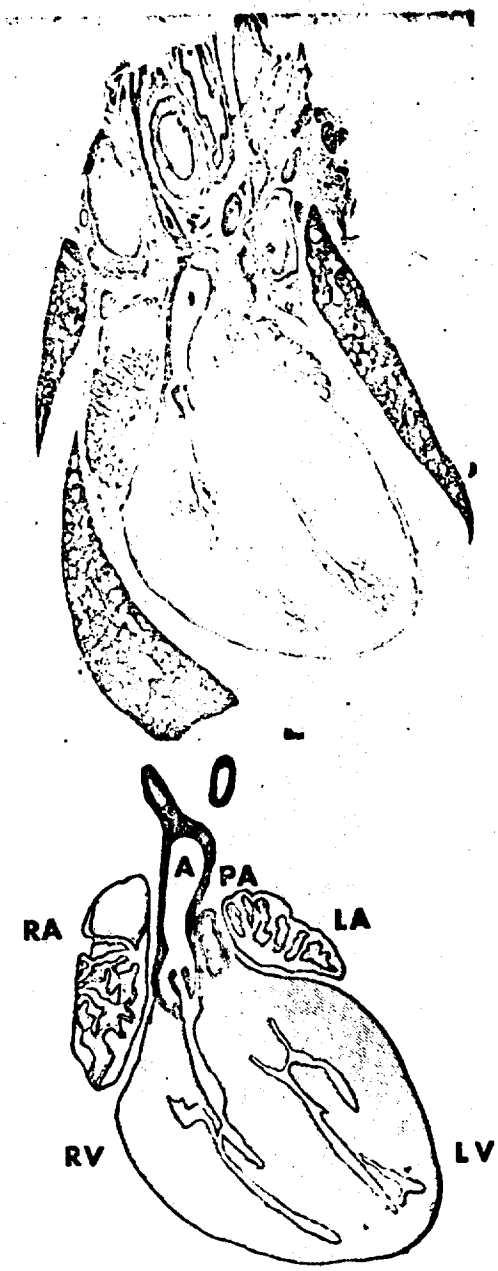

Figure 5

(Top) Frontal section through the heart of test rat with partial transportation and hypoplastic pulmonary artery. (Bottom) Diagram of the heart figure 5 (top).

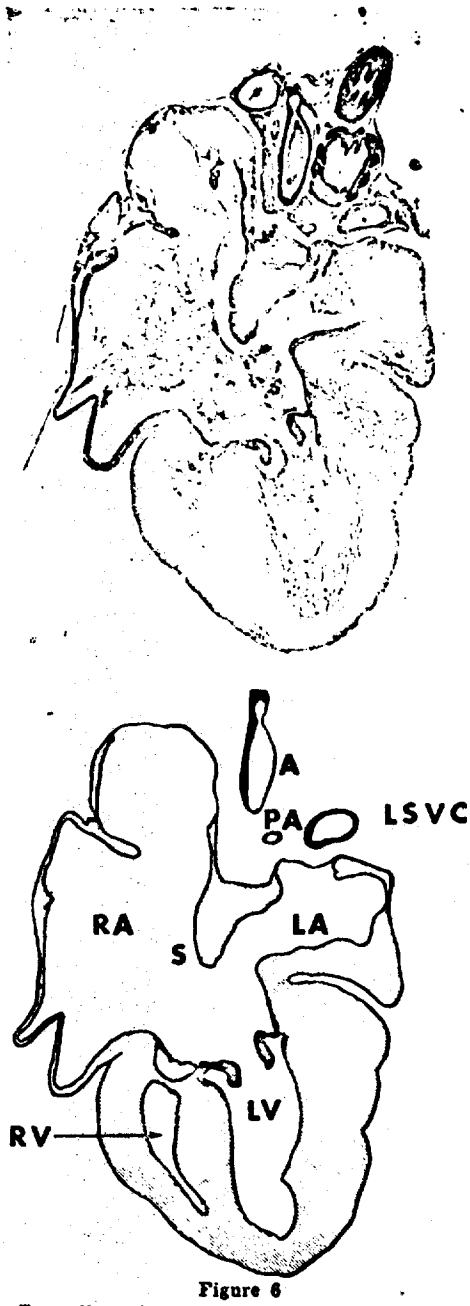

Figure 6

(Top) Frontal section through heart of test rat with common atrioventricular canal. (Bottom) Diagram of the heart in figure 6 (top).

tions showed that the pulmonary arteries from the major branches down to the arterioles were surrounded by a thick cuff of loose connective tissue, edematous in some instances but more frequently fibrous.

2. The second type of lung change which was found in 82 rats (18.8 per cent) probably represents a more advanced stage of the perivascular overgrowth mentioned above. In these rats, there was an internal thickening, in addition to the perivascular overgrowth, which caused narrowing of the vessel lumen. All of these animals had a concomitant cardiac malformation.

3. The third type of abnormality was a hypoplasia which was found in 11 rats (2.5 per cent). Eight of these had cardiac malformations, too, which were accompanied by absence of the main trunk of the pulmonary artery. The 3 other animals had normal hearts.

Thymus

On the average, the test animals showed a significant enlargement of the thymus.

Discussion

The experiments clearly demonstrate that pregnant rats subjected to an increased carbon dioxide tension for 24 hours produced offspring with a high incidence of cardiac malformations. The difference between test and control animals in the incidence of cardiac malformations was statistically significant and the types of malformations found in the test animals simply did not occur in the controls.

Carbon dioxide has not previously been studied alone as a teratogenic agent, although it has been used in combination with hypoxia. It is also possible that alterations in carbon dioxide tensions may have occurred in experiments where hypoxia alone was used as the teratogenic agent, but this was not demonstrated. To the author's knowledge, this is the first instance where carbon dioxide, unrelated to hypoxia, has been used successfully as a teratogenic agent.

The abnormal mechanism induced by increased carbon dioxide is not yet clear, but

there is some evidence in these experiments that it may be a temporary stimulation both of differentiation and of growth processes. At the conception of these experiments, the author had the idea that those malformations which could be due to overgrowth, such as subaortic stenosis or infundibular pulmonic stenosis, might be caused by increased tissue oxygen.[5] Just as pregnant animals exposed to hypoxia give birth to offspring with malformations characterized by arrested development,[6-10] hyperoxia might logically cause accelerated development resulting in malformations due to tissue overgrowth. If a tissue hyperoxia were to occur in a supposedly normal pregnancy, it would quite likely be secondary to an increase in carbon dioxide, for the increased carbon dioxide would cause an acidosis, a shift in the oxyhemoglobin dissociation curve, and thus an increase in tissue oxygen tension.

Though a tissue hyperoxia which might conceivably cause overgrowth has not been demonstrated in these experiments, there is evidence that overgrowth has occurred. There is evidence of localized tissue overgrowth found in the lungs, thymus and heart of the test animals. The thymus is larger than normal, the cardiac muscle in many test animals is thickened with reduction of the ventricular channel or narrowing of outflow passages, and the lungs have shown a perivascular overgrowth. These changes could be interpreted as manifestations of overgrowth. There is, in addition, evidence of periods of accelerated growth. Anomalies such as partial transposition, overriding aorta or defects in the muscular portion of the interventricular septum could be due to a disturbance of coordinated development caused by periods of acceleration of certain growth processes.[11-13] Transposition may be the consequence of early overgrowth of the ventricular sinusoids, followed by abnormal looping of the cardiac tube and a defect in separation. Overgrowth of some portions of the interventricular septum may produce a low ventricular septal defect or a high septal defect with overriding of the aorta. The CO_2 given during cardiogenesis

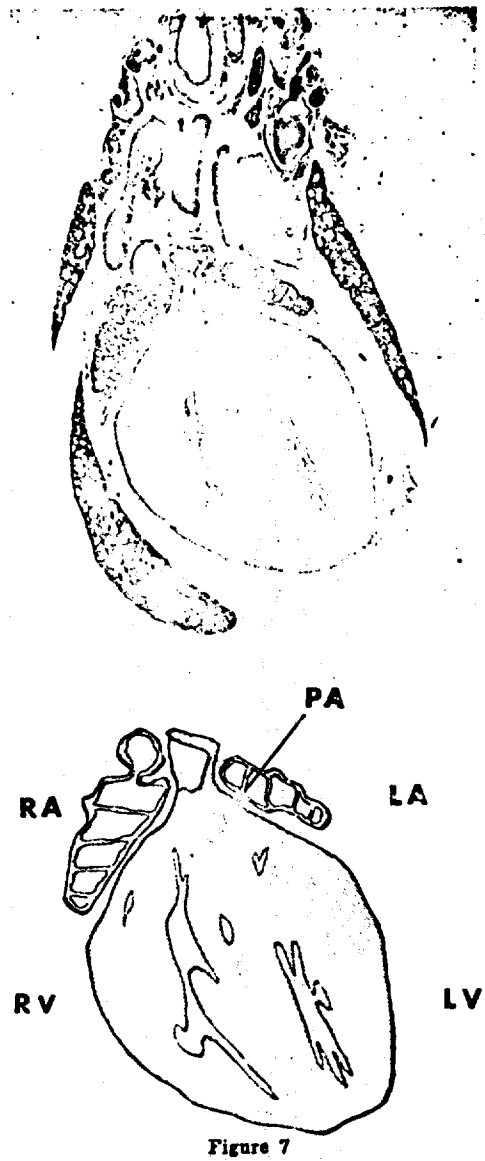

Figure 7

(Top) Frontal section through heart of test rat with myocardial hypertrophy and pulmonic stenosis. (Bottom) Diagram of the heart in figure 7 (top).

did not cause an increased incidence of the isolated high defect but rather a lower incidence than in the controls. In the entire test group, the incidence of the high defect was

only 1.5 per cent while it occurred with an incidence of 6.8 per cent in the controls. Perhaps through its stimulating effect on growth, the increased CO_2 actually caused a more ready closure of the interventricular foramen.

The defects which appear with highest incidence in this series (myocardial hypertrophy with outflow tract narrowing, partial transposition, overriding aorta) and which may be thought of as defects due to overgrowth are not found or at least found only infrequently in other studies of experimental teratogenesis.[11-17] It would appear that increased carbon dioxide produces an effect different from that of other agents studied.

The time of action of this teratogenic agent may at first glance appear confusing. Partial transposition, for example, was found to occur when carbon dioxide was administered at any time between the eighth and twelfth and again the fourteenth and sixteenth day of pregnancy. Yet this is consistent with possible mechanisms, for teratogenic agents may have either an immediate or a delayed action and carbon dioxide could work both ways.[18-22] Furthermore, changes may be due to specific conditions present at the time when the injurious agent is applied.

Exposure to increased carbon dioxide on days preceding cardiogenesis (fifth to tenth day) may affect implantation, chemodifferentiation or histodifferentiation[23, 24] by changing the pH of the surrounding tissues. During cardiogenesis (tenth to seventeenth day), tissue growth may be altered as a consequence of the influence of carbon dioxide on mitosis. The structural changes of the developing heart tube modify blood flow and this in turn can influence the ultimate fusion of growing septa.[25] After cardiogenesis, carbon dioxide can only influence further growth but not differentiation.

Summary

Seventy-one pregnant rats of a Sprague-Dawley strain were exposed for a single 24-hour period to a gas mixture of 6 per cent carbon dioxide, 20 per cent oxygen and 74 per cent nitrogen on different days during gestation. There were 530 offspring and these were compared with 159 newborn rats from 21 control dams. The incidence of cardiac malformations was 23.4 per cent in the test group and 6.8 per cent in the control group with the highest incidence in the test group occurring when the exposure was on the tenth day of gestation. Isolated ventricular septal defects, situated in the lower and posterior part of the muscle septum, were found in 8 cases, ventricular septal defects with overriding aorta in 24 cases and ventricular septal defects with partial transposition in 20 cases. Forty-seven of the abnormal hearts had a myocardial thickening accompanied by narrowing of the pulmonic or aortic outflow channel with or without other abnormalities, such as atresia of the mitral or tricuspid valves and hypoplasia of the aorta or pulmonary artery. There was a slight increase in perinatal mortality in the test group, and a lower frequency of male offspring. The average body weight was 18.9 per cent higher in the test group.

Summario in Interlingua

Septanta-un rattas pregnante de un racia Sprague-Dawley esseva exponite, in varie stadios del gestation, al effecto de periodos individual de 24 horas de respiration de un mixtura de 6 pro cento de bioxydo de carbon, 20 pro cento de oxygeno, e 74 pro cento de nitrogeno. Le prole total numerava 530 individuos, e istos esseva comparate con 159 neonatos ab 21 matres de controlo. Le incidentia de malformation cardiac esseva 23,4 pro cento in le gruppo experimental e 6,8 pro cento in le gruppo de controlo. In le gruppo experimental, le incidentia esseva le plus alte quando le exposition occurreva le decime die del gestation. Isolate defectos del septo ventricular, situate in le parte inferior e posterior del musculo, esseva trovate in 8 casos; defectos septal ventricular con supersalientia del aorta in 24; e defectos septal ventricular con transposition partial in 20. Quaranta-sept del cordes anormal habeva un spissification myocardial con restriction del canal de effluxo pulmonic o aortic in le presentia o absentia de altere anormalitates, como per exemplo atresia del valvula mitral o tricuspidic e hypoplasia del aorta o del arteria pulmonar. Esseva constatate un leve augmento del mortalitate in le gruppo experimental e un reducite proportion del prole masculc. Le peso corporee mediie esseva plus alte per 18,9 pro cento in le gruppo experimental.

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EFFECTS OF CARBON DIOXIDE ON THE ALPHA FREQUENCY AND REACTION TIME IN HUMANS[1]

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Carbon dioxide (CO_2) has been shown to have both excitatory and inhibitory effects on various measures of sensory and cortical activity in humans. Sensory function is generally impaired when subjects breathe high concentrations of CO_2 for short periods of time (0–10 min) (Gellhorn and Spiesman 1935a, b; Stokes *et al.* 1948; Schaefer and Carey 1954; Wyke 1963, p. 58). Studies which have investigated the effect of CO_2 on cortical activity in humans show conflicting results (see Discussion). These may be attributed to the different measures, CO_2 concentrations, and inhalation periods employed.

The present study sought to investigate the effects of various CO_2 concentrations on alpha frequency and reaction time in humans. The upper limit of 7.9% CO_2 was selected since it nears the maximum concentration permissible (approximately 10%) without loss of consciousness in man. The effects were also evaluated in terms of the length of CO_2 inhalation.

METHOD AND MATERIAL

Experimental design

Five male graduate students, experienced in the reaction time (RT) task, participated in two 50-min experimental sessions. An experimental session consisted of having each subject inhale five levels of CO_2 (0, 1.5, 3.5, 5.5 and 7.9%) mixed with air for a 5-min trial. To obtain control data, air was administered for the first 30 sec of each trial. A replicated 5 × 5 balanced Latin Square was used to determine the trial on which the various CO_2 conditions were presented to each subject for the two sessions. Six within-trial changes were obtained by sampling the data for the first two 30-sec intervals and then on alternate 30-sec intervals for the remaining part of the trial. Therefore, the five subjects, five CO_2 concentrations, two sessions, and six 30-sec within-trial samples resulted in a total N of 300.

Apparatus and data recording procedure

The various CO_2 concentrations were administered by having the subjects breathe through a rubber mouthpiece, attached to a Hans Rudolph valve, from a 60 l Douglas Bag. RT was measured to the nearest millisecond by a Hewlett Packard Model 522B electronic counter and Model 560A digital recorder. A Grass photostimulator Model PS2, set at intensity "16", was used to present visual stimuli of 10 μsec duration. A hood, attached to the flash tube, fixed the subject's eyes (which were closed) 6″ from the stimulus. Stimuli were presented at intervals varying from 3 to 7 sec. A model 220 California Technical Industries 6-channel tape reader triggered the photostimulator, counter, and polygraph RT monitor. Subjects reacted by pushing a micro-switch key with their right index finger which, in turn, sent a terminating pulse to the counter and polygraph monitor. A 180 g force was required to move the key the 7 mm excursion necessary to close the switch.

Alpha frequency was measured from EEGs recorded between the midline occipital-parietal (O_z–P_z) electrodes and from C_z referred to A_1 according to the International Standard 10–20 System. The procedure and apparatus used to record EEGs has been described elsewhere (Harter *et al.* 1964). The average alpha frequency for each subject and CO_2 concentration was determined by averaging twelve alpha frequency measures obtained from the twelve 30-sec samples of the EEG record (six within-trial samples for two sessions). Alpha frequency was measured from each 30-sec sample by selecting at least 3 sec of the largest amplitude alpha waves (any rhythmic activity between 9 and 13 c/sec) in the sample, counting the number of alpha waves selected, and dividing by their accumulated duration in seconds. Therefore, the average alpha frequency for each subject and CO_2 concentration was based on at least 36 sec of alpha activity.

RESULTS AND DISCUSSION

Analyses of variance were performed on the grouped data to determine the effects of time (within-trial effects) and CO_2 concentration inhaled on RT and alpha frequency.

Changes across time

Significant within-trial changes were evident in RT and alpha frequency recorded from O_z–P_z ($P < 0.05$). Alpha

[1] Adapted from a doctoral dissertation submitted to the University of Arizona, Tucson, Ariz., and supported by funds received from the U.S. Navy Electronics Laboratory under a National Academy of Sciences–National Research Council post-doctoral fellowship, National Aeronautics and Space Administration under a pre-doctoral fellowship, and National Science Foundation under Grant GB-1384.

frequency increased and RT decreased within the first 2 min of inhalation; throughout the remainder of the trial alpha frequency progressively decreased and RT progressively increased. A similar trend was evident in the alpha frequency from the C_z electrode although it did not approach statistical significance.

Effects of CO_2

Alpha frequency (O_z–P_z and C_z–A_1) and RT were significantly affected by CO_2 concentration administered ($P < 0.01$ and $P < 0.05$, respectively). Orthogonal comparisons indicated that RT was shorter and alpha frequency was higher during the 0–5.5% CO_2 conditions than during the 7.9% CO_2 conditions ($P < 0.01$). The differential effects of 0–5.5% CO_2 on RT and alpha frequency did not approach statistical significance.

The effects of CO_2 on the alpha frequency and RT of each subject are illustrated in Fig. 1. Consistent with past findings (Schaefer 1958), the subjects differed considerably in terms of their sensitivity to CO_2. The within-subject changes in RT and alpha frequency were negatively correlated across the CO_2 conditions investigated (rank-order correlations of −0.50, −0.60, −0.90, −0.90 and −0.00, respectively, for subjects EB, GH, HM, BF and GS). A relationship between alpha frequency and RT has been noted elsewhere (Lansing 1957; Callaway 1962; Surwillo 1963).

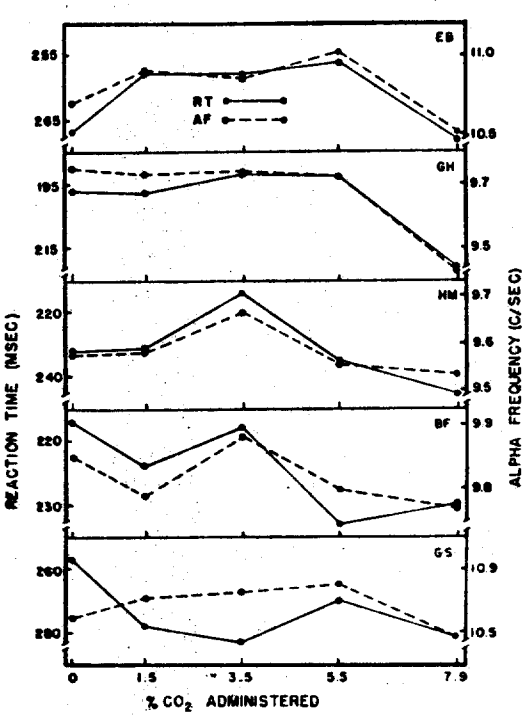

Fig. 1

Effects of CO_2 concentration administered on the reaction time (RT) and occipital-parietal alpha frequency (AF) of each subject (EB, GH, HM, BF, GS). Each plotted point is an average based on an N of 12.

In general, the threshold CO_2 concentration for "depressive" effects on both RT and alpha frequency appeared to fall between 3.5 and 7.9%. The threshold average value of approximately 5.5% CO_2 is comparable to those values found previously in sensory threshold studies (Gellhorn and Spiesman 1935a, b; Stokes *et al.* 1948). It is somewhat higher than the 3.3% CO_2 value for decreased critical flicker fusion and alpha blocking time found by Schaefer and Carey (1954). The latter discrepancy may be attributed to different inhalation periods (*i.e.*, 15 min in the latter and 5 min in the present study).

There was a suggestion that RT was shorter and alpha frequency was higher under the 3.5–5.5% CO_2 conditions as compared to the 0–1.5% CO_2 conditions. This effect, which did not approach statistical significance, has been noted previously (Gibbs *et al.* 1940; Schaefer 1949).

It has been proposed that the effects of CO_2 on the central nervous system depend on the concentration of CO_2 administered: 3–7% CO_2 causing direct depression of cortical neural activity only; 5–20% CO_2 causing generalized reticulo-cortical activation which overcomes the initial direct cortical depression; and 20% CO_2 and above causing narcosis or anesthesia and depression of reticulo-cortical activity (Wyke 1963). The results of the present study fail to support this hypothesis since (a) the changes in RT and alpha frequency suggesting an excitatory effect did not approach statistical significance; (b) there were considerable individual differences in reaction to CO_2 and (c) the CO_2 concentrations at which the second and third phases were evident in the present study were considerably lower than those proposed by Wyke (1963).

SUMMARY

The effects of acute exposure (5 min) to carbon dioxide (0–7.9%) on the EEGs and reaction times of five humans were investigated. Alpha frequency and alpha amplitude were recorded from the central and from the occipital-parietal areas of the scalp while subjects reacted with their right index finger to flashes of light. Variance analyses indicated that the percentage CO_2 inhaled significantly affected alpha frequency and reaction time. Alpha frequency and reaction time were significantly faster under the 0–5.5% CO_2 conditions than under the 7.9% CO_2 condition. A slight increase in alpha frequency and decrease in reaction time were evident under the 3.5 and 5.5% CO_2 conditions as compared to the 0 and 1.5% CO_2 conditions.

RÉSUMÉ

EFFETS DU CO_2 SUR LA FRÉQUENCE DE L'ALPHA ET LE TEMPS DE RÉACTION CHEZ L'HOMME

Les effets à une exposition aiguë (5 min) au CO_2 (0 à 7,9%) sur l'EEG et les temps de réaction de cinq hommes sont étudiés. La fréquence et l'amplitude de l'alpha dérivé des aires centrales et pariéto-occipitales du scalpe sont enregistrées, tandis que ces sujets réagissent avec leur index droit à des éclairs lumineux. Des analyses

de variance indiquent que le pourcentage de CO_2 inhalé affecte de façon significative la fréquence de l'alpha et le temps de réaction. La fréquence de l'alpha et le temps de réaction sont significativement plus rapide sous des concentrations de 0,5 à 5 % de CO_2 que sous des concentrations de 7,9 %. Une légère augmentation de la fréquence alpha et une diminution du temps de réaction apparaissent évidentes pour des concentrations de 3,5 % de CO_2 si on les compare à des concentrations de 0 et des 1,5 %.

The author wishes to express appreciation to Dr. R. W. Lansing for suggesting and supervising this project, and to Drs. N. R. Bartlett and R. H. Pool for their constructive suggestions in the preparation of the manuscript. Acknowledgment is also made to Dr. T. P. K. Lim for his medical supervision, and to other personnel of the Tucson Medical Center, Cardiopulmonary Laboratory, Tucson, Ariz., without whose support this project could not have been undertaken.

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Storage of Carbon Dioxide in Muscle

WHEN ventilation is suddenly arrested, the carbon dioxide produced by the body is stored within the body tissues as bicarbonate and dissolved carbon dioxide. It has been suggested that this storage of carbon dioxide does not proceed uniformly in all tissues. Rather it has been proposed that the body can be conceived as being composed of multiple compartments which store carbon dioxide at different rates.

In the multicompartment model of carbon dioxide stores proposed by Fahri and Rahn, during apnoea, muscle—because it has a low ratio of perfusion per unit volume—acts as a repository for carbon dioxide produced by other organs[1]. When there is no ventilation, therefore, the carbon dioxide content of the venous blood should be less than the carbon dioxide content of the arterial blood—the reverse of the usual situation.

To test this prediction, we measured the carbon dioxide and oxygen tension, the hydrogen ion concentration and the haematocrit of blood from the femoral artery and vein, and calculated carbon dioxide contents in nine anaesthetized and paralysed dogs during apnoea.

After the paralysed dogs had been ventilated with 100 per cent oxygen for 30 min, arterial and venous blood was obtained from one hind limb, and carbon dioxide production was measured. Apnoea was produced by stopping artificial ventilation. During the apnoeic period, blood samples were obtained at intervals of 1 or 2 min while the trachea of the dog was connected to a spirometer which contained oxygen so that full arterial saturation could be maintained. In three dogs, blood flow in the opposite limb was measured with a rotameter.

Table 1. FEMORAL ARTERIAL AND VENOUS CARBON DIOXIDE TENSIONS AND CONCENTRATIONS DURING APNOEA IN NINE DOGS (MEAN ± STANDARD ERROR)

Time (min)	CO_2 tension (mm Hg) Artery	Vein	CO_2 concentration difference (vol. per cent) Vein–artery
0	35 ± 4	40 ± 5	+2·1 ± 0·7
1	47 ± 4	44 ± 4	−1·4 ± 0·3
2	52 ± 5	47 ± 4	−2·5 ± 0·6
4	63 ± 6	53 ± 5	−3·3 ± 0·9
6	73 ± 6	60 ± 5	−3·3 ± 0·6

The carbon dioxide production of the dogs during the control period of artificial ventilation averaged 74 ml./min (standard error, ± 4). The mean blood flow in the femoral artery was 135 ml./min in the control period but decreased to an average of 92 ml./min during apnoea. Table 1 shows the carbon dioxide tensions and the venous minus the arterial carbon dioxide concentration differences observed during apnoea. During artificial ventilation the venous carbon dioxide tension exceeded the arterial by 5 mm of mercury while the carbon dioxide content of the vein was 2·1 vol. per cent greater than the artery. After 1 min of apnoea, these gradients were reversed and the venous carbon dioxide tension and concentration were less than those of the artery. At 6 min of apnoea, the average venous carbon dioxide tension was 13 mm of mercury less than that measured in the artery while the carbon dioxide concentration was 3·3 vol. per cent less.

The result of multiplying the average blood flow of the femoral artery by the mean venous–arterial carbon dioxide concentration differences shows that, during each minute of apnoea, muscle stores about 3 ml. of carbon dioxide produced elsewhere. This is about 4 per cent of the total production of carbon dioxide by the body each minute.

According to the multicompartment theory, carbon dioxide is stored in different tissue compartments, each with its own level of perfusion, metabolic rate and dissociation curve for carbon dioxide[2]. Ventilation changes produce rapid alterations in carbon dioxide content in richly perfused tissue such as the brain, but only slow changes in tissues with a large volume but a relatively poor perfusion such as muscle. The results of this study agree with the idea that muscle carbon dioxide reaches equilibrium sluggishly after a change in arterial carbon dioxide tension, thus producing the reversal of the usual venous–arterial concentration differences.

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7

*The Function of CO_2 in Cell Metabolism and Growth**

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A number of authors have described the carbon dioxide/bicarbonate system (CO_2/HCO_3^-) as essential for the propagation of animal cells in tissue culture,[4, 6, 17] and evidence has been presented that one of the chief functions of CO_2/HCO_3^- in growing cells is to provide specific precursors for syntheses of the purines, the pyrimidines, oxaloacetate and certain amino acids.[2, 5, 13]

Stimulating effects of low levels of carbon dioxide tension on embryonic growth and differentiation have been demonstrated by other investigators, while carbon dioxide tensions above 5 per cent atmospheric pressure have been reported to inhibit embryonic development and cell metabolism. These findings have recently been reviewed.[18]

It has been suggested that carbon dioxide may act as a physiological regulator of cell multiplication, and that cancer represents the *de novo* appearance of a cell that possesses metabolic mechanisms less susceptible to carbon dioxide inhibition than those of normal cells. It is the purpose of the present report, on the basis of some studies of the relationship between carbon dioxide tension and cellular energy metabolism, to discuss this hypothesis, which was first presented by Loomis.[11, 12]

Influence of Carbon Dioxide on Glycolysis

A stimulatory effect of CO_2/HCO_3^- on anaerobic glycolysis in slices of the Flexner-Jobling rat carcinoma was first demonstrated by Warburg *et al.*[10] We have studied the influence of carbon dioxide tension on aerobic glycolysis in slices of transplantable mouse tumors including a mammary carcinoma and a mast cell tumor carried in the DBA/2 strain of mice, a carcinosarcoma and a lymphatic leukemia carried in the Aka strain, and a chondroma carried in the St/Fh strain. The gas phase

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contained 20 per cent oxygen and 1, 5, 8, and 15 per cent carbon dioxide. Ringer's solution with an initial glucose concentration of 5 millimols (mM) served as experimental medium. Bicarbonate was added at concentrations corresponding to the carbon dioxide tension under investigation at

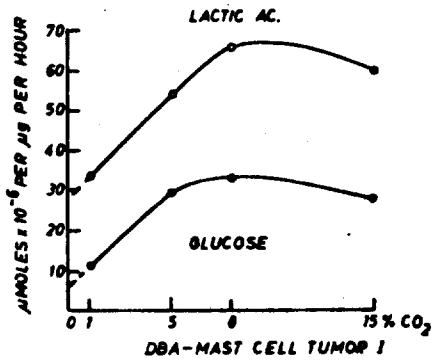

FIGURE 1. The influence of carbon dioxide on aerobic glycolysis in the DBA/2 mast cell tumor.

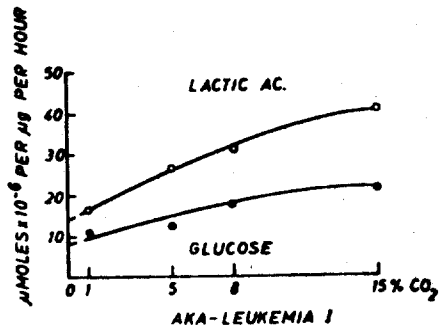

FIGURE 2. The influence of carbon dioxide on aerobic glycolysis in the Aka leukemia.

pH 7.4. Glucose and lactic acid concentrations were determined enzymatically.[5,6]

All the tumors responded to variation in carbon dioxide tension, showing maximal rates of glucose utilization and lactic acid production at 8 to 15 per cent carbon dioxide. When compared to glycolysis at 1 per cent carbon dioxide, an increase of 58 to 190 per cent was observed. Typical experiments are shown in Figures 1, 2, and 3.

In comparative studies of the glycolytic activity of tumor slices, variations in the relative amounts of stromal tissue may represent a serious source of error. Therefore, the DBA mast cell tumor, the Aka leukemia and the Aka carcinosarcoma were converted into ascites tumors.

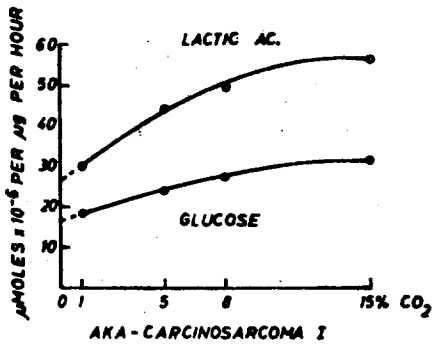

FIGURE 3. The influence of carbon dioxide on aerobic glycolysis in the Aka carcinosarcoma.

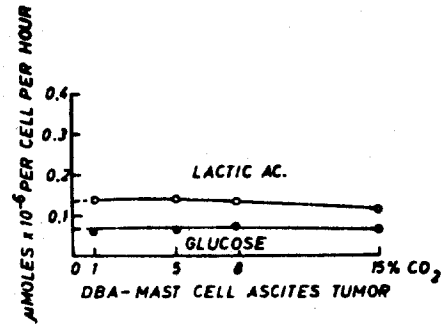

FIGURE 4. The influence of carbon dioxide on aerobic glycolysis in the DBA/2 mast cell ascites tumor.

When the influence of carbon dioxide on aerobic and anaerobic glycolysis was studied in these ascites tumor cells, no stimulation could be demonstrated (Figures 4, 5 and 6); similar results were obtained with a hyperdiploid cell line of the Ehrlich ascites tumor and with the Yoshida rat ascites tumor.

In order to test whether the conversion of solid tumors into the ascites form involved a selection of mutants resistant to carbon dioxide, ascites

tumor cells were inoculated subcutaneously into susceptible hosts. The solid tumors which subsequently developed were cut into thin slices, the aerobic glycolysis of which was studied at different carbon dioxide tensions. In all cases these secondary solid tumors responded to variations

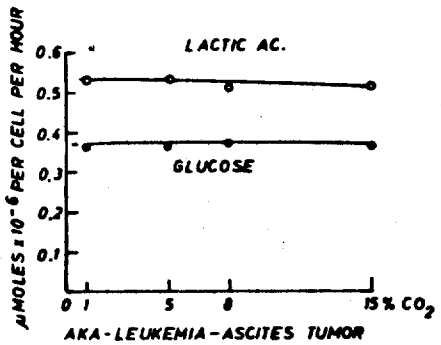

FIGURE 5. The influence of carbon dioxide on aerobic glycolysis in the Aka ascites leukemia.

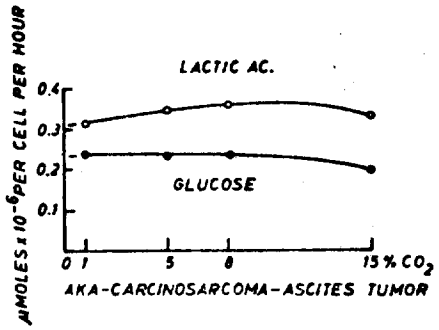

FIGURE 6. The influence of carbon dioxide on aerobic glycolysis in the Aka ascites carcinosarcoma.

in carbon dioxide tension in the same way as the primary solid tumors (Figures 7 and 8).

These observations do not support the assumption of a genetic basis for the difference between the effect of carbon dioxide on solid and ascites tumors. Therefore, the possibility was considered that the presence of stromal cells might be essential for the effect of carbon dioxide on glycolysis. This hypothesis was substantiated, when it was found that

the addition of strain-L mouse fibroblasts to the ascites tumor cells made the glycolytic metabolism of the latter sensitive to variations in carbon dioxide tension, as shown in Figures 9 and 10. A similar effect, although somewhat less pronounced, was obtained in the absence of fibroblasts,

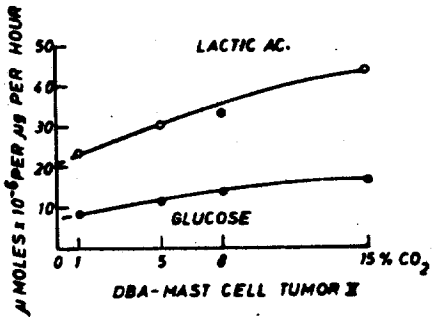

FIGURE 7. The influence of carbon dioxide on aerobic glycolysis in the secondary solid DBA/2 mast cell tumor.

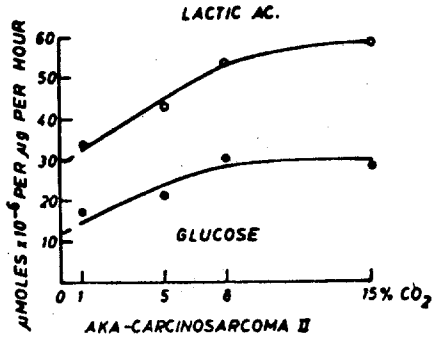

FIGURE 8. The influence of carbon dioxide on aerobic glycolysis in the secondary solid Aka carcinosarcoma.

when the ascites tumor cells were suspended in the supernatant fluid from fibroblast cultures which had been incubated for several hours in Ringer's solution. These observations indicate that fibroblasts produce a humoral factor which enables the tumor cells to utilize carbon dioxide in support of their glycolytic metabolism.

The results of these experiments raise two questions: (*a*) what is the mechanism of the effect of carbon dioxide on glycolysis; and (*b*) what

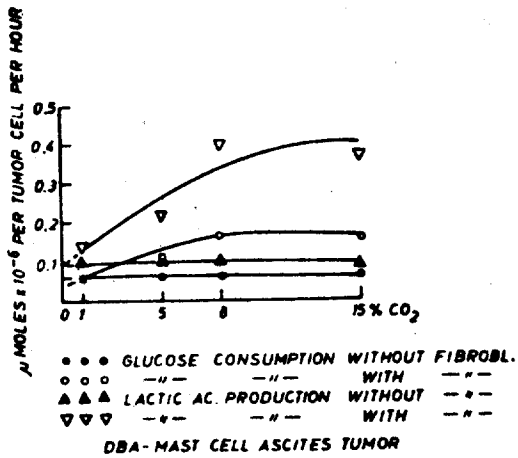

FIGURE 9. The influence of fibroblasts on aerobic glycolysis in the DBA/2 mast cell ascites tumor at different carbon dioxide tensions.

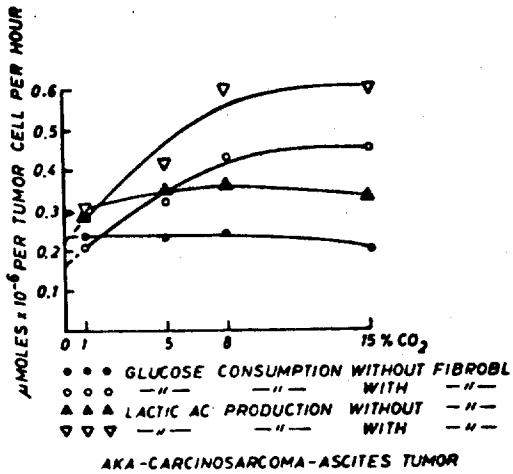

FIGURE 10. The influence of fibroblasts on aerobic glycolysis in the AKA ascites carcinosarcoma at different carbon dioxide tensions.

is the nature of the factor produced by fibroblasts which makes the tumor cells sensitive to the stimulatory effect of carbon dioxide?

It has been reported that C^{14}-labeled carbon dioxide is incorporated mainly into the purines, the pyrimidines and certain amino acids.[2, 13] In agreement with these observations tissue culture studies have shown that ribonucleosides are partially effective as a substitute for carbon dioxide in supporting cell multiplication, while a combination of ribosides and oxaloacetate provides conditions as good or better than carbon dioxide for multiplication of carbon-dioxide-depleted conjunctival cells.[2]

These observations led to a study of the effect of purines, pyrimidines, ribosides and oxaloacetate on aerobic glycolysis at 1 per cent and at 8 per cent carbon dioxide in Aka carcinosarcoma in its original solid form and in its ascites form with and without fibroblasts. It was found that adenosine at a concentration of 1 millimol was partially or completely effective as a substitute for carbon dioxide in supporting glycolysis in the solid tumor as well as in the ascites tumor. The presence of fibroblasts was not essential for this effect of adenosine. Guanosine, uridine, cytidine and thymidine and their corresponding purine and pyrimidine bases were not effective, and the same applied to adenine and to oxaloacetate.

The effect of adenosine as a substitute for carbon dioxide points to the concentration of coenzyme I (DPN) and adenosinediphosphate (ADP) and adenosinetriphosphate (ATP) as the limiting factor in glycolysis at low carbon dioxide tensions. In support of this assumption we have found that carbon dioxide has no effect on glycolysis in tumor homogenates fortified with ATP and DPN. How the concentration of these cofactors is influenced by carbon dioxide remains to be shown. The function of carbon dioxide as a precursor in the purine ring indicates that the carbon dioxide effect may be due to an increased rate of synthesis of the cofactors. This would be the most simple explanation, but experimental proof is still lacking.

Studies by Shaw and Stadie[15] indicate that phosphofructokinase, which catalyzes the formation of fructose-1,6-diphosphate, is inactive in the absence of bicarbonate. In order to test whether this step of glycolysis might be the one which is influenced by variations in carbon dioxide tension, we have studied the production of lactic acid in tumor homogenates fortified with ATP and DPN at 1 per cent and at 8 per cent carbon dioxide, using glucose-6-phosphate and fructose-1,6-diphosphate as substrates. In none of these experiments was carbon dioxide tension found to be essential for lactic acid production.

The function of the fibroblasts has not yet been studied in detail. The fact that adenosine does not require the fibroblasts for its effect on glycolysis points to a level below adenosine as the site of the fibroblast

effect. It is conceivable that the incorporation of carbon dioxide into the purine ring is dependent on a factor produced by the fibroblasts, but the possibility also exists that the fibroblasts are supplying the pentose component which is required for nucleoside synthesis. The pentose shunt may be more active in stromal cells than in tumor cells.

The Influence of Carbon Dioxide on Respiration

When the influence of carbon dioxide on cellular energy production is discussed, not only its effect on glycolysis but also its effect on cell respiration should be considered. A number of authors have shown that CO_2 HCO_3^- has a stimulating effect on the respiration of various tissues,[8] while an inhibitory effect has been demonstrated in other systems. Studying the effect of 0 to 5 per cent carbon dioxide on the respiration in Earle's strain-L fibroblasts, the Yoshida rat ascites tumor cells, and normal and malignant human leukocytes, we have found maximal respiration at 0.5 to 2 per cent carbon dioxide.

Carbon dioxide tensions between 3 and 5 per cent of atmospheric pressure tended to inhibit cellular respiration. Ehrlich ascites tumor cells, on the other hand, showed moderate inhibition at all carbon dioxide tensions tested. These results have previously been reported.[1, 8, 9, 10, 14]

Evidence has also been presented that stimulation of cellular respiration is due to an increased rate of oxaloacetate synthesis.[10]

From a physiological point of view the effect of carbon dioxide tensions above 5 per cent of atmospheric pressure is more interesting. Therefore, using the Cartesian diver technique, as previously described,[10] we studied the effect of 0 to 15 per cent carbon dioxide on the respiration in Earle's strain-L fibroblasts and Aka carcinosarcoma in its ascites form. These studies showed a stimulating effect of 1 per cent carbon dioxide, but inhibition at higher carbon dioxide tensions.

In the Aka tumor cells the inhibitory effect reached its maximum at 5 per cent carbon dioxide, above which concentration no further inhibition was seen. In the fibroblasts there was a progressive inhibition from 5 per cent through 15 per cent carbon dioxide. At 15 per cent carbon dioxide all respiration had stopped (Figures 11 and 12).

The mechanism of the inhibitory effect of carbon dioxide on cellular respiration is not known. It has been suggested that in the presence of carbon dioxide the equilibrium between fatty acid oxidation and fatty acid synthesis may shift toward synthesis,[3] but experimental evidence of this is still lacking.

In mitochondrial preparations from *Ricinus* endosperm, succinic oxidase has been found to be markedly inhibited by carbon dioxide concentrations of 10 per cent and above. Whether a similar inhibition occurs in animal cells remains to be shown.

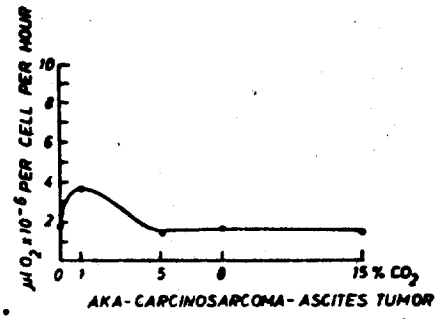

FIGURE 11. The influence of carbon dioxide on respiration in the Aka ascites carcinosarcoma.

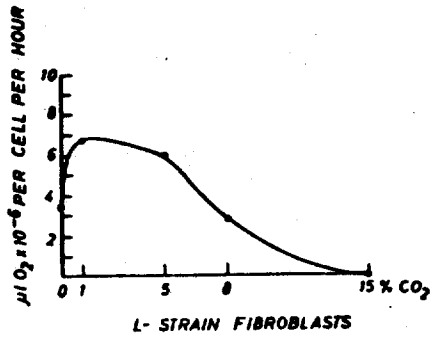

FIGURE 12. The influence of carbon dioxide on respiration in the L-strain mouse fibroblasts.

Influence of Carbon Dioxide on Total Energy Production

The carbon dioxide/bicarbonate system is known to influence cell metabolism and biosynthesis in numerous ways. Some reactions are stimulated and others are inhibited. The demonstration of a correlation between the sum of these effects and carbon dioxide tension would point to carbon dioxide as a physiological regulator of cell multiplication. On the other hand, if a balance exists between the stimulating and inhibiting effects of carbon dioxide irrespective of physiological variations in carbon dioxide tension, then carbon dioxide is not likely to play a regulating role in cell growth.

In order to illustrate the effect of various carbon dioxide tensions on total energy metabolism, a calculation has been made of the amount of

adenosinetriphosphate which may be produced by the glycolytic and the respiratory metabolism in the strain-L fibroblasts and in the Aka carcinosarcoma. Respiratory energy production was calculated on the basis of the observed oxygen consumption, and glycolytic energy production on the basis of aerobic lactic acid accumulation. The latter probably represents a minimum value, since part of the lactic acid produced during the experimental period may have been oxidized. However, since in our experiments the ratio of glucose consumption to lactic acid production was almost the same under anaerobic and aerobic conditions, it was assumed that lactic acid combustion represented only a minor source of error.

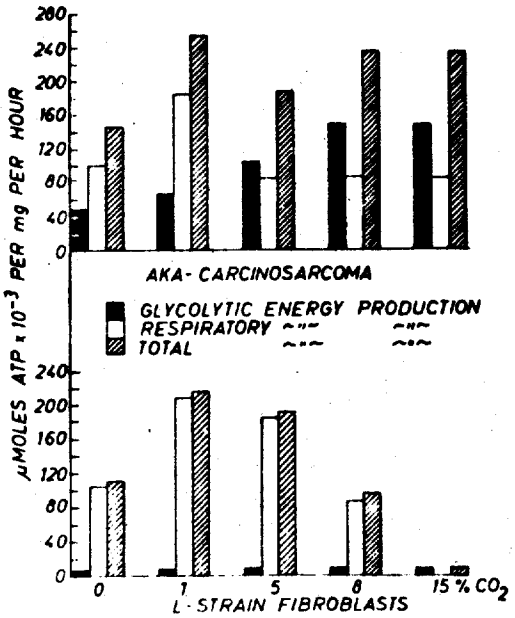

FIGURE 13. The influence of carbon dioxide on energy production in L strain mouse fibroblasts and Aka carcinosarcoma.

The results of these calculations are shown in Figure 13. It is seen that energy production in the fibroblasts is predominantly respiratory at all carbon dioxide tensions. A maximum occurs at 1 per cent carbon dioxide. At 5 per cent carbon dioxide energy production is still close to

maximum, but above 5 per cent a rapid decrease is seen. Thus, these cells seem to possess a feedback mechanism by which total energy production is regulated by the end product of their respiratory metabolism.

In the Aka carcinosarcoma carbon dioxide has a similar effect on respiration, although the inhibition is more pronounced at 5 per cent carbon dioxide and less pronounced at higher carbon dioxide tensions. However, the effect on total energy production is completely different due to the simultaneous stimulation of glycolysis. At low carbon dioxide tensions the metabolism of these cells is predominantly respiratory, although glycolysis plays a much greater role than in the fibroblasts. Maximal energy production occurs at 1 per cent carbon dioxide as in the fibroblasts. At 5 per cent carbon dioxide a depression of respiration is seen and glycolysis becomes the most important source of energy. The gain in glycolytic energy production does not completely compensate for the loss of respiratory energy, but at higher carbon dioxide tensions total energy production approaches the maximum due to the increased rate of glycolysis. Thus, at physiological concentrations carbon dioxide has no inhibiting, but rather a moderate stimulating effect on total energy production in the Aka carcinosarcoma cells.

The present observations and considerations lend support to Loomis's theory. Through its effect on respiration carbon dioxide may act as a physiological regulator of energy metabolism and cell multiplication. The high glycolytic capacity of tumor cells, however, represents a metabolic mechanism that makes cancer cells less susceptible to carbon dioxide inhibition than most normal cells. If the glycolytic capacity is sufficiently high, carbon dioxide may even have a stimulatory effect on total energy production. Under these circumstances stromal cells may stimulate the growth of tumor cells by their production of carbon dioxide and, in addition, of a yet undetermined factor which enables tumor cells to utilize carbon dioxide in support of their glycolytic metabolism.

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USE OF CARBON DIOXIDE AND LIQUID NITROGEN FOR PRESERVING MEATS[1]

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Future development of centralized packaging for frozen or fresh meats may emphasize problems associated with keeping quality and possible health hazards of meat cuts and cured meat items. At this Conference in 1968, Naumann emphasized that current knowledge should be better utilized for centralized packaging. Further advantage should be taken of existing methods for preservation; among these the use of carbon dioxide and vacuum packaging has been known for many years. The work of Moran et al. (1932), Callow (1932) and Haines (1933) lead to additional investigations on the use of CO_2 for prolonging shelf-life of red meats, chicken, and cured meats (Ogilvy and Ayres, 1951a,b,1953). Carbon dioxide was shown by Callow (1932) to result in improved color retention of bacon as compared with an atmosphere of air. Since Jensen (1945) reviewed vacuum packaging of cured meats, many workers have reported that removal of oxygen aided in preserving color and flavor (Urbain and Ramsbottom, 1948; Ulrich, 1949; Kraybill, 1951; Tonge et al., 1964). The effect of vacuum packaging on growth of microorganisms is not as well defined. Ingram (1962) suggested that factors such as permeability of packaging materials to gases, type of meat, and numbers and kinds of organisms all played a role in keeping quality.

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The role of CO_2 and vacuum packaging on color retention and bacterial counts during refrigerated storage of bacon is described in the first phase of the present work. Although discussion of centralized packaging is concerned with red meats, information concerning prolonged keeping qulaity of cured meats should also be exploited because they are packaged in processing plants. Increased interest in the use of cryogenic processing of meats for air transport stimulated the second part of our investigations; the initial results of the use of liquid nitrogen are also reported herein.

Vacuum and Carbon Dioxide Packaging of Bacon

Bacon obtained from a packing plant in Iowa was packaged in Curpolene (Curwood, Inc., New London, Conn.), a laminate of polypropylene, polyethylene, saran-coated cellophane and another layer of polyethylene. The film had very low oxygen and water vapor permeability. Bacon was stored in the dark for 48 hr. after vacuum packaging or packaging in an atmosphere of CO_2 to simulate marketing conditions prior to display in a refrigerated case. During this period, CO_2 was absorbed by the meat to form a vacuum-type package. Differences in color retention, as compared with color photos made from freshly packaged bacon, are given in Table 1.

Table 1. Color Evaluations of Freshly Packaged Bacon[a]

	Days stored at 5°C							
Packaging treatments	0	3	5	7	10	14	21	Total
Without 48 hr. dark storage								
Vacuum	1	1	1	1	1	1	1	7
CO_2	2	2	2	3	2	3	3	17
0.5% residual O_2	3	3	3	2	3	2	2	18
3.5% residual O_2	4	4	4	4	3	4	4	27
Air	5	5	5	5	4	4	5	33
With 48 hr. dark storage	0	3	7	12	21	30		Total
CO_2	1	1	1	1	1	1		6
Vacuum	1	1	1	1	1	2		7
Air	1	2	2	2	2	3		12

[a] Lowest score = most desirable product: evaluated by a panel of 4 judges.

With dark storage for 48 hr. both vacuum and CO_2 packaging preserved the desirable red cured meat color, whereas bacon packaged in air became faded within 48 hr. in the dark. Small amounts of residual oxygen in packages produced rapid discoloration of bacon displayed under lights immediately after packaging. Color retention was very good for bacon subjected to either vacuum or CO_2 packaging with storage as long as one month at 5°C under soft white fluorescent light. CO_2 had an effect similar to that of vacuum packaging on color retention, presumably resulting from exclusion of oxygen in the packages. After 130 days, no outward indications of spoilage were evident, although some fading of CO_2-packaged bacon occurred. Some condensation was observed on the "cold" side of the package, indicative of possible air transfer into the package.

Median counts of aerobic bacteria from seven replicate samples are shown in Figure 1. Probably one of the most striking features was the prolonged lag phase induced by treatment with CO_2. Total aerobes on vacuum-packaged bacon had a lag period of about one week; this time was approximately doubled when CO_2-packaging was used. Numbers of aerobes never became as great with CO_2 treatment as with vacuum packaging or packaging in an atmosphere of air. CO_2 was more effective than vacuum packaging in control of aerobic bacteria. Similarly, lactobacilli did not attain as high populations in bacon packaged in CO_2 as in vacuum-packaged samples or air controls.

No Clostridium perfringens were recovered from bacon during storage for 30 days at 5°C, regardless of treatment. Other investigators have reported similar results (Ulrich, 1949; Cavett, 1962; Ingram, 1962). Bacon inoculated with C. perfringens showed no growth of the organism after 18 days at 5°C (Table 2). However, C. perfringens persisted on inoculated bacon during the storage period. Bacon did not provide a good medium for growth of C. perfringens; whether this was due to the fat content and unsaturated fatty acids or other factors such as curing salts was not determined. Wynne and Foster (1948) noted that C. perfringens spores did not germinate in the presence of CO_2. Foster and Wynne (1948) also stated that the unsaturated C_{18} fatty acids, particularly oleic, inhibited many types of bacteria and retarded germination of Clostridium spores.

Since packaged bacon may not be consumed in one sitting in the home, the effects of various packaging treatments should be known when packages are opened and then closed and stored again in the refrigerator. Sealed packages were stored, opened at intervals up to 1 month, and sampled after being left loosely closed for 3 days in the refrigerator.

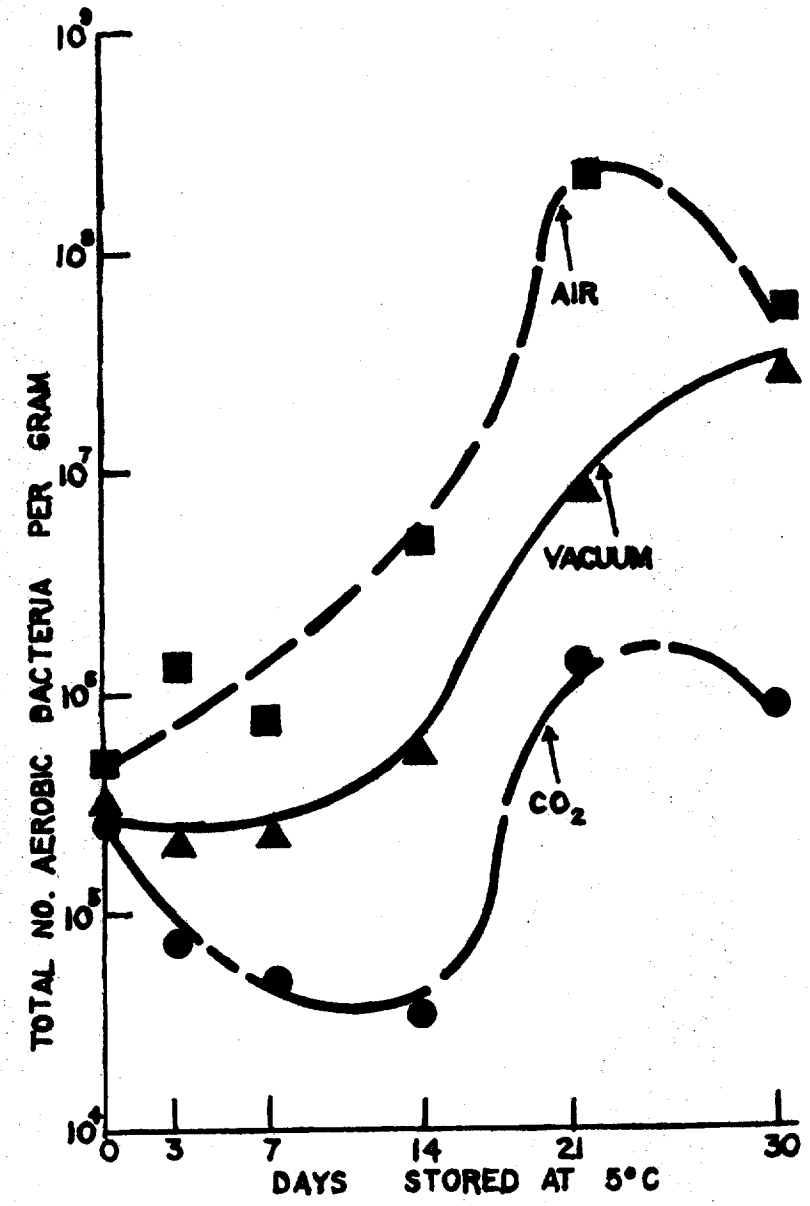

Figure 1. Aerobic bacteria recovered from bacon packaged under vacuum, in CO_2, or in air.

Table 2. Clostridium perfringens Recovered From Inoculated Bacon[a]

Packaging treatment	Days stored at 5°C					
	0	3	5	7	13	18
Uninoculated controls						
Vacuum packaged	$<10^2$	$<10^1$	$<10^1$	-	-	-
Air packaged	$<10^2$	$<10^1$	$<10^1$	-	-	-
Inoculated						
Air packaged[b]	41	19	6.2	15	2.9	0.3
Vacuum packaged[b]	20	16	4.8	6.2	1.5	5.4

[a] Inoculum = 3.3×10^7 cells per ml.

[b] No. of cells x 10^4 per gram.

Bacterial counts indicated that CO_2 inhibited growth of microorganisms, whereas bacon in vacuum and air packages showed more pronounced increases in bacterial numbers. Meat treated with CO_2, even after packages were opened, still possessed enough absorbed gas so that the CO_2 exerted a residual effect on microorganisms. Ogilvy and Ayres (1951 a, b) also noted an extension of keeping time of CO_2-treated chicken and frankfurters after the products were later exposed to air.

This information and results obtained in earlier investigations point out the beneficial effects of use of CO_2 for extending keeping time of bacon and other cured meats. The application of these findings to commercial production remains to be realized. Nevertheless, the technology of such packaging methods is sufficiently well developed that the procedures appear feasible.

Liquid Nitrogen Freezing of Beef

It is well established that for the transportation of fresh meat, time and temperature must be controlled to reduce the health hazard and to keep meat quality at a high level. Advances in cryogenic technology have resulted in improved quality of defrosted meat by increasing the rate of freezing and consequently decreasing shrinkage, tissue damage, drip loss and color loss (Meryman, 1956). General principles of cryogenic freezing and their relation to food quality changes were reviewed at this Conference by Fennema in 1968.

The inherent low temperature of cryogenic systems, coupled with rapid air transportation could make feasible the delivery of meat in well-insulated containers to distant points without further refrigeration during transport. With the arrival of the jumbo jet, increasing amounts of perishable, high value foods may be moved by air (Walrath and Konicek, 1967). At least one airline is currently carrying fresh meats to Europe, and this business appears to be growing. Centralized packaging near producing centers, combined with air shipment offers interesting possibilities for the meat industry. Current systems make use of vacuum packaging in Cryovac, rapid freezing and holding in insulated boxes until arrival. In view of the interest of Iowa meat packers in this operation, and the lack of information on the microbiology of meat handled in this manner, a study was initiated on the keeping quality of beef cuts frozen with liquid nitrogen and stored to stimulate conditions of air transport and later refrigerated holding. In practice, wholesale cuts may be shell-frozen for shipment without further refrigeration. However, this initial investigation was concerned with retail beef steaks with the idea that there may be possible application of these findings to centralized cutting and packaging.

Three methods of low temperature preservation were used for the steaks: (1) air freezing, in which steaks were frozen until their internal temperature reached -29°C in a cold room having mechanical air circulation and maintained at -37°C (±3°C); (2) cryogenic freezing, by immersing packages in liquid nitrogen and obtaining an internal meat temperature of -188°C (±2°C); and (3) refrigeration, by storing packages in a display case at 5°C (±2°C) as unfrozen controls for comparison of the progress of spoilage. All samples were vacuum packaged in a frozen food type of polyethylene film(0.0025 in.).

Frozen samples were stored in pre-cooled styrofoam boxes in the display case at 5°C or in an incubator at 30°C, and examined periodically. These temperatures were selected to represent favorable and unfavorable conditions, respectively, during transport or holding.

Rate of freezing, total freezing time and internal temperature of the meat were summarized (Table 3 and Figure 2). The use of liquid nitrogen increased the freezing rate to reduce the total freezing time by approximately 96% or 30 times, and to a much lower internal temperature. Time and temperature relationships during freezing (Figure 2) and post-freezing storage (Figure 3) were compared. The time for

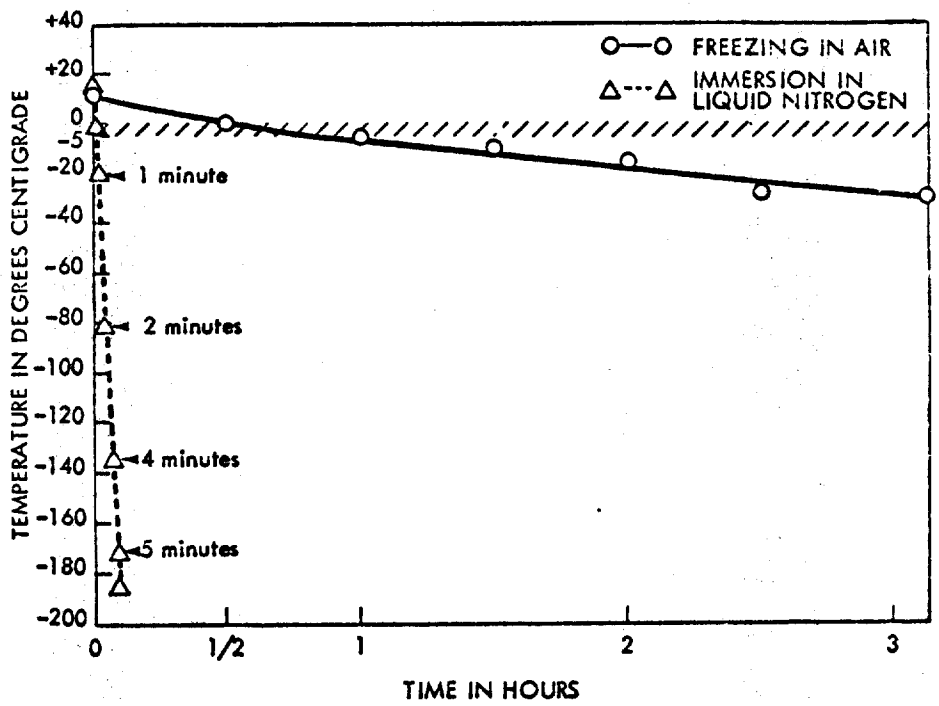

Figure 2. Decrease in internal temperature of round steaks during freezing.

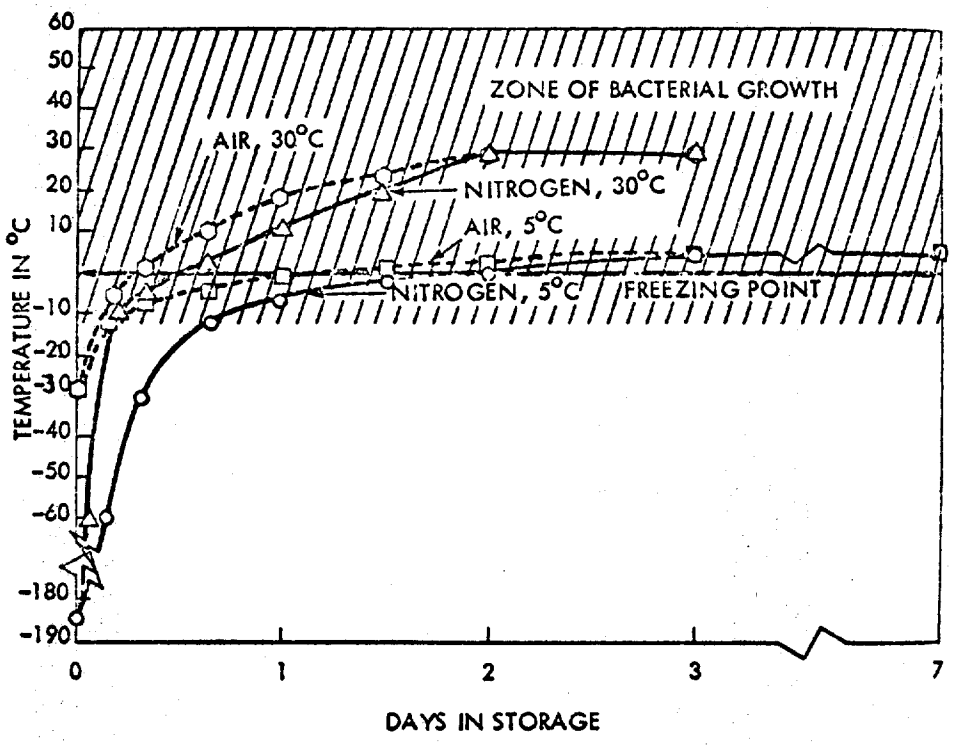

Figure 3. Increase in temperature of steaks during storage.

Table 3. Comparison of Time-Temperature Measurements of Round Steaks Frozen with Liquid Nitrogen or Conventional Air-Freezing Methods

Method of freezing	Rate of freezing	Total freezing time	Final temperature of thermal center
Air	550 mμ/sec	180 min	-29°C
Liquid nitrogen	23,000 mμ/sec	6 min	-190°C

equilibration of the temperature of the meat was influenced by the storage temperature but not by the initial temperature at the time the meat was placed into storage. Although thawing rates were different during the early phases of storage, steaks frozen in liquid nitrogen (initial temperature of -190°C) or in air (initial temperature of -29°C) reached the temperature of storage at the same time (2 days at 30°C and 3 days at 5°C). This situation is analagous to that of heat transfer in canning, in which the initial temperature of the food in the can has little influence on come-up time because the rate of heating is affected by the temperature gradient. During thawing and later storage, the average temperature of the meat depended on its initial temperature, and was higher for steaks frozen in air than in liquid nitrogen. Air-frozen steaks were in the temperature zone favorable for bacterial growth for a longer time than were steaks frozen in liquid nitrogen. As may be expected, storage at 30°C also provided higher average temperatures than did the lower holding temperature.

An analysis of variance (Table 4) showed a significant effect of temperature of storage on numbers of total aerobes, fluorescent Pseudomonas and enterococci developing on pre-frozen steaks. With storage of meat at 30°C, liquid nitrogen was not as effective for preservation as it was when the frozen meat was kept at 5°C. Salmonellae and coagulase positive staphylococci were frequently recovered, and the analysis of variance indicated that method of freezing had no significant effect on the recovery of these two types of organisms. However, incidence of Salmonellae was greater on meat frozen by air blast than by liquid nitrogen during later storage at either 5° or 30°C.

Table 4. Analysis of Variance of Bacteria Recovered from Prefrozen Steaks

Source of variation	d.f.	Mean squares: Total aerobes	Fluorescent Pseudomonas	Enterococci	Salmonella	Coagulase-positive Staphylococcus
Days in storage (A)	5	107.36*	142.67*	49.35*	509.64	4.12
Storage at 5° and 30°C (B)	1	169.41*	176.10*	162.70*	251.27	2.08
A x B	5	50.72*	55.16*	55.45*	76.66	- -
Air vs. liquid nitrogen (C)	1	1.46	2.86*	3.18*	11.61	1.22
A x C	5	1.00	0.78	1.45	- - -	- -
B x C	1	0.87	0.51	0.21	- - -	0.33
Error	164	0.50	0.42	0.47	221.79	1.66

* P = .01

The statistical analysis (Table 5) gave evidence of a significant effect of prefreezing on numbers of bacteria present on the surface of meat that was subsequently held at 5°C. An early logarithmic growth phase occurred with fresh refrigerated meat, so that bacterial numbers reached much higher levels by the third and fourth days of storage than those on packaged beef that had been previously frozen (Figure 4). Conversely, the freezing treatments lengthened the lag phase of organisms on pre-frozen samples.

Table 5. Analysis of Variance of Bacteria Recovered from Fresh and Prefrozen Steaks

Source of variation	d.f.	Mean squares: Total aerobes	Fluorescent Pseudomonas	Enterococci
Days in storage (A)	5	109.52*	150.21*	16.56*
Fresh vs. prefrozen steaks (B)	1	39.91*	24.33*	11.25*
A x B	4	11.31*	5.88*	5.08*
Error	219	1.29	1.21	1.15

* P = .01

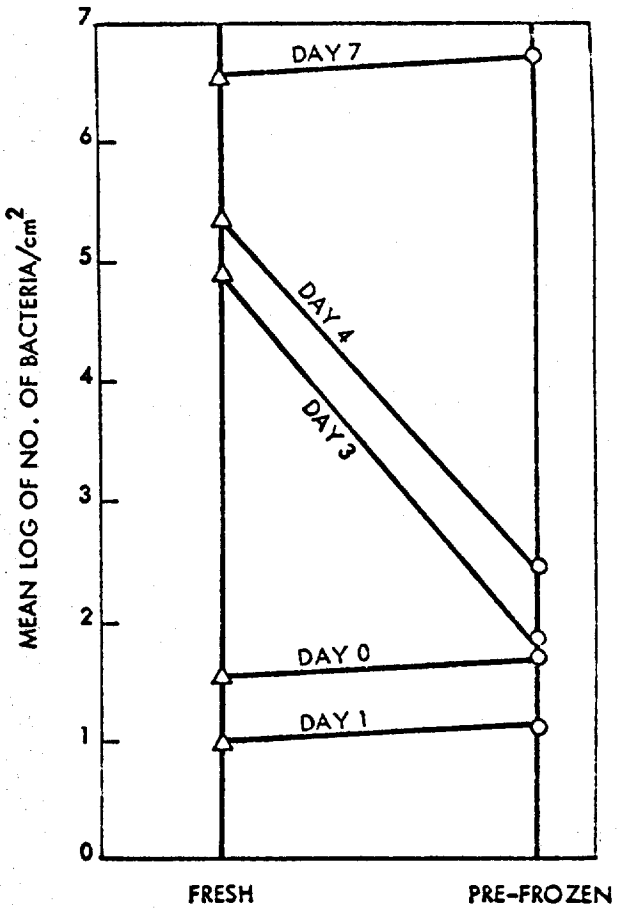

Figure 4. Comparison of numbers of total aerobes on fresh and pre-frozen steaks during storage at 5°C.

The analysis of variance did not show a significant effect of method of freezing on total aerobes developing on pre-frozen steaks. A comparison of these bacterial counts on the steaks during storage is given in Figure 5. Little difference was noted between freezing methods for storage at 30°C. However, bacterial numbers were appreciably greater on meat frozen in air and then stored for 3 or 4 days at 5°C than on liquid nitrogen frozen samples. A sequential T-test (Table 6) indicated that the first significant increase in bacterial populations occurred earlier, between the third and fourth days of storage, on air frozen steaks than on steaks frozen in liquid nitrogen. The difference in bacterial numbers observed on the fourth day of storage, shown in Figure 5, was also significant, as noted in Table 7.

Table 6. Sequential T-test for Significance of the Difference in Levels of Bacterial Populations Developing on Prefrozen Steaks During Storage at 5°C

	Method of freezing	
Difference	Air	Liquid nitrogen
Between day 0 and day 1	0.40	0.44
Between day 1 and day 3	0.62	0.17
Between day 3 and day 4	0.93*	0.51
Between day 4 and day 7	3.58*	4.19*

* P = .05

Table 7. Sequential T-test for Significance of the Difference Between the Two Methods of Freezing on Numbers of Total Aerobes Developing on Steaks During Storage at 5°C

Storage time	Difference between air and liquid nitrogen freezing
Day 0	0.08
Day 1	0.04
Day 3	0.41
Day 4	0.83*
Day 7	0.21

* P = .05

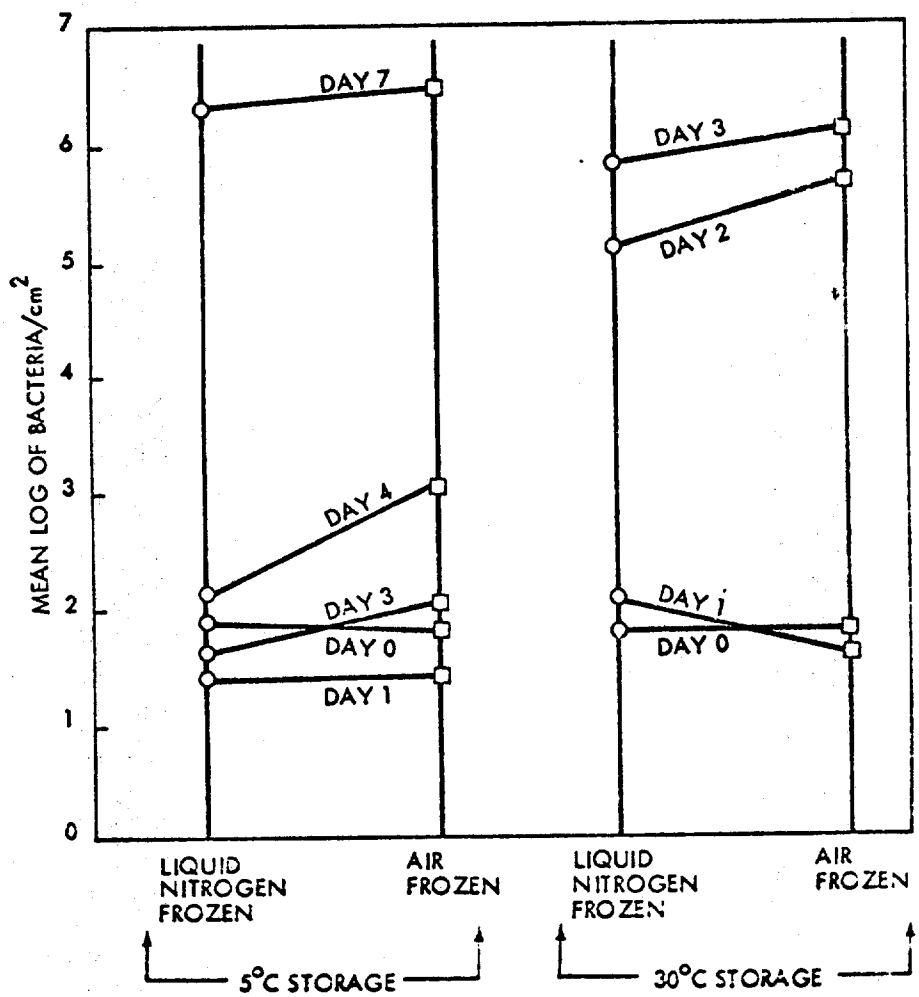

Figure 5. Comparison of effect of freezing methods on numbers of total aerobes on steaks during storage at 5° and 30°C.

Numbers of enterococci followed trends similar to those of total aerobes. However, freezing treatments appeared to allow for greater growth of enterococci than that occurring with unfrozen meat after samples were held for 7 days at 5°C.

Percentage of metmyoglobin was measured in accordance with the procedure described by Snyder (1965) and Zimmerman and Snyder (1969). Metmyoglobin was calculated from reflectance readings on the absorbancy scale (R_A) of the Beckman DK-2A spectrophotometer. Formation of metmyoglobin was slowest on meat frozen with liquid nitrogen (Figure 6). Visual examination of the samples, as well as reflectance measurements, confirmed that the red meat pigment was best preserved for 4 days at 5°C in beef frozen with liquid nitrogen. Freezing in air afforded relatively little color protection as compared with fresh refrigerated meat.

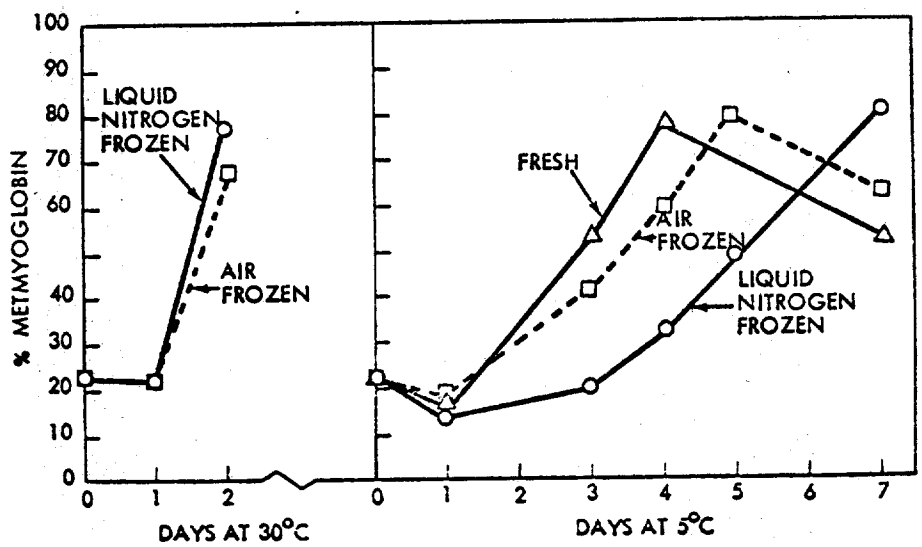

Figure 6. Changes in percent metmyoglobin on fresh and prefrozen steaks during storage at 5° and 30°C.

From this work, the following trends, if not conclusions, may be noted: (1) prefrozen steaks have better keeping quality than fresh steaks under equivalent storage conditions (this is an important consideration for centralized packaging); (2) liquid nitrogen reduced total freezing time of steaks by a factor of 30 compared with freezing in air; (3) with the same environmental temperature and type of container, steaks frozen with liquid nitrogen could be shipped with a greater safety factor against bacterial spoilage and loss of red color than meat frozen in air; (4) steaks frozen with liquid nitrogen and shipped by air in well-insulated containers would not offer greater hazards from Salmonellae and staphylococci than fresh refrigerated meat.

Since this portion of the work was completed, a liquid nitrogen spray freezing tunnel was placed into operation. Beef loins, vacuum packaged in Cryovac, and shell-frozen in liquid nitrogen, are being investigated for aerobic and anaerobic bacteria, color changes, and shrinkage under various holding conditions. With additional testing for effect of temperature fluctuations to simulate air transport and storage, it is believed that more complete information will be gained regarding use of liquid nitrogen for preservation.

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MEASUREMENT OF BLOOD GASES BY GAS CHROMATOGRAPHY[1]

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Abstract. A method using gas chromatography to measure blood gases has been developed. Equipment and procedure are described. Respiratory gases, O_2 and CO_2, and N_2, can be measured, although these analyses should be performed separately. For all three gases the calibration is linear and passes through zero. The maximum deviation from linearity was found to be 0.1 vol % for C_{O_2}, 0.3 vol % for C_{CO_2} and 2.2 mm Hg for P_{N_2}. The average reproducibility tested on many samples was within ±0.06 vol % for O_2, ±0.18 vol % for CO_2 and ±2. mm Hg for P_{N_2}.

Gas chromatography
Measurement of respiratory gases in blood
Measurement of dissolved gases in blood

The major difficulty in measuring blood gases by gas chromatography is obtaining rapid and complete release of the gases from the blood, including those bound to the respiratory pigment.

The methods available to extract gases from blood, or any other liquid, are of three types: (1) the "vacuum extraction" method (RAMSEY, 1959; LUCAS and AYRES, 1961; FARHI, EDWARDS and HOMMA, 1963). In this method the gases are extracted from the blood prior to being introduced into the gas chromatograph, thus fast elution and good resolution of the peaks are easily obtained. It requires, however, in addition to the gas chromatograph, a Van Slyke apparatus, which is itself a source of problems. Also a fraction of the gases always is retained in the liquid remaining in the Van Slyke extraction chamber. (2) The "washout" or "gas flow-through" method (WILSON *et al.*, 1961; GALLA and OTTENSTEIN, 1962; WILSON, 1964). In this method the sample is injected directly into the carrier gas stream. The procedure is very simple and all gases eventually will be released from the blood. However, resolution is poor and elution is slow. Since the peaks cannot be measured accurately in terms of peak height, it is necessary to determine peak surface area by integration or approximation. (3) The "diffusion process" or "equilibration" method (HAMILTON, 1962). This method combines the advantages of the two preceding techniques.

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A version of this latter method is described here and its accuracy is appraised.

Method

PRINCIPLE

A blood sample, with gas-free reagents added, is stirred in an extraction chamber isolated from the carrier gas line. If this chamber contains an atmosphere of He, or any other carrier gas different from those to be extracted, a vacuum exists relative to the gases contained in the blood sample. The gas molecules then diffuse from the blood into the surrounding gas phase until equilibrium is reached. At this point the carrier gas is allowed to flow through the extraction chamber, to the chromatograph columns and detector, transporting with it both the extracted gases and all gases which are still retained in the blood at the end of the equilibration period.

This method offers the following advantages: all gases are extracted with certainty, even those with a high partition coefficient, and sharp narrow peaks are produced making possible simple quantitative measurements in terms of peak height.

EQUIPMENT

A Beckman gas chromatograph Model 2A and a Bristol 1 mV full scale recorder are used. The chromatograph is equipped with two parallel columns, one packed with silica gel and the other with molecular sieve 5A. The length of the columns is adjusted to provide good resolution and fast elution. When only N_2 is measured, it is preferable

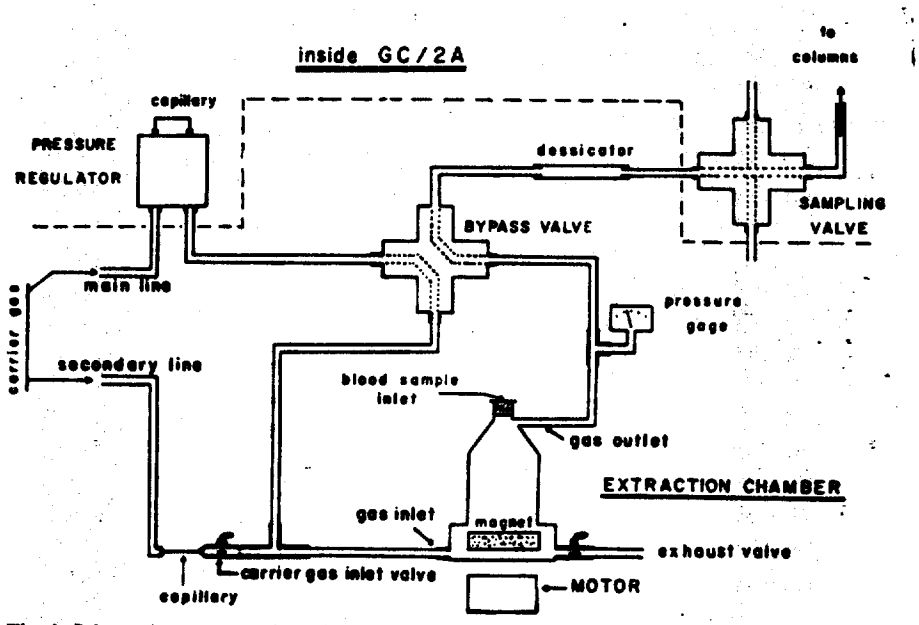

Fig. 1. Schematic representation of the blood gas extraction chamber and accessory apparatus.

to eliminate the silica gel column. Either column can be reactivated by baking it overnight at 210 °C.

The blood extraction chamber and accessory apparatus (fig. 1), built in our laboratory, consist of the following components:

(a) The extraction chamber is made up of two parts. A glass cover (Fisher #6.390-10) is hermetically sealed on a brass reservoir. The cover has a sample inlet and a gas outlet. The reservoir has a carrier gas inlet and an exhaust for discarding the analyzed blood. The extraction chamber contains a magnetic stirrer activated by a small phonograph motor mounted beneath it.

(b) There are 3 valves in the accessory apparatus: the isolating or bypass valve, the carrier gas inlet valve, and the exhaust valve. The isolating or bypass valve (Republic valves 330 series or Beckman #23800) is a four way valve designed to isolate the extraction chamber from the main carrier gas line. The carrier gas inlet valve (toggle valve—Hoke 490 series) is opened only to free the reagents from their dissolved gases and to permit the pressure in the extraction chamber to equilibrate with that of the main carrier gas line after the sample has been introduced. The flow of carrier gas through this valve is regulated by a capillary tube interposed just ahead of the valve. The exhaust valve (toggle valve—Hoke 490 series) is opened to flush out the analyzed blood and to wash out the chamber.

(c) The pressure gauge, graduated in ½ pounds up to 30 pounds is used to measure the pressure in the carrier gas line and in the extraction chamber, and to monitor the system for leaks.

(d) The dessicator is filled with silica gel and is used only when N_2 is measured. It is essential that the dessicator be inserted beyond the bypass valve.

(e) These various components are connected with Swagelok fittings of brass or nylon when they are in contact with glass. Nylaflow tubing (¼" and ⅛" o.d.) is used to convey the carrier gas from the gas cylinder to the various parts.

(f) A "gas tight Hamilton syringe" with a Chaney adaptor, and a ¾ inch, 24 gauge needle are used to introduce the blood or gas samples into the extraction chamber. Our measurements were made with 0.5 and 1.0 ml syringes.

REAGENTS

The reagents used differ according to the kinds of gas to be measured. To analyze for N_2 the blood is stirred with twice its own volume of a previously prepared reagent containing the following ingredients: 1 part powder, containing sodium hydrosulfite (10 g) and sodium anthraquinone betasulfonate (1 g), and 5 parts 1 N potassium hydroxide solution. To analyze for O_2 and CO_2, the blood is stirred with twice its own volume of each of the following solutions:

a	Potassium ferricyanide	20 g
	Saponin	2 g
	Water	100 ml

b	Sodium sulfate	30 g
	Sulfuric acid	7 ml
	Water	100 ml

A drop of octanol is added to all reagents.

PREPARATION FOR ANALYSIS

Several successive steps are identical both for calibrating with standard samples and for analyzing unknown samples.

(a) Since the gas chromatograph is never turned off, the equipment is made ready simply by turning on the recorder, the stirrer and the detector filament. The carrier gas flow, which is kept low when the instrument is not in use, is increased to 30 lbs equivalent to a flow of approximately 50 ml/min. Stabilization is obtained within half an hour. For our measurements, the following settings were used: temperature 70 °C, current 400 mA. The isolating valve is now opened (flow-through position) and the carrier gas inlet and exhaust valves closed. In our experiments the pressure in the extraction chamber read 17 lbs. In order to simplify the description of the nextsteps, this figure of 17 lbs will be used, although it must be borne in mind that this pressure depends upon the arrangement of the equipment. At this time, the isolating valve is closed again (bypass position), and the carrier gas inlet valve and the extraction chamber sample inlet are opened.

(b) The measured reagents and octanol are introduced into the extraction chamber through the sample inlet which is then sealed with a rubber cap. A ¾" 24 gauge needle is stuck into the chamber through the rubber cap. The carrier gas, entering the chamber at the bottom, bubbles through the reagents and exits through the needle until the reagents are gas free (about 2 min).

(c) The needle is rapidly removed and the carrier gas inlet valve closed. These two movements must be done quickly so that the pressure in the extraction chamber does not rise more than 1 pound.

PREPARATION OF BLOOD SAMPLES

An anaerobic blood sample is drawn directly from the blood vessel, or from a tonometer, into a Luer-Lok syringe. In the first instance the syringe dead space is filled with heparin. After the sample has been collected, the tip of the syringe is submerged in mercury, a drop of which is drawn into the syringe. The opening is then covered with a cap tightly fitting the lock (B-D #411 AC) in such a manner that no bubble is forced into the syringe. The syringe is stored at room temperature for N_2 analysis and in ice for O_2 and CO_2 analysis.

At the time of analysis, the blood is transferred into the Hamilton syringe by thrusting its needle through the cap into the Luer-Lok syringe. Then the two syringes are firmly held together and vigorously shaken to mix the blood which is then pushed into the Hamilton syringe. The same sample volume and same needle must be used for each series of measurements, including calibration. For each analysis the Hamilton

syringe is rinsed and freed of bubbles with a small amount of the blood which is then discarded. When the sample is taken, a small excess volume is injected into the Hamilton syringe.

Before introducing the blood sample into the extraction chamber, a drop of the excess blood is placed on top of the rubber cap covering the sample inlet to prevent microscopic bubbles from being trapped in the needle tip. If replicate analyses of the same sample are desired, the Hamilton syringe is immediately reinserted in the Luer-Lok syringe; otherwise it is washed with lukewarm saline.

ANALYSIS

(a) The blood sample prepared in the Hamilton syringe is introduced into the extraction chamber through the rubber cap. A timer is set for a time predetermined to assure equilibration of the extracted gases. The carrier gas inlet valve is opened to reinstate the 17 lbs pressure in the extraction chamber.

(b) At the end of this time, the bypass valve is opened to let the carrier gas sweep the extracted gases to the columns where they will be separated. When the first peak appears on the recorder, the bypass valve is closed.

(c) The carrier gas inlet and the exhaust valves are opened, the rubber cap is removed and the extraction chamber is cleaned several times with tap water which is flushed out through the exhaust by simply placing a finger tip on the sample inlet. The exhaust valve is then closed, and more reagents are introduced for the next sample. Each analysis requires 5½ to 6 min.

CALIBRATION OF THE INSTRUMENT

Standard samples, different for each gas, are injected into the gas chromatograph and the response is measured in terms of peak height.

(a) For measuring oxygen, the instrument is calibrated with gas mixtures. Since the relationship between F_{O_2} and peak height is linear, only two mixtures are necessary. One is room air which contains 20.93% oxygen, and the other is a mixture of 10% O_2 and atmospheric nitrogen (argon 1.185% and nitrogen 98.815%. Handbook of Chemistry and Physics). For both gases it is important to moisten the Hamilton syringe in order to saturate the sample with water vapor. The syringe must also be rinsed many times with the standardizing sample before collecting the final sample.

Two corrections must be made to arrive at a true calibration. First the volume percentage of O_2 must be expressed in STPD conditions, thus the O_2 concentration of the standard sample must be multiplied by the following factor:

$$(1) \qquad F_1 = \frac{P - P_{H_2O}}{760} \times \frac{273}{273 + t}$$

in which P = the ambient barometric pressure, P_{H_2O} = the pressure of water vapor at the ambient temperature, and t = the ambient temperature in °C.

Second, the contribution of argon to the peak common to both O_2 and A must be subtracted. If the response, D, of the instrument is directly proportional to the volume of gas analyzed V_G:

$$D = \text{Sensitivity} \times V_G$$

or, since the volume of gas, V_G, equals the volume of the sample, V_s, times the fraction of gas,

$$D = \text{Sensitivity} \times V_s \times F .$$

Thus, if O_2 is completely absorbed in a sample of room air after it has been injected into the extraction chamber, the first peak, D_A, is proportional only to the volume of argon in room air. Since the product (Sensitivity $\times$ V_s) is the same in all samples, D_A depends only on F_A which varies as F_{N_2} in any gas mixture containing atmospheric nitrogen. Then

$$F_A/F_{N_2} = D_A/D_{N_2} = \text{constant} .$$

Once this ratio has been measured (it remains the same during successive days), the contribution of argon to the O_2 and A peak is given by:

$$F_2 = \frac{D_A \text{ room air}}{D_{N_2} \text{ room air}} \times D_{N_2} \text{ any gas mixture}$$

and true $D_{O_2} = D_{O_2+A} - F_2$.

It is the plot of the corrected concentrations versus the corrected deflections which passes through zero and is used to calculate the O_2 concentration in the blood samples. Since argon, as nitrogen, is only dissolved in blood, the argon correction need not be applied to the blood O_2 peaks.

(b) To calibrate the instrument for CO_2 bicarbonate solutions in distilled water are used. As for O_2, the relationship between peak height and CO_2 concentration is linear and passes through zero. The range of CO_2 concentrations used for calibration depends on the expected values in the unknown samples. Simple calculations show that 0.378 g of dry, purified, bicarbonate must be dissolved in 1 liter of distilled water to make up a solution releasing 10 vol % of CO_2. Any volume of CO_2 determined by this method of calibration is expressed in vol % STPD.

(c) To calibrate for nitrogen equilibrated blood samples are used. The details pertaining to good equilibration have been described by FARHI *et al.* (1963). The only difference in our procedure is the introduction of a drop of mercury into the Luer-Lok syringe containing the equilibrated blood. As in Farhi's experiments, the dead space of this syringe is not filled with heparin, but the syringe is filled and rinsed many times before the sample is collected. Two kinds of tonometers have been used with equal performance (FINLEY *et al.*, 1960; FARHI, 1965), but that described by Farhi greatly simplifies the procedure.

Results and discussion

The results presented in this paper constitute an evaluation of the method. Two

criteria were used: linearity of the gas chromatograph response to increasing concentration of a gas in a constant volume and reproducibility of a single analysis.

RESPIRATORY GASES (O_2 AND CO_2)

The system's linearity can be demonstrated by using one known gas sample as a standard to calculate the gas concentration in other known samples. Any deviation from linearity is shown by the difference between the calculated and known values.

Typical results are shown in table 1 for O_2 and CO_2. The values given for O_2 are those obtained after correction for STPD conditions and for argon. If the samples with the lowest concentration of O_2 and CO_2 are used as standard, the differences between the actual and measured concentration are higher when expressed in vol % STPD but they would be the same when expressed in percentage of the actual concentrations. The values of 1.1% for O_2 and 1.0% for CO_2 were the highest ever measured in our experience. The mean difference in percentage of actual concentration for a series of 25 calibration curves was 0.54% for O_2 (1.10 to 0.12) and 0.60% for CO_2 (1.00–0.03). These results indicate an extremely small deviation from perfect linearity.

The reproducibility of O_2 and CO_2 measurements in a given sample was assessed by determining the coefficient of variation (C.V. = standard deviation divided by the mean) in numerous gas, bicarbonates solutions and blood samples, all of which were analyzed from 3 to 6 times. As shown in table 2, the deviation from the mean is consistently less than 1% of the absolute value. The mean coefficient of variation

TABLE 1

Evaluation of linearity, with one sample used as a standard[1]

Standard vol % STPD	Actual Concentration vol % STPD	Measured Concentration vol % STPD	Difference vol % STPD	Difference in percentage of actual concentration
Oxygen				
18.79	9.04	9.097	+.057	.63
18.65	8.97	8.990	+.020	.22
18.79	9.04	9.124	+.084	.92
18.84	9.18	9.079	−.101	1.10
23.20	13.15	13.066	−.084	.64
23.20	13.15	13.192	+.042	.32
Carbon Dioxide				
60.00	40.00	39.83	−.17	.42
60.00	40.00	40.27	+.27	.67
60.00	20.00	19.97	−.03	.15
45.00	30.00	29.99	−.01	.03
45.00	30.00	30.21	+.21	.70
45.00	15.00	14.85	−.15	1.00

[1] All values are the mean of a duplicate or triplicate determination. In all cases the volume of sample was the same (0.4 ml).

TABLE 2

Reproducibility of O_2 and CO_2 measurements

Nature of sample	Measurement	Range of concentrations vol %	Number of samples	Volume of sample	Mean coefficient of variation percentage	Range of coefficient of variation percentage
Gas	O_2	23.8–18.5	20	0 4	0.22	0.71–0.09
Gas	O_2	11.7– 8.8	20	0.4	0.31	0.78–0.12
Blood	O_2	22.5–15.7	20	0.4	0.28	0.83–0.08
Blood	O_2	8.0– 0.2	20	0.2–0.4	0.34	0.92–0.07
Bicarbonates	CO_2	60.0–15.0	40	0.4	0.27	0.67–0.11
Blood	CO_2	70.0–10.0	40	0.2–0.4	0.35	0.87–0.16

indicates a good reproducibility. The less satisfactory results were obtained with the smaller blood samples. These were samples of blood from fishes and amphibians which have large red cells that precipitate rapidly.

INERT GAS (N_2)

The linearity of the nitrogen calibration was assessed in the same manner as for O_2. The difference between the known and the estimated nitrogen partial pressure was always within ± 2.2 mm Hg in blood samples equilibrated with a gas mixture containing from 8.2 to 91.73% nitrogen. In all cases air was used as the standard.

The reproducibility in a single sample was established by repeating the analysis from 3 to 8 times for 120 samples equilibrated with standard gas mixtures. Fig. 2 shows the coefficient of variation (C.V.) for these samples as a function of the mean deflection. It can be seen that C.V. is inversely related to the mean indicating that the error must be larger in small blood samples and/or in samples equilibrated with a low P_{N_2}. The relationship between P_{N_2} and C.V. is demonstrated in table 3 in which our results have been grouped according to a relatively narrow range of F_{N_2} in the standard gas. Although the coefficient of variation increases, the absolute error in terms of mm Hg remains the same over the complete range of P_{N_2}. No significant relationship was established between the coefficient of variation and blood samples of various sizes (1 ml to 0.6 ml) equilibrated with the same gas mixture. Since small blood samples are more difficult to handle, we believe it important to use as large a sample as possible; the lower the expected P_{N_2} the more mandatory this rule becomes.

Absorption of the respiratory gases is absolutely necessary since by this method the extracted gases are diluted in a large volume of carrier gas. Failure to absorb the respiratory gases leads to overlapping peaks whose shape may be distorted (FARHI *et al.*, 1963).

Some details of calibration and analysis were found to be extremely critical in obtaining satisfactory results for O_2, CO_2 and N_2 measurements.

(a) All gas mixtures used for the O_2 calibration, especially the compressed gas mixtures, must really be at ATPS.

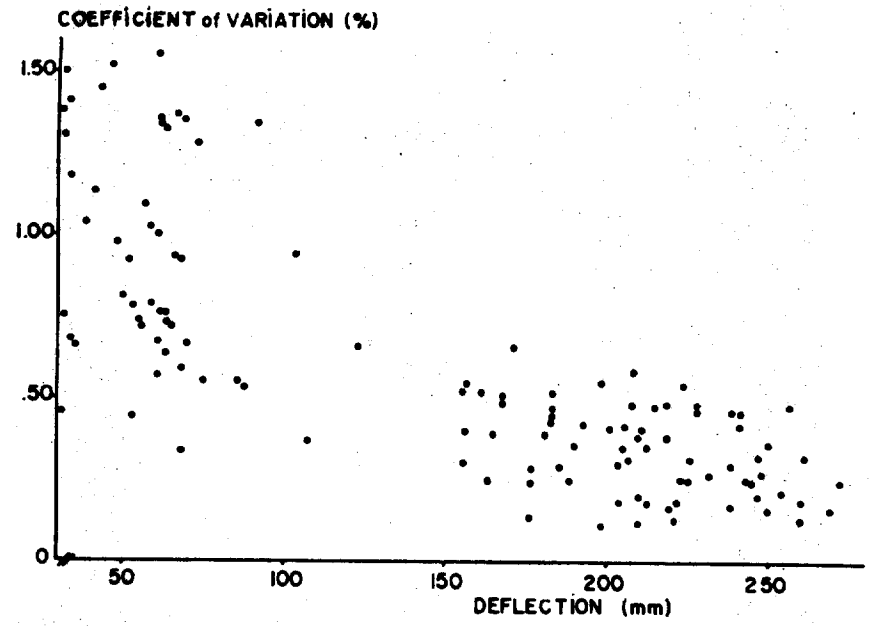

Fig. 2. Coefficient of variation as a function of the mean deflection of 3 to 8 replicates. The deflection depends on one or a combination of the following factors; P_{N_2} of the sample, size of sample, age and condition of the column and setting of the gas chromatograph (flow of carrier gas, temperature, current through the detector filament and attenuation).

TABLE 3

Reproducibility of P_{N_2} measurements

Fraction N_2 in standard gas	0.93–0.90	0.82–0.75	0.60–0.50	0.401	0.26–0.20	0.16–0.08
Number of samples	9	56	5	4	20	26
Range of coefficient of variation %	0.45–0.16	0.59–0.11	0.66–0.40	1.34–0.37	1.55–0.54	1.52–0.33
Mean C.V. %	0.32	0.33	0.54	0.73	0.92	0.96
Absolute error[1] mm Hg	2.02	1.78	2.08	2.00	1.59	0.9

[1] Calculated for the mean F_{N_2} in each group, and in the case of $P - P_{H_2O} = 700$ mm Hg.

(b) When filled with standard gases the Hamilton syringe barrel must not be held too long in the hands or volume may vary due to heat transfer.

(c) Precise weighing and dilution of bicarbonates are very important.

(d) The temperature of equilibration as compared to that of the unknown samples is essential to calibrate the instrument for N_2 measurements (FARHI *et al.*, 1963).

(e) Pressure in the extraction chamber must be low when the samples are injected, and must remain the same for all the gas samples, otherwise unequal volumes of gas are injected.

(f) Pressure in the extraction chamber during the bypass must be the same for all samples, gaseous or liquid, since the peak shape depends on the volume of carrier gas in which the sample is diluted.

(g) Duration of bypass must be the same for all samples and must not be shorter than a predetermined value (3 min in our experiments).

(h) Contamination of the gas samples and microscopic bubbles in the blood samples are by far the most important causes of error and the easiest to make.

(i) The blood samples must be well mixed in the Luer-Lok syringe before the Hamilton syringe is filled which, in turn, should be emptied without delay into the extraction chamber. For O_2 and CO_2 measurements the reason for this is evident, for measurement of N_2 it is necessary because of the difference in N_2 coefficient of solubility between red cells and plasma (VAN SLYKE, DILLON and MARGARIA, 1934; FARHI *et al.*, 1963).

(j) Leakage through the rubber cap on the sample inlet was observed following repeated puncture. It is advisable to change the cap after every other analysis.

In conclusion, this method provides an easy means of measuring O_2 and CO_2 concentration and the nitrogen tension in small blood samples. The procedure can be mastered with reasonable training and does not exceed in difficulty or time required that demanded for any other technique. Calibration of the instrument is simple; it reveals in samples of same volume a linear relationship between gas concentration and the peak height. The calibration line passes through zero for all gases. The reproducibility of a single analysis in the same sample is very satisfactory and is not affected by the concentration of gas in the sample.

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pCO_2 Inhibition of Normal and Malignant Growth [1,2]

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Summary

Evidence is presented for the theory that normal adult cells limit their own growth by producing inhibitory levels of free CO_2 consequent to their own respiration. It is suggested that embryonic and malignant cells achieve the ability to grow continuously by using an adenosine triphosphate (ATP) generating system—glycolysis—that does not result in the production of CO_2. Embryonic cells lose this auxiliary system as they mature into adult cells, but cancer cells develop glycolytic potential as they become malignant. Evidence from several sources suggests that free CO_2 is a physiologically produced, nontoxic, reversible inhibitor of cell growth. Experiments by Root suggest that this effect may be achieved by limiting the supply of ATP consequent to the self-inhibition of respiration by its end product, CO_2. If normal respiring cells are prevented from growing by a deficiency in their supply of metabolic energy, then embryonic and cancer cells may escape this physiological control by producing additional amounts of ATP through glycolytic mechanisms. To paraphrase Armin Braun's description of the crown-gall tumor cell, this suggests that, as a result of the transition from a normal cell into a fully malignant cancer cell, a specific growth-substance synthesizing system—glycolysis—becomes progressively activated. This leads to the production by the cancer cell of greater than regulatory amounts of this growth factor—ATP. The continued production of this substance by the cancer cell could account for the unregulated proliferation of such a cell.—J. Nat. Cancer Inst. 22: 207-217, 1959.

Homeostasis has been defined by Walter Cannon as the tendency to stability found in such normal body states as blood sugar, temperature, and alveolar CO_2 (*1*). One aspect of this over-all concept may be termed cytostasis, *i.e.*, the tendency to stability of both the total number and different types of cells that constitute an adult organism. Cytostasis, in other words, refers to the self-inhibitory equilibrium existing between metazoan cells, which prevents *net* growth except after injury. Unlike a population of protozoa, metazoan cells do not grow indefinitely but reach a stable condition of cytostasis in the adult organism.

[1] Received for publication September 17, 1958.

[2] One of a series of speculative papers received in response to the open invitation which appeared in previous issues.

Little is known of the forces responsible for cytostasis. What biochemical factors initiate growth after injury and yet control it so that growth ceases once the injury has been repaired? Why is the growth of cancer cells not controlled by the same forces that maintain cytostasis in normal tissues?

One possible mechanism was suggested by Armin Braun *(2)*. He found that plant cells would not grow in a basic medium of mineral salts and sucrose unless 7 specific growth substances were added: glutamine, asparagine, inositol, cytidylic and guanylic acids, 6-furfurylaminopurine, and naphthaleneacetic acid. In contrast, crown-gall tumor cells would grow in the absence of all these growth factors.

This result suggests that differentiated plant tissues have lost the power to synthesize their own growth substances. Their rate of growth is apparently controlled by the regulated amount of these growth substances carried to them from distant parts of the intact plant. As Braun states, "It thus appears that, as a result of the transition from a normal cell to a fully altered, rapidly growing crown-gall tumor cell, a series of quite distinct, but well-defined growth-substance-synthesizing systems becomes progressively activated. This leads to the production by the affected cell of greater than regulatory amounts of these growth-promoting substances. The continued production in greater than regulatory amounts of these substances by the tumor cell could and most probably does account for the continued unregulated proliferation of such a cell." *(2)*.

Is such a mechanism responsible for cytostasis and malignancy in animal tissues? The answer would appear to be negative on first consideration, because feeding excess vitamins, amino acids, or nucleic acids to animals does not alter their adult form. Growth hormones appear to be special rather than general regulators of tissue growth because they control the growth of only certain tissues, and some animals, such as hydra, demonstrate cytostasis even though they contain neither endocrine cells nor a circulatory system. No animal cancer cell, moreover, has ever been found that can manufacture its own growth hormone.

If lack of externally supplied growth substances is not responsible, perhaps cytostasis results from one or more self-produced inhibitors. Meryl Rose has postulated a whole hierarchy of organ-specific inhibitors *(3)*. In a recent review of inhibitor theories *(4)*, Rose suggested that ". . . malignant tumors may fail in self-limitation because excessive cell death prevents them from achieving limiting concentrations."

The present paper presents evidence for a new theory, namely, that cytostasis results from an insufficient supply of phosphate bond energy—adenosine triphosphate (ATP)—consequent to the *in vivo* inhibition of respiration by its end product, CO_2.

pCO_2

Writing over a generation ago, Haywood and Root *(5)* stated, "In two respects carbon dioxide claims a place of peculiar interest for the physiologist. First, as a metabolic product, it is present wherever there are active

cells. Secondly, as many workers have observed, it has the effect of very promptly depressing certain cell activities." Despite this and similar statements in the literature, tissue pCO_2 has been curiously neglected for many years. Perhaps this neglect stems from the fact that ". . . pCO_2 is the most troublesome of all biological factors to measure" (*6*), and any variable that is hard to measure is likely to be neglected.

Loomis has surveyed the chemistry and biology of pCO_2 (*7, 8*), and has emphasized that three new methods of measuring this important feedback variable have recently been devised [3] (*8, 9*), so that, today, pCO_2 can be measured and controlled experimentally with considerable precision.

pCO_2 refers to the pressure of CO_2 gas dissolved within a tissue, just as pO_2 refers to the pressure of dissolved oxygen gas. It differs from total CO_2 in that it is not concerned with such nongaseous products as H_2CO_3, HCO_3 and CO_3 except as they buffer the existing concentration of free, unhydrated CO_2 molecules. CO_2 gas is physiologically unique because, being un-ionized, it is more soluble in fat than in water (*10*) and hence is able to pass through nuclear and cell membranes faster than any other known substance (*11, 12*). Photosynthesizing plants, for example, are known to absorb free CO_2 molecules rather than the almost impermeable bicarbonate ion (*13*). Since all respiring tissues generate CO_2, they automatically create gradient fields of pCO_2 that permeate tissues almost as if they were acellular. Any particular cell within a respiring tissue, therefore, is under the influence of the over-all pCO_2-gradient of that tissue.

Some idea of the physiological importance of pCO_2 is given by the care with which the respiratory center of the brain regulates pCO_2 rather than oxygen. Powers (*14*) has pointed out that lung-breathing animals have the dual advantage of living in air that contains a fixed percentage of CO_2 (0.03%) and of possessing closed alveolar spaces within which alveolar CO_2 can be closely regulated at 5.3 percent CO_2. Fish, he pointed out, have gills that are essentially open systems; they can control their arterial pCO_2 only by *a*) regulating their level of muscular activity or by *b*) migrating to water of higher or lower pCO_2. He suggested that this explains why active brook trout inhabit aerated brooks where the pCO_2 approximates 0.03 percent atmosphere, while lethargic lake trout inhabit the depths of partially stagnant lakes where the pCO_2 may be ten times higher.

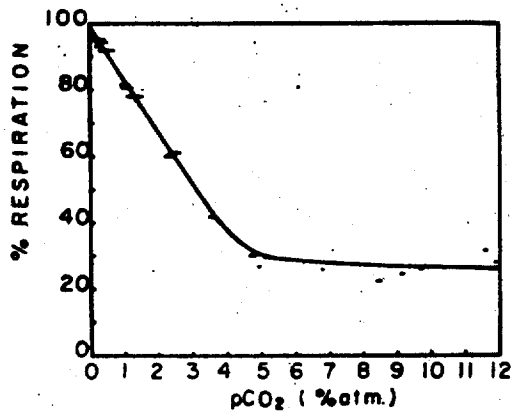
Given the importance of tissue pCO_2 as a feedback variable, what is its level in human tissues? Venous and capillary blood have a pCO_2 of about 46 mm. Hg or 6 percent atmosphere, so that human tissues contain gradients of pCO_2 that begin at about 6 percent atmosphere and rise progressively the farther a cell is from an open capillary (to perhaps 15-20% atm.). The height of this gradient depends *a*) on the respiratory rate of the tissue and *b*) on the number of open capillaries within it. Since tissue respiration and vascularity often increase at the same time,

[3] Direct pCO_2 measurements may be made with the new CO_2-pH Duometer manufactured by Advanced Instruments, Inc., 45 Kenneth Street, Newton Highlands 61, Mass.

it may be that tissue pCO_2 is homeostatically regulated in the same manner as alveolar pCO_2.

pCO_2 Inhibition of Respiration

Root (*15*) has shown that pCO_2 specifically inhibits respiration in fertilized *Arbacia* eggs (text-fig. 1).



TEXT-FIGURE 1.—pCO_2 inhibition of respiration in fertilized *Arbacia* eggs. Data from figure 3 of (*15*).

Since similar experiments with tissues from higher animals do not seem to have been carried out,[4] this curve may be used to obtain some indication of how pCO_2 affects cellular respiration in general. The danger in such an extrapolation may not be as great as it first appears, for many years of work have shown that the respiratory and glycolytic enzymes of cells are surprisingly alike throughout the plant and animal kingdom. Yeast and pigeon-breast muscle, for example, are both classical sources of respiratory enzymes.

Text-figure 1 presents several interesting points. First, a pCO_2 of 6 percent atmosphere is seen to inhibit respiration by over 70 percent. Since cell survival requires only about one fifth the energy needed for maximum cell growth (*16*), this suggests that cytostasis results from the 6 to 15? percent atmosphere pCO_2 present in adult mammalian tissues, for 70 to 80 percent inhibition of respiration would generate only enough ATP to maintain tissues without providing them with enough ATP for growth.

Second, any lowering of tissue pCO_2 would strongly increase the supply of ATP available to a cell and hence tend to initiate growth. A surface abrasion of the skin, for example, would allow CO_2 to be lost directly to

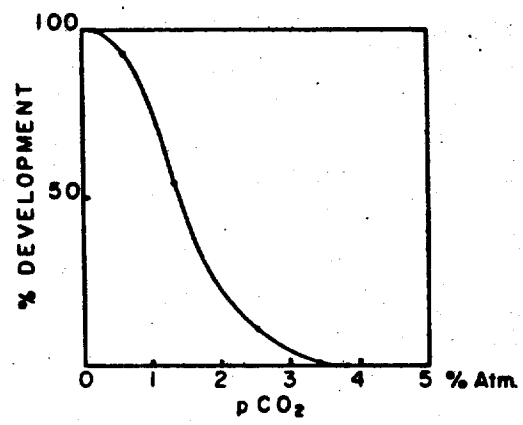
[4] Added in proof: In subsequent experiments, Root found that increased levels of pCO_2 inhibited the respiration of both nerve and muscle. See Root, W. S.: The influence of carbon dioxide upon the respiration of nerve. J. Cell. & Comp. Physiol. 1: 239-252, 1932; and Root, W. S.: The influence of carbon dioxide upon the respiration of muscle. Physiol. Zool. 6: 137-149, 1933.

the air and hence would tend to lower the pCO_2 of the newly exposed cells. Alternatively, any increase in vascularity to an area would tend to lower its pCO_2 and thus stimulate growth. Removing cells to the environment of a tissue culture would also tend to initiate growth, if the pCO_2 of the tissue-culture air space were lower than it had been *in situ*. Finally, text-figure 1 is interesting in that the curve flattens out above about 6 percent atmosphere, and thus allows maintenance levels of ATP to be generated even under very high pressures of CO_2.

Maintenance of Cytostasis by Regulated Levels of pCO_2

Direct evidence that CO_2 is a physiologically produced, nontoxic, and reversible inhibitor[6] of cell growth is available from work on lower animals. Goldin (*17*), for example, found that appropriate levels of pCO_2 can cause complete inhibition of growth in regenerating *Tubularia* tissues. Smith and Clowes (*18*) found that pCO_2 progressively inhibits cell division in both sea urchin (*Arbacia*) and starfish (*Asterias*) eggs (text-fig. 2).

Haywood and Root (*5*) confirmed these findings, including the important point that complete inhibition of growth was obtainable by sufficient elevation of the pCO^2. Salisbury and VanDemark (*19*) showed that a pCO_2 of 100 percent atmosphere reversibly inhibited both the glycolytic activity and motility of mammalian sperm, the reversible nature of such inhibition being shown by finding that sperm stored in a glass ampoule



TEXT-FIGURE 2.—pCO_2 inhibition of cell division in fertilized *Asterias* eggs. Data from figure 3 in (*18*).

[6]Evidence from several sources (*7, 8*) indicates that low levels of pCO_2 stimulate cell differentiation, even though high levels are inhibitory to growth and respiration. This finding may explain the morphological inferiority of glycolytic to respiratory ATP as seen, for example, in the dedifferentiation of cancer cells and in the anaerobic versus aerobic culture of yeast. Warburg (*16*) believes that structural differences explain this phenomenon, *i.e.*, respiratory ATP is generated within mitochondria, while glycolytic ATP is generated within the fluid protoplasm. Since ATP from any source probably equilibrates rapidly throughout the cell, it seems more likely that the morphogenetic superiority of respiratory ATP stems from the fact that it alone is accompanied by the generation of CO_2.

under CO_2 was fully fertile after the rigors of a week at room temperature (*20*). Normally, of course, sperm remains fertile only when stored under refrigerated conditions.

Taken together, these different experiments suggest that adult tissues may achieve cytostasis by limiting their supply of ATP through pCO_2 inhibition of their respiratory mechanism. It is unfortunate that similar studies have not been made on mammalian tissue cultures, a fact that is due perhaps to the mistaken notion that pCO_2 affects only pH (*21*).

Glycolytic Freedom From pCO_2 Inhibition

Phosphate bond energy can be produced in two alternative ways: 1) by respiration which yields CO_2 as its end product and 2) by glycolysis which does not yield CO_2 but lactic acid instead. If each of these enzyme systems tends to be specifically inhibited by its own end products, then glycolysis should be more resistant to pCO_2 inhibition than respiration. This prediction has been confirmed in recent experiments by Salisbury and Van-Demark (*19*) who showed that sperm maintains 95 percent of its glycolytic activity at a pCO_2 of 5 percent atmosphere. Cells with well-developed respiratory *and* glycolytic machinery, therefore, should be able to obtain maintenance levels of ATP from their pCO_2-controlled respiration and, in addition, should be able to obtain additional amounts of ATP for growth by glycolysis. If cytostasis, therefore, results from an insufficient supply of ATP in self-inhibited respiring cells, uninhibited growth should occur in cells containing the auxiliary power supply known as glycolysis. The interesting fact is that, except for a few tissues,[6] normal adult tissues have low rates of glycolysis (*i.e.*, 1–8), while both embryonic and cancer tissues have high rates (*i.e.*, 16–70) (*22*, *23*). Both embryonic and cancer cells, of course, are continuously growing tissues.

Glycolysis and Cancer

Warburg has long maintained that cancer cells originate in two phases: an irreversible injury to their respiratory apparatus, followed by a slowly developed increase in their glycolytic potential (*16*). Weinhouse (*24*) and others have argued that often there is no detectable reduction of respiratory activity in tumors. All workers are agreed, however, that malignant and embryonic cells characteristically show rates of glycolysis far above normal adult tissues. Warburg (*16*), in fact, found glycolytic rates as high as 70 in ascites tumor cells and remarked that with ". . . such high values we come very close to the fermenting values of wildly proliferating *Torula* yeasts." Weinhouse believes that ". . . high glycolysis occurs *despite* quantitatively and qualitatively normal occurrence of (respiratory) carbon and electron transport, . . ." concluding, "I believe it would be more accurate to state that anaerobic glycolysis is so high in tumours that a

[6] Special factors may operate in such tissues. For example, some may have lost the power to grow under any circumstances (brain and retina), others may translate their growth into special channels (myeloid and placental tissues), while still others may have lost so much of their respiratory capacity that their net rate of ATP synthesis is not sufficient for growth, despite their high glycolytic rate.

normal respiration and a normal Pasteur effect are incapable of eliminating it."

Much of the confusion surrounding the metabolism of tumors derives from the fact that the respiratory and the glycolytic *potential* of cells are operationally measured by the usual techniques, rather than their actual rates as they occur in the intact animal. Thus, respiration is usually measured in a Warburg vessel containing NaOH in a center well so that the pCO_2 approximates 0.0 percent atmosphere rather than the 6 to 15? percent atmosphere actually present in living tissues. All such studies, therefore, measure maximum, uninhibited respiratory rates rather than the 70 percent inhibited rates existing in normal tissues. Glycolysis similarly is measured under unphysiologically anaerobic conditions so that it, too, is measured under maximal, uninhibited conditions. Actual rates of glycolysis *in vivo* would be far lower, consequent to end-product inhibition by accumulated lactic acid and by the inhibition of glycolysis by respiration known as the Pasteur effect. Even the latter is a feedback entity, for respiration itself *in vivo* is not maximal but self-inhibited in nature, so that its effect on glycolysis is less than if it were running at full speed. Warburg today believes that *in vitro* measurements of aerobic glycolysis are meaningless ". . . because aerobic fermentation is too labile and too dependent on external conditions." (*16*).

What is needed today, therefore, is a study of the respiratory and glycolytic rates of normal and malignant tissues under as physiological conditions of pCO_2, pH, pO_2, and pNH_3 (*8*) as possible. Respiratory studies of this nature may be made either with the indirect method of Warburg (*in 25*) or with chemical rather than manometric methods of measuring oxygen uptake (*26*).

Respiration and Glycolysis in Normal and Malignant Tissues

Burk (*27*) has compared the respiratory and glycolytic potential of four homologous rat tissues: *i.e.*, normal, regenerating, embryonic, and cancerous (table 1).

TABLE 1.—*Comparison of respiration and glycolysis in homologous rat hepatic tissues* (24)

Tissue	Respiration	Glycolysis
	Q_{O_2}	$\frac{N}{Q_{A}2}$
Normal	6.0	1.0
Regenerating	6.4	1.8
Embryonic	6.0	8.0
Hepatoma*	6.4	12.1

*Induced by feeding 4-dimethylaminoazobenzene.

Summarizing his results, Greenstein remarked, "What is indeed striking is the similar metabolic behavior of resting and regenerating liver, and the dissimilar behavior of the hepatoma. It is evident that, in the case of regenerating liver, it is possible to have rapid growth in the absence of

appreciable glycolysis. This is, however, controlled growth. In the case of the hepatoma, there is presented an ostensibly uncontrolled growth accompanied by extensive glycolysis. The four homologous rat hepatic tissues thus fall, metabolically, into two main categories, resting and regenerating liver on the one side and hepatoma and fetal liver on the other." (*23*). The conclusion seems to be that cells achieve cytostasis if they are powered by respiration alone, while they avoid cytostasis and grow continuously if they are powered by both respiratory *and* glycolytic mechanisms. Since normal cells obtain 99 percent of their ATP from respiratory sources (*16*), such cells achieve cytostasis by damping their furnaces with a blanket of CO_2. Embryonic and malignant cells, on the other hand, possess an auxiliary motor that is not inhibited by CO_2 and so is available to supply them with the extra energy needed for growth.

Why Does Carcinogenesis Occur in Normal Tissue Culture?

Biesele (*28*) has written that the enclosed environment of a tissue culture provides ". . . just the conditions needed to transform the metabolism of normal epithelial cells to that of malignant cells." Cancer, in other words, tends to arise spontaneously in normal cells grown for prolonged periods of time in tissue culture. Goldblatt and Cameron (*29*) suggested that this malignant change may be due to the intermittent anoxia that accompanies serially transplanted tissue cultures. Since incubating tissue cultures for 7 days at a time automatically exposes them to increasing conditions of anaerobiosis, long-continued cultures are repeatedly exposed to increasing levels of pCO_2 as well as to decreasing levels of pO_2. The operative variable in the malignant change therefore may actually be high pCO_2 rather than anoxia.

Earle *et al.* (*30*) have observed that the size of the inoculum in a culture has no effect on its final amount of growth. Growth is limited in such cultures by several conditions, including the size of the physical container and the accumulation of metabolites. Since the only way in which the size of container could affect the process is by limiting the diffusion of gases, it may be that self-limitation of growth results from the gradual accumulation of inhibitory levels of pCO_2.

If respiratory self-inhibition controls the growth of normal cells in tissue culture, what would happen if a cell should progressively mutate so that it possessed additional powers of glycolysis? Because any increase in the supply of ATP would increase the rate of growth of such a cell, one can predict that such respiring and glycolyzing cells would gradually dominate the culture, a process akin to natural selection operating to increase steadily their glycolytic potential with the continued passage of time.

Since small pieces of an old culture are used to start new ones, fortuitous selection of a mutated area could serve to speed this process by a mechanism akin to genetic drift. Tissue-culture routine, therefore, contains almost all the elements needed for rapid selection of mutant varieties.

For example, mutations themselves are fostered during the repeated bursts of growth that follow successive transplantations, for mutations chiefly occur during periods of active mitosis. Like a chronically irritated sore or mammary gland, bursts of growth alternate with periods of increasing cytostasis, the net result being a rapid increase in glycolytic potential and eventual malignancy.

Burk (in *16*) has actually observed this process in two sublines, derived from the same identical normal cell that developed high and low malignancy at the same time that they developed high and low glycolytic potential. Strain 1742, for example, today produces cancer in 97 percent of mice injected and has a glycolytic potential of 70. Strain 2049, on the other hand, is apparently still precancerous, for it produces cancer in only 1 percent of mice injected and has a glycolytic rate of 25.

Whether glycolytic potential is gained by a process akin to somatic mutation (*31*) or by the "infection" of the strain with an extrinsic "virus" is immaterial to the argument, just as long as the altered cell is capable of transmitting its increased glycolytic potential to its descendents. In this connection, Burk (*32*) found that the Rous sarcoma 1 virus, which can induce malignancy within 7 days (*33*), confers both malignancy and increased glycolytic potential to the cells on which it acts.

Cancer as a Normal Biological Consequence of Cytostasis

It is a general biological truth that whenever a population is chronically suppressed by an inhibitor for prolonged periods, then eventually some members of the population become immune to the effect of the inhibitor and take over biologically. Just as new rusts continually appear in wheat-producing areas, and DDT-resistant flies appear in areas treated for several years with DDT, so perhaps the cancer cell represents the successful evolutionary response of cells grown under conditions of cytostasis where high levels of pCO_2 prevent their growth by starving them of metabolic energy. Just as streptomycin-resistant bacteria appear in cultures chronically suppressed with streptomycin, so cancer may represent ". . . a process as inevitable as evolutionary progress and of the same general quality." (*31*). It should be remarked in passing that the suppressive agent in observed instances of this phenomenon seems to act solely by selecting the favored mutant rather than by causing the mutation to occur in the first place.

Burnet has emphasized that the logarithmic relationship existing between cancer and increasing age bears all the earmarks of a series of progressive somatic mutations. "From the age of 20 or 30, it appears that we are subject to a random process becoming progressively more evident with age and with inexorable regularity . . . . The only way in which an observable effect can result is when mutants with a proliferative advantage can emerge. Every significant somatic mutation, whether affecting a normal cell or one that has already mutated, must necessarily be along the road to malignancy. The age incidence of cancer is precisely what would be expected from such a situation. In fact I

fancy that the log-log relationship between cancer incidence and age could be foreseen from the beginning as an inevitable consequence of the evolution of multicellular animals." (*31*).

In conclusion, if the biochemical uniqueness of the cancer cell resides in its dual mechanisms of ATP production, then any treatment that inhibits glycolysis, but not respiration, should be in the right direction. Total inhibition of glycolysis, of course, would have to be accompanied by the administration of lactic acid or some other source of respiratory substrate such as glutamate or acetate. Even then, glycolysis may be too fundamental a mechanism to inhibit safely, so that palliation rather than cure should be expected from all such methods. Actual cure of a cancer cell may be defined as its conversion into a normal cell, not by inhibiting its extra titer of glycolytic enzymes, but by preventing the synthesis of these enzymes in the first place. Since the synthesis of a high titer of glycolytic enzymes takes place in embryonic cells but not in adult ones, some normal process occurs *in vivo* that effectively inhibits the synthesis of these enzymes during maturation. If the mechanism of normal maturation can be elucidated, it may be possible to apply this knowledge to the problem of converting a malignant cell into a normal one.

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QUANTITATIVE DETERMINATION OF $^{14}CO_2$ BY SCINTILLATION SPECTROMETRY IN METABOLIC STUDIES IN VIVO

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Because of the key position of carbon dioxide as a central catabolite, a number of techniques has been evolved for the quantitative determination of $^{14}CO_2$ (Abt et al. 1961, Hagenfeldt 1967, Franc et al. 1965, Bach et al. 1967, Runyan et al. 1967, Saba and Di Luzio 1966). These techniques are based on the release of $^{14}CO_2$ by an acid and its absorption into alkaline medium, e.g. in Warburg vessels. The quantitative transfer of absorbed $^{14}CO_2$ to scintillation measuring vials is attended by various technical problems, however. For in *vitro* experiments, Saba and Di Luzio (1966) suggested a simple apparatus which has the advantage that the released $^{14}CO_2$ is trapped directly in the scintillation measuring vial. The present report describes a simplified modification of this method for in vivo experiments, which raises the rate of $^{14}CO_2$ determination while conserving the accuracy of the original technique. It also describes the construction of a simple metabolic chamber allowing long-term metabolic studies with laboratory animals up to the size of an adult guinea pig.

Methods

The quantitative determination of $^{14}CO_2$ *in vivo* comprises three linked processes:

a) the absorption of expired $^{14}CO_2$ into KOH,
b) the release of $^{14}CO_2$ from KOH into a suitable absorbent,
c) the actual measurement of $^{14}CO_2$ by scintillation spectrometry.

For effecting these processes we have two separate apparatuses. The first, which is used to collect expired $^{14}CO_2$, is illustrated in Fig. 1. It is a hermetically sealed glass metabolic chamber with on inlet and outlet tube and a stools and urine separator. The outer part of the metabolic chamber consists of a desiccator with an inner diameter of 29 cm and a height of 40 cm. On a tripod inside the desiccator there is a conical funnel made of steel sheeting 0.5 mm thick and lined with a layer of teflon.* The funnel has a gradient of about 45 degrees and at the bottom it ends in a ring with four grips attaching it to the glass separator. Conical narrowing of the stools and urine separator ensures that the fluid running down the sides of the funnel and the separator is separated from the solid matter, which drops through the middle. On top of the teflon-coated funnel there is a grid with round openings 7 mm in diameter and 4 mm apart, with sufficient room for food and water. The grid is surmounted by the campanulate part of the lid of the desicator, leaving a space of about 5.5 dm^3 for the experimental animal. Fresh air is let into the chamber through a glass tube introduced through a rubber stopper in the roof of the desiccator and extending to the floor of the chamber. Expired air is led out by a tube ending just below the closure of the desiccator. The difference in the distance of the inlet and outlet tube ensures that the air is kept moving. The air pumped into the chamber passes through two filters, one of which is filled with soda lime for drying the air and the other with 2.5 N aqueous KOH solution for absorbing non-active atmospheric CO_2. The expired $^{14}CO_2$ is bubbled through two successive filters filled with 2.5 N KOH. In the first, sintered glass for dispersing the outgoing $^{14}CO_2$ is attached to the outlet tube, so as to permit maximum absorption in the KOH. The outlet tube in the second filter has a capillary ending. Circulation of the air is assured by a CH-03 surgical suction flask or by a laboratory oil pump. The rate at which the air was drawn off was set at 400 to 600 ml/min by means of clips.

* Provided by the KOMEX cooperative, Prague

The simple apparatus for the release and absorption of $^{14}CO_2$ collected into KOH is illustrated in Fig. 2. It consists of three parts:

1) a 50-ml Erlenmayer flask with a rubber stopper, a short burette and a welded-on scintillation vial,
2) a connection with a rubber washer which can be turned out from teflon or made from two scintillation vial closures, and
3) a scintillation measuring vial for the absorption and actual measurement of $^{14}CO_2$ activity.

The quantitative determination of $^{14}CO_2$ is done as follows: A sample of 2.5 N KOH (0.5—1 ml according to presumed activity) with absorbed $^{14}CO_2$ is pipetted into the Erlenmayer flask, the flask is sealed with the rubber stopper, which holds the burette, and the scintillation vial, containing 0.5 ml concentrated distilled ethanolamine, is screwed on by means of the connection. When the whole

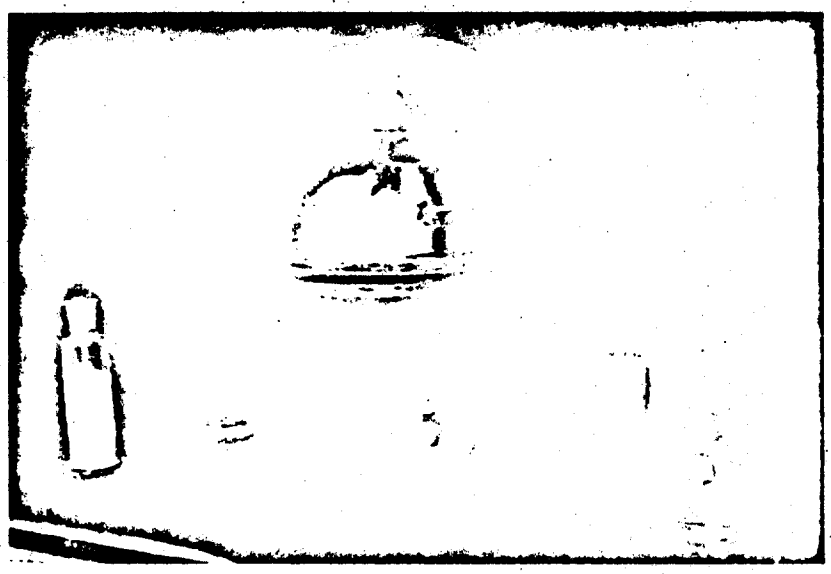

Fig. 1. Metabolic chamber suitable for animals up to size of adult guinea pig

system has been completely sealed, 0.5 ml—1 ml 5 N sulphuric acid (according to the size of the sample) is added under mild pressure by means of a hypodermic needle attached to rubber tubing on the burette, so as to leave a drop of the acid in the stopcock of the burette. After mixing the solution in the vial, it is left to react one hour at 37°C. The scintillation vial with ethanolamine is then unscrewed, and 5 ml 96% ethanol and 10 ml scintillator liquid toluene (SLT) are added. The prepared sample

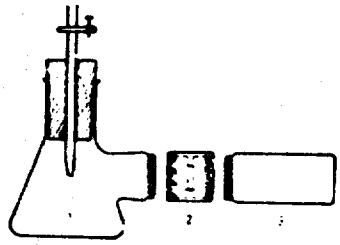

Fig. 2. Apparatus for release and absorption of $^{14}CO_2$. 1 — Erlenmayer flask with welded-on part of scintillation vial, 2 — connection, 3 — scintillation measuring vial.

is measured at 0°C with a Mark I (Nuclear Chicago) counter, using an external standard to correct quenching.

This technique was tested under different conditions with $Na_2{}^{14}CO_3$ standards* and with $^{14}CO_2$ samples obtained in preliminary metabolic experiments on guinea pigs to which acetate-1-^{14}C was administered intraperitoneally**.

Results and Discussion

a) *Absorption of expired $^{14}CO_2$ into KOH:* It was found that for the quantitative absorption of expired $^{14}CO_2$ by our method, it was sufficient to link up two successive filters each containing 200 ml 2.5 N KOH. Of the total activity expired, 98.5—99% was present in the first filter (evidently as a result of very fine dispersion of the outgoing $^{14}CO_2$ by the sintered glass). The second filter contained 1—1.5% activity; no activity could be determined in the other filters.

Coating the funnel in the metabolic chamber with teflon proved to be an excellent measure, as seen from the results obtained when checking the adhesion of active urine and stools and cholesterol-26-^{14}C standards** to the teflon-coated surface of the funnel. In the case of urine with 7,980 DPM activity we found no activity after eight-fold washing with a total volume of 25 ml distilled water. In a smear test using methanol tampons we found 181 DPM, i.e. 2.3% of the activity measured in the urine. On employing aqueous cholesterol-26-^{14}C solution with Tween 80, with which we wetted the surface of the teflon-coated funnel, left it to stand 24 hours and then washed it twice with a total volume of 25 ml distilled water, we found no difference between the activity of the washing water and the activity applied to the teflon-coated surface of the funnel.

b) *Release and absorption of $^{14}CO_2$:* In their original method for *in vitro* experimental conditions, Saba and Di Luzio (1966) recommend as the absorbent Whatman paper saturated with aqueous KOH solution and placed round the circumference of the scintillation vial so as to increase the absorption area. This entails leaving the scintillation vial over night in a desiccator, as the water in the filter paper would have a negative effect on scintillation determination of the activity of the samples. We therefore chose ethanolamine as the absorbent, as it has better properties for scintillation analysis (it combines well with the toluene scintillator and does not cause large quenching). Since concentrated ethanolamine forms a film on the wall of the scintillation vial, and hence a large absorption area, we compared, under different conditions, the results obtained by using Whatman paper saturated with ethanolamine and ethanolamine without Whatman paper. These results, which include the influence of the volume of the analysed sample and of the incubation temperature, are given in Tab. 1. They demonstrate that a sample volume of 0.2—1 ml does not influence the results of analyses. Incubation at a constant temperature of 37°C in a water bath is better than incubation at laboratory temperature, which is very variable and influences the results of measurement of activity. In all the experiments, the activity found in the flasks containing ethanolamine without Whatman paper was higher than in those containing Whatman paper impregnated with ethanolamine (the differences were in the region of 20%). This difference is evidently due not to differences in $^{14}CO_2$ absorption, but to optical non-homogeneity of the scintillation flasks containing Whatman paper, which cannot be completely corrected by an external standard, so that data on the disintegration count are inaccurate. We therefore carried out further experiments entirely with scintillation vials containing ethanolamine without Whatman paper as the carrier.

The quantitative character of our technique was verified by a recovery test using standard aqueous $Na_2{}^{14}CO_3$ solution, whose activity was measured directly (after mixing the aqueous solution with toluene scintillator by means of NCS* and after the release and absorption of $^{14}CO_2$ by our technique. In agreement with the data of the authors of the original method, recovery in 10 samples ranged from 96 to 97%.

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* Nuclear Chicago Solubiliser

Table 1

Measurement of $^{14}CO_2$ activity in correlation to the volume of the sample and to the incubation temperature. The correlations were done in samples with different activity. The results are given as the DPM/ml sample. n = 10

Measuring method	Volume of active sample		
	0.2 ml	0.5 ml	1 ml
Without paper	68,647 ± 736	69,241 ± 908	68,855 ± 870
With paper	55,837 ± 209	57,130 ± 877	56.290 ± 1,127

Measuring method	Incubation temperature	
	laboratory	37°C
Without paper	20,328 ± 904	21,749 ± 657
With paper	15,277 ± 1,115	19,263 ± 382

Table 2

Measurement of $^{14}CO_2$ activity in correlation to the length of the incubation time and the amount of ethanolamine. t = 37°C; n = 5

Type of sample	Incubation time in hours			
	½	1	1 ½	2
Active sample (DPM/ml)	2,423 ± 153	2,535 ± 113	2,567 ± 117	2,548 ± 159
Solution of standard (DPM/ml)	2,837 ± 205	2,824 ± 173	2,856 ± 176	2,841 ± 156

Type of sample	Amount of ethanolamine in ml			
	0.25	0.5	0.75	1
Active sample (DPM/ml)	2,293 ± 113	2,360 ± 73	2,452 ± 36	2,377 ± 47
Solution of standard (DPM/ml)	2,796 ± 67	2,854 ± 36	2,826 ± 139	2,725 ± 91

Tab. 2 gives the results of a study of the influence of the length of the incubation time on the degrye of $^{14}CO_2$ release from the sample. Like the authors of the original method, we found that quantitative release occurred after only 30 minutes' incubation. We further studied the influence of the amount of ethanolamine on the quantitative capture of $^{14}CO_2$. Within a range of 0.25 to 1 ml, the amount of ethanolamine did not influence the final activity measured. We also found that KOH samples with absorbed $^{14}CO_2$ could be kept in the sealed for several weeks without any marked change occurring in their activity.

c) *Actual measurement of $^{14}CO_2$ by scintillation spectrometry.* The actual measurement of ^{14}C activity in samples does not encounter any serious obstacles, since the degree of quenching of ethanolamine is small. We recommend redistilling the ethanolamine at 171°C prior to use. The advantage of our technique is that it provides instantaneous information on the activity of the samples, whereas with the original technique the samples have to be dried over night.

The above technique was used with success to study acetate-1-^{14}C and ascorbic acid-1-^{14}C metabolism in guinea pigs in different nutritional situations (Ginter et al. 1969, 1971). Fig. 3 gives the results of a preliminary experiment, not yet published, which show the pronounced stimulant effect of vitamin C on the oxidation of cholesterol-26-^{14}C to $^{14}CO_2$ in guinea pigs in a state of acute C avitaminosis.

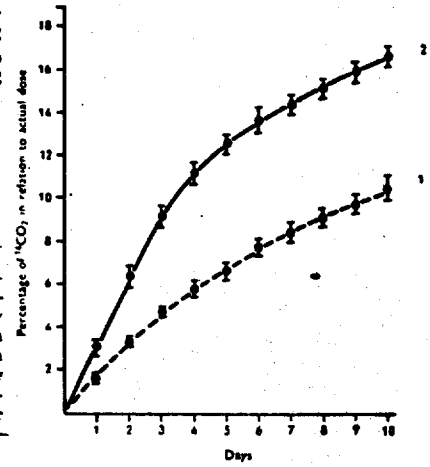

Fig. 3. Stimulant effect of vitamin C on oxidation of cholesterol-26-^{14}C in guinea pigs. 1 — guinea pigs with acute vitamin C deficiency (mean from four animals). 2 — guinea pigs in state of acute C avitaminosis, resaturated with large doses of ascorbic acid (mean from four animals). The vertical lines at each point represent the standard error of the mean. The differences are highly significant ($P < 0.01$—0.001).

Summary

NEMEC, R., J. ČERVEŇ, E. GINTER (Radioisotope Lab., Inst., Human Nutr., Bratislava). *Quantitative Determination of $^{14}CO_2$ by Scintillation Spectrometry in Metabolic Studies in vivo.* Physiol. bohemoslov., 20 (3): 281—286, 1971.

A simple and reliable method for determining the amount of expired $^{14}CO_2$ in metabolic studies *in vivo* was evolved. The method is based on the absorption of $^{14}CO_2$ into ethanolamine directly in the scintillation vials and on subsequent measurement of activity by means of liquid scintillation spectrometry. The construction of a simple metabolic chamber suitable for work with laboratory animals up to the size of an adult guinea pig is described.

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Determination of carbon dioxide content of blood by infrared analysis[1]

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ORTEGA, F. GIMENO, S. A. M. ORIE, AND G. J. TAMMELING. *Determination of carbon dioxide content of blood by infrared analysis.* J. Appl. Physiol. 21(4): 1377-1380. 1966.—This paper deals with an adaptation of an infrared analyzer for the determination of the carbon dioxide content of blood. The apparatus has been tested with various carbon dioxide tensions and the results have been compared with those obtained with the conventional Van Slyke-Neill technique. The reproducibility of the infrared method is better than that of the Van Slyke-Neill method under comparable operating conditions. The infrared method requires practically no skill. A complete analysis can be performed within 5 min. The main disadvantage of the method is its limitation to carbon dioxide. It should not be used when anesthetic gases are present.

blood gas analysis; Van Slyke-Neill technique

Since 1924, the manometric technique of Van Slyke and Neill (10) has been the standard procedure for the estimation of the carbon dioxide content of blood. This method gives satisfactory results but has the disadvantage of being time consuming, while accuracy fully depends on the skill of the technicians.

Nowadays, many alternative techniques are available. These methods are either modifications of the original Van Slyke-Neill technique (5) or are based on completely different principles: equilibration techniques (8), interpolation techniques (1), gas chromatography (7, 12), membrane electrodes, colorimetric methods (14), etc.

Recently, the application of infrared analysis for the measurement of the carbon dioxide content of blood has been described by Oehmig and Sommerkamp (6). The results with this method, however, were not quite as good as those obtained by the manometric technique.

It seemed worthwhile to explore in more detail the possibilities of the infrared analyzer since this apparatus is available in many cardiopulmonary function laboratories and, with a low-cost modification, may be used for the determination of carbon dioxide content of blood.

The present paper deals with the accuracy of the infrared method at various carbon dioxide tensions in blood. The results are compared with those obtained by the conventional Van Slyke-Neill technique both under "routine" and "optimal" laboratory conditions.

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METHODS

Blood samples were drawn from systemic arteries of patients with pulmonary disease in whom blood gas analysis was indicated. The aliquots were collected anaerobically in 10-ml glass syringes, the dead space being filled with heparin solution (2,000 U/ml) and a mixing ring. All determinations were performed within 4 hr after sampling and the syringes were stored in a refrigerator (ca. +3 C) until analysis. Since it concerned a comparative study, the values were not corrected for dilution of blood by the anticoagulant.

Equilibration of blood with gas mixtures of known carbon dioxide concentrations (4.0, 7.8, and 13.6%, respectively, carbon dioxide in oxygen), was performed with a Lauê twin tonometer (3). Time of equilibration amounted to 60 min, flow rate of the gas stream through the tonometer was 50 ml/min, the quantity of blood was 3 ml, temperature 38.0 C. The gas cylinders were analyzed by the Scholander technique with an accuracy of better than 0.1 vol %.

The Van Slyke-Neill technique was applied in the conventional way, using aliquots of 1.0 ml of blood, according to the instructions given by Bartels et al. (2). A magnetically stirred manometric apparatus was used (A.H. Thomas, Philadelphia).

For the measuring principle of infrared analysis we refer to the original publication of Luft (4). The infrared analyzer (Godart, Utrecht; Capnograph, type CG/58003) is modified in the following way: the measuring head is connected by Tygon tubing to a membrane pump and a Lucite blood cuvette (Fig. 1). The gas volume of the closed circuit, including the cuvette filled with reagents, amounted to 10.0 ml. The flow rate of the circulating gas can be regulated and is adjusted to about 30 ml/min. The interchangeable cuvette is connected airtight to a Teflon support by an O ring. Overflow of liquids from the cuvette to the measuring cell is prevented by a small gauze-filled tube.

By means of a selector switch the direct-reading meter of the Capnograph is disconnected and the amplifier output fed into a precision micro ammeter with a 15-cm mirror scale allowing accurate readings to 0.5% of full scale. The sensitivity can be adjusted by means of coarse and fine potentiometers.

Before starting analysis, the cuvette is filled with 2 ml of a 3% solution of lactic acid which releases the carbon dioxide from the blood. One drop of octyl alcohol is added as antifoam. Next, the cuvette is connected to the Teflon support. The closed circuit is now filled with room air and the microammeter set at zero. The blood sample is transmitted, either from the 10-ml syringe or from the tonometer, into an 0.5-ml tuberculin syringe with 100 scale divisions. An injection needle (external diameter, 1.2 mm) is soldered to the tuberculin syringe. The first loading with blood is discarded, the rest of the blood being used to fill up the dead space of syringe and needle. The second

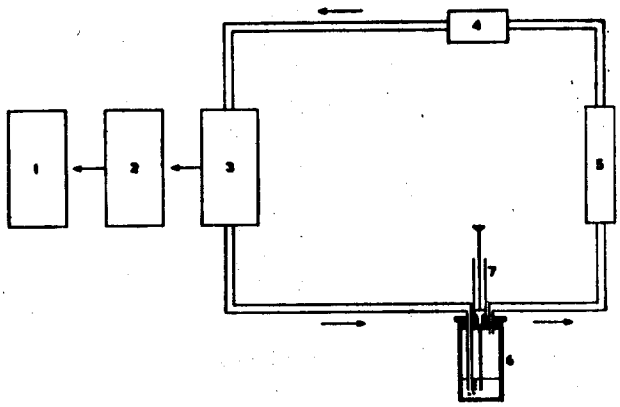

FIG. 1. Diagram of the closed circuit for the determination of carbon dioxide in blood by the infrared method. 1, microammeter; 2, amplifier; 3, infrared-measuring head; 4, membrane pump; 5, gauze-filled tube; 6, Lucite cuvette; 7, 0.5-ml tuberculin syringe with fixed needle.

load is adjusted exactly to the 0.5 ml division. Accuracy of the sample size is better than 1%. The conus of the tuberculin syringe is now plugged into a cone-shape hole in the cuvette support, the tip of the needle being immersed in the releasing agent (Fig. 1). The zero position of the meter is checked and readjusted if necessary and 0.5 ml of blood is injected into the cuvette; the syringe is not removed from the support. The carbon dioxide concentration in the circulating gas is increased by the release of CO_2 from the blood.

After 3 min, equilibrium is attained and the final reading can be made. A complete analysis, including cleaning of the cuvette and syringes, may easily be done within 5 min.

Since the electrical output of the apparatus does not vary linearly with the carbon dioxide content, a calibration curve must be made. This can be done by applying the above-mentioned analytic procedure to solutions with known concentrations of sodium bicarbonate (e.g., 10, 20, 30, 40 mM/liter). A calibration curve needs to be made only once because alinearity is constant, variations in background gases and volume of the closed circuit being negligible.

A check on the sensitivity with a sodium bicarbonate solution (for instance 20 mM/liter) is sufficient for daily use. Sensitivity of the apparatus is adjusted so that a 20 mM/liter sodium bicarbonate solution gives a deflection of 50 scale divisions. The bicarbonate solutions were stored in 5-ml ampoules.

RESULTS AND COMMENTS

First we tried to establish the reproducibility of the Van Slyke-Neill technique under different conditions. In the present study, by "reproducibility" is meant agreement between two determinations of the same specimen. Under optimal conditions the reproducibility for the carbon dioxide content in blood is about 0.2 mM/liter (2). Our experience is in accordance with this (Table 1; first line). These determinations were performed by one well-trained technician who worked exclusively with the Van Slyke apparatus. The blood samples were equilibrated in the tonometer until analysis.

Under routine conditions, reproducibility of the manometric technique was disappointing and significantly worse than under optimal circumstances ($P < 0.05$) (Table 1, second line). In this case the samples were analyzed in duplicate without special precautions by three laboratory technicians who also carried out other routine work. In both series all measurements were accepted, even when duplicates showed considerable differences. These findings clearly demonstrate that the accuracy of the Van Slyke-Neill technique depends on the laboratory conditions and should not be overestimated for daily routine work.

Reproducibility of the infrared method was tested by 28 blood samples. Each sample was equilibrated with three different gas mixtures (see METHODS) in a Lauê tonometer. The results are presented in Table 1 and in Fig. 2. At the lower carbon dioxide tensions (mean P_{CO_2} = 28.4 and 55.4 mm Hg), the standard deviation of the differences between duplicates (Sv), measured by the infrared method and by the manometric technique (optimal conditions) do not differ significantly ($P > 0.05$).

At a high carbon dioxide tension, however, reproducibility of the infrared method is worse than in the lower ranges and is about the same as the reproducibility of the Van Slyke-Neill procedure under routine conditions in the lower ranges.

In a third series of experiments, 26 blood samples were analyzed once by both methods under routine laboratory conditions (Table 1, Fig. 3). The mean values do not differ significantly. The standard deviation of the differences is about the same as found between the duplicates with the Van Slyke-Neill method under routine conditions.

The results of repeated measurements on a single blood sample and on standard known solutions are presented in Table 2. During the 6 hr of the experiment the blood sample was kept almost constant by continuous equilibration in a Lauê tonometer. The Van Slyke technique and the infrared method provided nearly identical results on blood, even though

TABLE 1. *Carbon dioxide content (mM/liter) of blood determined by the Van Slyke-Neill method and infrared method under various conditions*

		n	M_1	M_2	$\bar{v}$*	Sv
Van Slyke-Neill method	Optimal conditions	29	21.96	21.98	−0.02	0.26
	Routine conditions	35	22.70	22.73	−0.03	0.37
Infrared method	Mean tonometer P_{CO_2} = 28.4 mm Hg	28	18.21	18.30	−0.09	0.23
	Mean tonometer P_{CO_2} = 55.5 mm Hg	28	23.60	23.61	−0.01	0.25
	Mean tonometer P_{CO_2} = 96.9 mm Hg	28	30.02	29.70	+0.32	0.46
Van Slyke-Neill (M_1) versus infrared method (M_2)		26	21.75	21.84	−0.09	0.41

n = number of samples, M = arithmetic mean, $\bar{v}$ = mean difference, Sv = standard deviation of the differences between duplicates. * No significant differences.

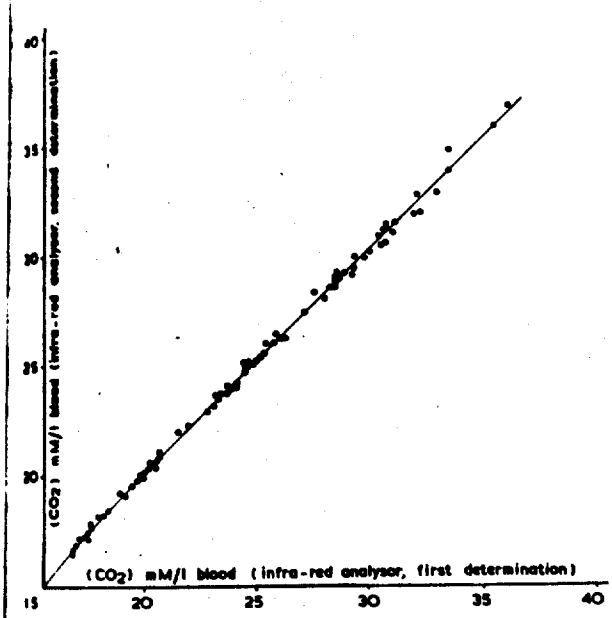

FIG. 2. Carbon dioxide content of 84 blood samples determined by the infrared method in duplicate.

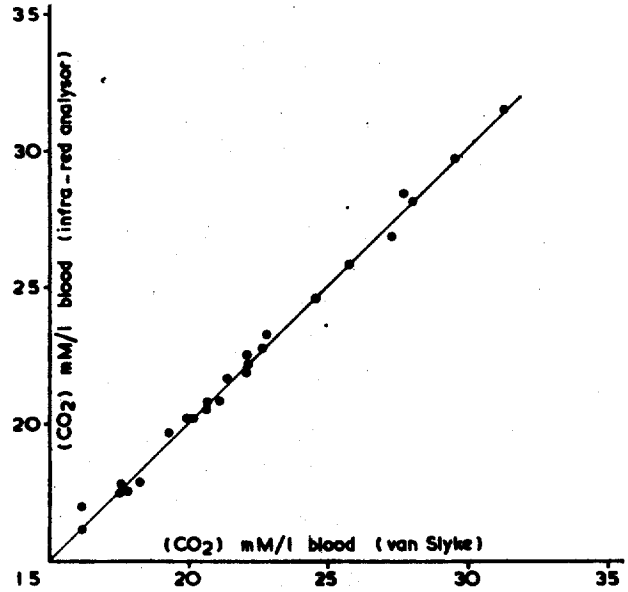

FIG. 3. Carbon dioxide content of 26 blood samples determined by the infrared method and compared with the CO_2 content as determined by the Van Slyke-Neill method.

the standard solutions were measured too low on the Van Slyke apparatus.

The infrared analyzer was calibrated with sodium bicarbonate solutions, their carbon dioxide content being calculated on the base of the weight of dry sodium bicarbonate substance (analytic grade B.D.H.; contains not less than 99.5% $NaHCO_3$). The purity of the sodium bicarbonate was checked flame-photometrically and also by heating it at 300 C in order to evolve the removable carbon dioxide and water. Calibration with sodium carbonate thus obtained yielded results which were only slightly different from those obtained by calibration with sodium bicarbonate (Table 2).

Low values for the Van Slyke technique have also been described in the case of sodium carbonate (12) and potassium carbonate (13) being used as standard solutions. The present data agree very well with those of Wilson et al. (12), who in a comparative study showed that the Van Slyke technique and gas chromatography provided identical results on blood, even though the standard sodium carbonate solution was measured too low with the Van Slyke technique.

Determination of the carbon dioxide content of blood by the infrared method provides several advantages over the Van Slyke-Neill procedure under comparable operating conditions. For reason of its simplicity, the results obtained are practically independent of the skill of technicians and on laboratory conditions.

Calibration of the apparatus requires only a few minutes and needs be performed but once a day. The apparatus is stable and sturdy and needs practically no maintenance. Our instrument has been in daily use for more than 2 years without any repairs.

The most important disadvantage of the infrared method, however, is its limitation to the analysis of carbon dioxide, whereas the Van Slyke-Neill method is more versatile (CO_2, O_2, and other gases). Furthermore, the infrared method should not be used for samples obtained during surgery because anesthetic gases may interfere.

TABLE 2. *Repeated measurements of carbon dioxide content of standard known solutions and of a single blood sample by the Van Slyke technique and infrared method*

Sample	Van Slyke Technique, mM CO_2/liter	Infrared Method (Scale Deflection)	Sample	Van Slyke Technique, mM CO_2/liter	Infrared Method, mM CO_2/liter
$NaHCO_3$	39.57	90.5	Blood	19.32	19.00
(40.0	39.45	90.0		18.98	18.85
mM/liter)	39.66	89.5		19.25	18.95
	39.54	89.5		19.21	19.00
	39.54	90.5		19.18	18.95
				19.14	19.05
Mean	39.55	90.0		19.40	19.60
				19.37	19.40
Na_2CO_3 (40.0	39.76	93.0		20.76	21.00
mM/liter)	39.76	93.0		20.81	20.95
	39.70	92.5		20.89	21.10
	39.71	92.5		20.76	21.05
	39.69	91.5		20.76	21.20
Mean	39.72	92.5	Mean	19.83	19.85

The authors are grateful to Prof. Dr. W. G. Zijlstra and coworkers for critical review of the manuscript. The technical assistance of Miss P. Degenhart and Miss B. G. M. Remkes is gratefully acknowledged.

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Brit. J. Psychiat. (1973), 122, 171–3

ABSTRACT

Augmented Respiratory Relief

A New Use for CO_2 Therapy in the Treatment of Phobic Conditions: A Preliminary Report on Two Cases

By A. ORWIN

Respiratory relief (R.R.) appeared to be a simple and rapid treatment for specific phobias and for the phobic components of more complex neurotic conditions (Orwin, 1971). The basic hypothesis was that the R.R., satisfying an intense need to breathe, if simultaneous with the presentation of a phobic stimulus, would inhibit the degree of anxiety previously provoked and that this inhibition would persist. The need to breathe was induced by voluntary restriction up to maximum voluntary respiratory arrest (M.V.R.A.). As in conventional desensitization (Wolpe, 1958), a hierarchy was drawn up and the patients were desensitized using R.R. instead of relaxation. Carbon dioxide-oxygen mixtures were introduced because CO_2 is known to stimulate respiration, and it was expected that by intensifying the need to breathe the speed and effectiveness of treatment would be increased by the augmented respiratory relief (A.R.R.).

Inhalations of CO_2 as a non-specific treatment for neurotic anxiety were introduced by Meduna (1947) who used 30 per cent CO_2. Others, including Wolpe (1958) advocated 70 per cent CO_2 with 30 per cent O_2. In the present study it was found, after some experimentation, that this range of 30 to 70 per cent CO_2 with O_2 to 100 per cent produced a marked respiratory response after M.V.R.A., precise percentages depending on individual tolerance. The gases came from separately monitored cylinders, the total flow being 10 litres per minute.

Method

The rationale of the technique was explained to the patients, and they were assessed for physical fitness before treatment. After familiarizing the patients with the effects of CO_2 inhalation, a simple procedure was learned: mask in preferred hand; deep inspiration and full expiration of air (reducing dilutent effect of alveolar air); mask to face and maximum inhalation of previously determined CO_2/O_2 mixture; mask removed and nose pinched by other hand with mouth closed; and signalling with free hand the imminence of forced inspiration, that is A.R.R., allowing time for stimulus presentation. A maximum of eight trials were used in a session and the interval between trials was two minutes.

Results

Ten phobias were rapidly removed in an uncontrolled series in which A.R.R. was used either as an adjunct or as sole treatment. Two illustrative cases are described:

A volunteer, a clinical psychologist aged 24 years with spider phobia was shown a large house spider (maximum leg spam 4 cm.) in a gold-fish bowl. Initially very anxious, after inhalation of 50 per cent CO_2/O_2 mixture and on R.R. he not only touched the spider but allowed it to run freely over his hand. Two minutes later he repeated this without R.R.; six months afterwards he remained phobia-free.

A woman aged 26 had for ten years suffered from a severe obsessional compulsive neurosis, with marked fear of faecal substances. Near approach to these caused compulsive washing of hands and clothes, while imagined contact led to minute examination of clothes and shoes probably followed by washing. To avoid such contacts she gazed constantly downwards, examining the ground, and was unable to look another person in the face; this again was linked to feelings of guilt and inferiority connected with adolescent masturbation. Treatment by desensitization, aversion therapies,

prolonged exposure and modelling as well as hypnotic suggestion and abreaction over 14 months produced considerable benefit. She had married, and managed limited household and clerical work, but was never free of her fears and still eyed the ground if out of doors. Marital problems had caused depression with deterioration, and for one year she had had implosion and desensitization on an out-patient and domiciliary basis with limited progress, when A.R.R. was commenced.

Typical progress through a hierarchial items is illustrated in the table.

After fifteen sessions of A.R.R. (plus four sessions of simple R.R.) she has held dog faeces, inspected an underground sewer, slept between unclean sheets and so on, all with minimal washing. She was also able to look another person in the face for the first time since the beginning of her illness and she became calm and self-possessed. She still avoided aspects of her home environment and had feelings of inferiority about being overweight and having facial and mammary hair; these were reality-based. There was need for further support because of marital difficulties and the personality deformity caused by her condition, but persistent and incapacitating symptoms had been more rapidly controlled than before with much less expenditure of time and effort.

Discussion

Carbon dioxide is known to stimulate the ventilatory response and to have a complex effect on the higher brain centres. Continuous inhalation of 10 per cent CO_2 increases the responsiveness of the hypothalamic-cortical system (Gellhorn, 1953), the excitation originating in the reticular formation (Wyke, 1963), and this activity is believed to play an important role in conditioning (Smythies, 1970). There is experimental evidence that CO_2 in concentrations of less than 5 per cent can act as a specific chemical brain stimulus which produces 'facilitation of the learning of adaptive somatic motor response' in birds and mammals (Weinstein, 1967). In A.R.R. there is a marked ventilatory response, and for a time during peak CO_2 stimulation, that is around A.R.R., it is possible that subcortical excitation occurs which facilitates the association of visceral relief with phobic stimulus. (So far electroencephalographic studies have produced equivocal evidence of excitation.) This association causes the disappearance of the stimulus-produced anxiety reaction, which may be inhibited or rendered undetectable by the ongoing autonomic excitation. Moreover, since cognitive factors may play a large role in the emotional labelling of sympathetic activity (Schachter, 1971), it follows that if the excitation was detectable it could be interpreted as a

Table I

Desensitization using A.R.R.: Session 6: Contamination by cow manure using:

(a) Specimen of cow manure			(b) Therapist's hand after touching specimen	
Trial no.	Concentration CO_2/O_2		Response at A.R.R.	Comments
1	70	30	(a) Viewed	No anxiety
2	70	30	(a) Touched	No anxiety
3	70	30	(a) Held	Minimal anxiety expressed fear that clothes might become contaminated
4	70	30	(b) Several cm. from patient's jumper	No anxiety
5	70	30	(b) 1·0 cm. from patient's jumper	No anxiety
6	70	30	(b) 0·5 cm. from patient's jumper	No anxiety
7	70	30	(b) Touched and held patient's jumper	No anxiety

manifestation of the progressing relief response.

Wolpe (1969) suggested that if the inspiration of CO_2/O_2 mixtures caused a powerful anxiety-inhibiting excitation it could be employed as a specific for deconditioning, and he referred to a claim by Philpott (1967) that this had been achieved while the patient was hyperventilating from continuous inhalations, but there were no details provided. It would appear that the intensification of response and the precision allowed by exact timing of presentation and relief in A.R.R. would be more effective.

A.R.R. is simple to apply, and therapy time has been less and the effects more rapid than with R.R., but there are disadvantages; for example the treatment must be medically supervised. This restricts nurses in the clinic, and prevents domiciliary work by nurses, social workers and relatives. However, if A.R.R. proves to be reliable—and this will depend as with simple R.R. on further clinical investigation and controlled trials—it could be a valuable adjunct to the standard treatment of phobias.

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AN ELECTROCHEMICAL TECHNIC FOR MEASURING CARBON DIOXIDE CONTENT OF BLOOD

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FOREWORD

This work was accomplished in the Physiology Branch under task No. 775802, between September 1965 and March 1966. The paper was submitted for publication on 12 January 1967.

Alton J. Rahe made the statistical analysis. Carlos Tamez and A/1C Dennis J. Watson gave technical assistance. Roland Dornes constructed the glass electrode holder.

The membrane material was obtained from Dr. Ethel G. Mulleson of Dow Corning Center Aid to Medical Research.

This report has been reviewed and is approved.

James B. Nuttall

JAMES B. NUTTALL
Colonel, USAF, MC
Commander

ABSTRACT

An electrochemical technic for measuring carbon dioxide content in whole blood has been devised and evaluated. This technic has been extended to measure the Bunsen solubility coefficient of carbon dioxide. The method requires a membrane-covered pH electrode. The electrode incorporates tris(hydroxymethyl)aminomethane buffer in the isolated electrolyte and is covered with a Silastic membrane. These features provided good stability between readings which proved to be of advantage in carrying out the technic. Two-thirds of the full electrode response time was reached in 30 seconds. This electrode system permits rapid determinations of carbon dioxide content in blood samples of less than 1 ml. The measurement of carbon dioxide content is performed by hemolyzing and acidifying a blood sample in such a manner that the released carbon dioxide goes into physical solution. The increase of tension caused by this physically dissolved carbon dioxide is measured by exposing the sample to the previously calibrated electrode. Results obtained using this technic compare favorably with the Van Slyke manometric technic. While use of the technic apparently requires some compromise with accuracy (standard deviation of replicate samples = 0.76 vol. % compared with 0.12 vol. % for the Van Slyke), its convenience may outweigh this consideration in certain routine applications.

AN ELECTROCHEMICAL TECHNIC FOR MEASURING CARBON DIOXIDE CONTENT OF BLOOD

I. INTRODUCTION

Since 1954, when Stow et al. (18, 19) first described their membrane-covered electrochemical cell for measuring carbon dioxide in blood, extensive application has been made of the concept of inserting a permeable barrier between a sample being measured and an electrode system. In such a system, the constituent of interest is permitted to diffuse across the barrier to affect the electrical characteristics of an electrochemical detector cell. In 1956 Clark (5), and later Severinghaus and Bradley (16), reported on the use of this concept and related systems for the measurement of oxygen and carbon dioxide in blood. Neville (12, 14), who was initially interested in the problem of measuring oxygen in the gas phase introduced several modifications designed to permit operation of such devices in aerospace environments. Actually, the solution to the problem of measuring gases in blood and other tissues by this technic had been made obvious by Clark (4), who described the use of cellophane, polyethylene, and several other semipermeable materials for separating blood from a sensitive platinum electrode used to measure oxygen. Clark made use of polarographic technics in his work. His paper cites several earlier uses (3, 7) of covered electrodes for measuring oxygen. Other investigators have described electrochemical structures which incorporate the idea of separating both electrodes from the sample being measured with a barrier. Thus, Jacobson's (9) paper illustrates a device for measuring oxygen in gases that makes use of the electrochemical reduction of oxygen on a small active surface of a carbon tube electrode, the outside of which is in contact with an electrolyte containing a reference electrode. The inactive inner wall of this tube electrode separated his cell from the gas sample being measured. Connelly et al. (6) also described an instrument incorporating a platinum microelectrode in an electrochemical cell which was separated from the sample being measured by a nerve membrane or blotter paper. These authors illustrated the use of their device by measuring oxygen utilization in respiring nerve tissue and also by directly measuring the oxygen concentration in gas mixtures.

It is interesting to note that before artificial membranes became easily available Blinks and Skow (1, 2) in 1938 used electrochemical cells in conjunction with the natural outer membrane or cuticle of plants to measure oxygen and carbon dioxide changes during photosynthetic activity. These authors also mention the use of a covering of "green cellophane" for comparing and testing the response of their electrode systems. Among other advantages listed by Blinks and Skow for this instrumental combination are the decreased diffusion time and the small volume of solution restricted to a very thin film between the cuticle membrane and the indicator electrode. In view of the wide use of this arrangement in modern electrochemical gas analysis, credit for its original exploitation belongs to these investigators.

A shortcoming of the commercially available electrodes of the type discussed above for measuring carbon dioxide has been the instability of these devices. Our own experience indicated that drift of these units was a continual source of annoyance and delay because of the constant need for recalibration. Present

interest in this type of device relates to our desire to utilize the instrument in a method for determining carbon dioxide content of blood in conjunction with a previously described (11, 13) electrochemical method for oxygen content. Developments related to both the improvement of the electrochemical detector and the validation of the carbon dioxide content technic are described in this report.

After considering the geometry and chemistry of several CO_2 electrode systems with which we had worked, modifications were made to provide not only a fast-responding electrode but also an electrode having improved stability. Our experience has indicated that the system described here requires calibration only once a day, at the most, and all the results reported here were obtained on the basis of a single daily calibration.

The technic for measuring the concentration of carbon dioxide uses less than 1 ml. of blood and involves the conversion of bound gas into physically dissolved gas and the measurement of the developed carbon dioxide tension electrochemically. Severinghaus (17) described a method similar in concept but did not present validating data. During the preparation of this communication, work by Linden et al. (10) came to our attention which utilizes the technic described by Severinghaus to measure CO_2 content of dog blood. This technic will be discussed and compared to the present one.

Our results were obtained with human blood and plasma and compared with the manometric method of Van Slyke and Neill (20). Favorable results were also obtained by applying this technic to the measurement of the CO_2 solubility in acid solutions.

II. METHOD

Electrode system

In evaluating CO_2 electrode systems, primary consideration should be given to the stability of the uncovered glass electrode by testing the electrode with standard pH buffers. Proper evaluation of the characteristics of the pH electrode itself is significantly related to the success obtained with the covered system for carbon dioxide.

Saturated calomel and Ag-AgCl reference electrodes have been used and they worked equally well. Although a calomel reference electrode was used in obtaining the data for this report, we now favor the Ag-AgCl reference electrode because of the convenience of its preparation and use.

Figure 1 is a cross-sectional view of the CO_2 electrode assembly used in these experiments. A glass electrode is enclosed in a Lucite holder, these two units being housed in a stainless steel sheath. The electrode and holders are placed in a water bath in a slanted position approximately 60° with respect to the horizontal. A sample cuvette, which has a Plexiglas window, threads onto the steel sheath and permits all the active surface of the pH glass to project into the chamber of the cuvette. The glass active surface is separated from the sample by a Silastic membrane which allows CO_2 and other gases to diffuse to the pH electrode.

In figure 1, two O-rings *(A, B)* hold the membrane in place and also help to position the glass electrode within the Lucite holder. An O-ring *(C)* at the upper edge of the cuvette chamber seals the chamber when the electrode is inserted. A small piece of 0.005-inch thick Silastic membrane is stretched tightly over the tip of the glass electrode. An electrolyte space is provided on the glass electrode by interposing two layers of surgical cotton gauze. Tris(hydroxymethyl)aminomethane buffer of 0.005 *M* and pH 7.8 was used as the electrolyte fluid in the glass electrode compartment of the carbon dioxide detector cell. The buffer system has proved very satisfactory from the standpoint of both sensitivity and stability but no exact comparison with the usual bicarbonate-potassium chloride electrolyte mixture has been attempted.

The volume of electrolyte in the space between the outer membrane and the pH active surface of the glass electrode is very critical

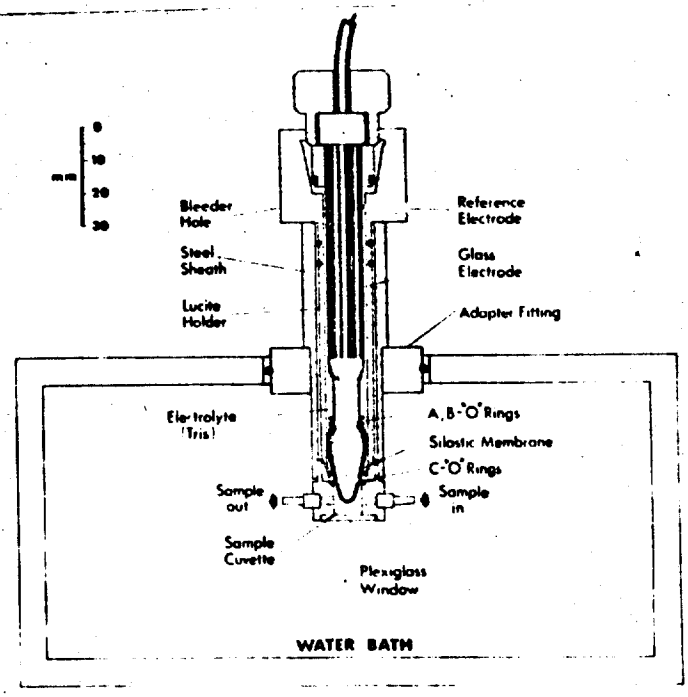

FIGURE 1

Sectional view showing the glass electrode, connection for fiber junction calomel electrode, plastic and metal cuvette, sample chamber, with Plexiglas window and water bath.

to the proper operation of the cell, particularly with regard to the response time, the stability, and the accuracy. Compared with small fluid spaces, large fluid spaces, obviously, will require greater amounts of carbon dioxide to diffuse across the membrane in order to equilibrate with the sample. This affects not only the response time, but also leads to a systematic error due to the change in the true tension of the sample caused by the equilibration process. The latter is significant, of course, only when small amounts of liquid samples are being measured. An additional consideration with comparatively large electrolyte spaces is that, other things being equal, there exists a larger cross-sectional area of contact with the solution forming the bridge to the reference electrode. As a consequence, a proportionately greater flux of carbon dioxide into and out of this bridge space occurs, depending on the carbon dioxide concentration gradients existing at any given moment. Attempts to use a vanishingly small electrolyte space between the glass electrode and the membrane (by applying the membrane directly to the electrode surface without a spacer), generally leads to unstable recordings because of the tendency for the electrolyte to pull away from the adjacent surfaces of the glass and the membrane. Both surface tension effects and the mechanical pressure exerted by the stretched membrane play a role in causing this undesirable result.

Thus the response time, the stability, and the accuracy of these electrode systems are interrelated and it is usually necessary to trade-off between these functional characteristics in order to best accommodate particular application problems.

The use of a gauze spacer beneath the membrane, with O-rings holding both of these

elements tightly against the glass electrode, gave good results. The O-rings not only hold the membrane in place, but also assure a minimum cross-sectional area for diffusion of carbon dioxide into and out of the bridge space. Conductivity adequate to insure proper action of the cell was obtained in spite of the exceedingly small contact area since the glass electrode itself undoubtedly contributes most of the resistance in any cell of this type.

Although long-term stability of the device is enhanced by use of a spacer, good short-term service with shorter response times could be obtained by using only a thin membrane over the electrode without a spacer. One such trial using 0.5 mil polyethylene with a small amount of a commercial preparation of carbonic anhydrase (Calbiochem) added to the electrolyte, gave a response time of six seconds for 90% of a step change (2% and 5% carbon dioxide). A closer evaluation of the use of carbonic anhydrase preparation to enhance the response time is currently underway.

The two O-rings shown on the Lucite holder in figure 1 act to insure proper alignment of the electrode within the assembly. A bleeder hole for excess electrolyte and an insertion hole for the calomel reference electrode are also shown.

Figure 2 shows schematically the electronic equipment used. The high impedance signal conditioner was a laboratory pH meter. The time required for two-thirds equilibration with the electrode described here (using cotton gauze as a spacer) was 30 seconds for changes of 20 mm. P_{CO_2} in the range of 14 to 60 mm. P_{CO_2} at 23° C.

Carbon dioxide technic

Carbon dioxide content in blood is measured by hemolyzing and acidifying a blood sample in the following manner: 10 ml. of 1.0 *M* NaH_2PO_4, pH 4.3, is placed in a 10 ml. glass-tipped syringe, being careful to avoid bubbles. This solution also contains a hemolytic agent of approximately 0.6 mg. % digitonin. Figure 3 shows the syringe with an unfolded needle-puncture stopper. The outer cavity of the stopper is also filled with solution B to prevent air bubbles from getting into the syringe when the blood sample is introduced. Two glass beads of known volume are placed in the syringe to aid in the mixing of sample and acid. Mixing was accomplished by rotating slowly by hand for one or two minutes until hemolysis was complete.

Figure 4 shows a sample being introduced into the acid mixture. A 0.75 ml. blood sample is introduced into solution B by means of a 25-gage, 2-inch needle. After solution B and the sample are mixed, the sample is ready to be introduced into the 1 ml. sample chamber of the CO_2 cell.

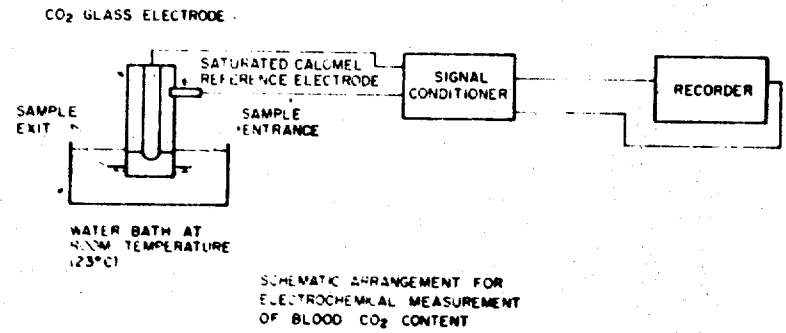

FIGURE 2

Block diagram of equipment. The high impedance signal conditioner used was a pH meter connected to a potentiometric recorder.

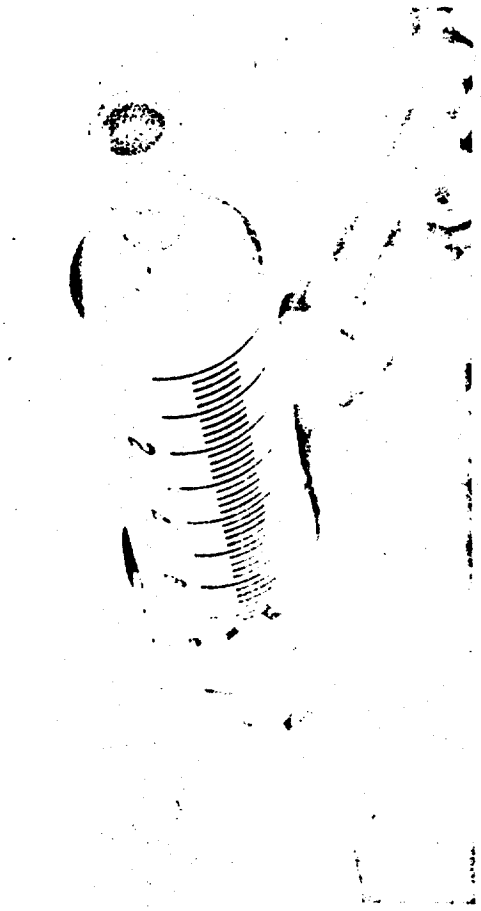

FIGURE 3

The 10-ml. syringe contains solution B with two glass beads used for mixing. The cavity of the stopper shown is also filled with solution B.

When introducing the solution B – blood mixture into the sample chamber, care should be taken to avoid the emergence of bubbles. This can be accomplished by holding the syringe upright and allowing gravity to push the syringe plunger down. The volume of this mixture is more than sufficient to flush the sample chamber of the electrode assembly and smaller sample volumes can be used if desired.

Additional care should be taken to insure that no gas bubbles are formed after the bound CO_2 is released by acidification. Body heat transferred to the syringe because of handling can cause a local rise in temperature of the sample mixture. The subsequent lowering of the gas solubility, in turn, can cause enough supersaturation to permit the evolution of bubbles from the solution. Pressure waves caused by forcing the plunger also lead to bubble formation.

The results reported here were obtained with freshly drawn whole human blood, both heparin and citrate being used as anticoagulants. All the blood samples, except two which were collected anaerobically and measured without further treatment, were equilibrated at room temperature (23° C.) with gases of known tensions.

Van Slyke technic

The results obtained with the electrochemical technic were compared with the Van Slyke and Neill (20) method using 1 ml. blood samples. The computed CO_2 contents of the sodium bicarbonate standards used in the determination of solubility and for standardization purposes (see below) were also checked by direct measurements of the Bunsen solubility coefficient of carbon dioxide in solution B by means of the Van Slyke method (21).

Solubility technic and electrode calibration

Determination of the Bunsen solubility coefficient for CO_2 in solution B was made by acidifying standard sodium bicarbonate solutions and measuring the CO_2 tension developed. This is carried out in the same manner as described for blood. The sodium bicarbonate concentrations used were 9.3, 14.0, and 18.6 mM./liter.

In the solubility determinations with the manometric technic, solution B was equilibrated with known CO_2 tensions in a tonometer system. The sample was then transferred to the Van Slyke apparatus by means of a previously cooled Oswald pipet. Cooling the pipet prevented loss of CO_2 from solution during handling of the samples. The water jacket

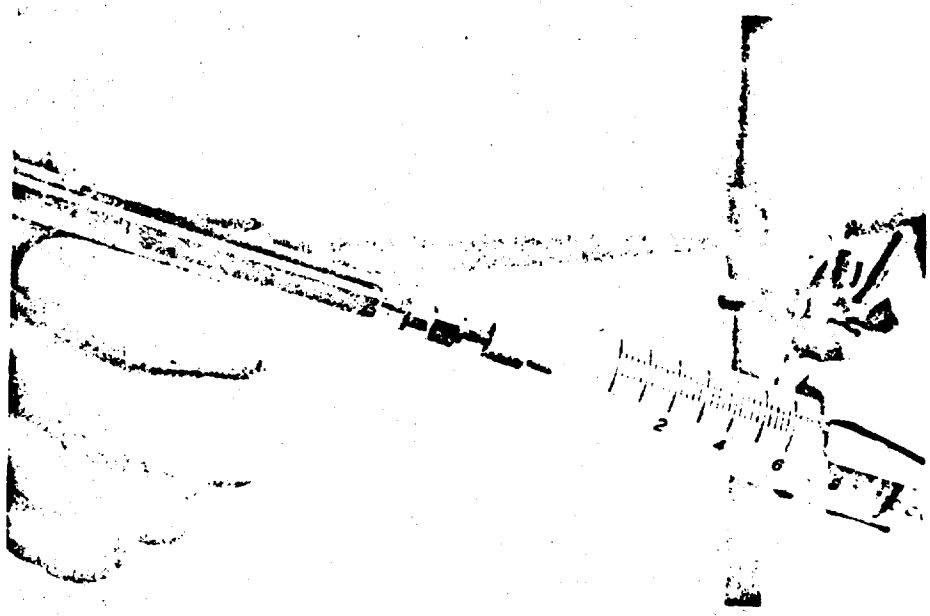

FIGURE 4

The sample is shown being introduced into the syringe containing solution B.

chamber of the Van Slyke was connected to the water bath of the tonometer to maintain the same temperature in both units.

Calculations

From the measured partial pressure of carbon dioxide in the solution B–sample mixture, the volume of carbon dioxide in the original sample can be computed from Henry's law if the dilution factor and solubility coefficient are known. The average Bunsen solubility coefficient of CO_2 in solution B was found to be 62.4% of the comparable water value in a temperature range of approximately 21° to 27 C. The following relation was used for the content determinations:

$$\text{Vol. \%} = (\alpha)\left(\frac{P_{CO_2}}{760}\right)\left(\frac{V+v}{v}\right)(100) \quad (1)$$

where

α = Bunsen solubility coefficient (cc./cc. = 0.624 α H_2O),

P_{CO_2} = carbon dioxide tension of solution B sample,

V = volume of solution B,

v = volume of sample.

The Bunsen solubility coefficient is calculated from the data developed with the standard bicarbonate solutions in similar fashion:

$$\alpha = (M\ NaHCO_3)\ (22.26)\left(\frac{760}{P_{CO_2}}\right)\left(\frac{v}{V+v}\right) \quad (2)$$

III. RESULTS

Electrode calibration

The electrode can be calibrated with either solution B saturated with known CO_2 concentrations or with water-saturated gases. Figure 5 shows a typical calibration comparing the two methods of standardizing the electrode. While the results have been consistently similar, most of our calibrations carried out in connection with this work were obtained with

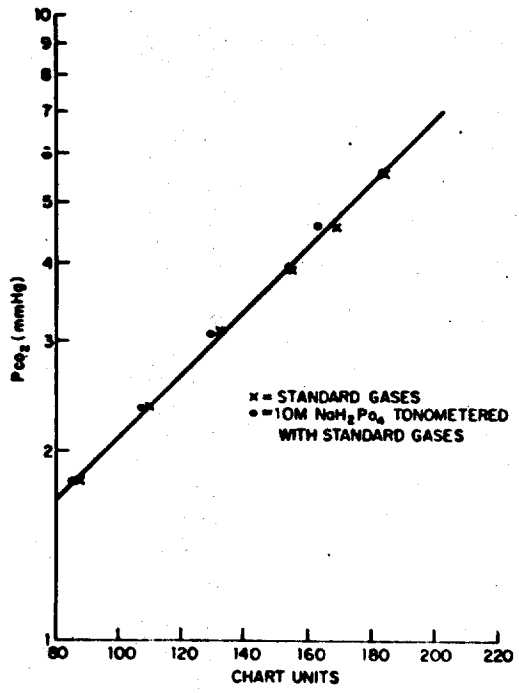

FIGURE 5

Calibration showing relationship between CO_2 electrode output and CO_2 tension using both gases and equilibrated solution B.

gas-saturated solutions. For routine purposes, the use of wet gases is probably more convenient. The percent CO_2 in the gas mixtures used was analyzed by the micro-Scholander (15) technic.

Solubility

One hundred thirty-four Van Slyke solubility measurements were made on solution B between 22.2° and 27.2° C. and compared with 70 electrode determinations made at temperatures from 20.6° to 24.0° C.

Because the data for the two methods were not comparable on the basis of temperature and also because of the nonlinear relation of the change in solubility with temperature, the results were normalized by converting each measurement to a percentage of the water solubility (8) at an equivalent temperature. The electrode determinations yielded a mean value of 63.4% of the water solubility (S_M = 2.93) and the Van Slyke 61.6 (S_M = 2.27). Since the group means determined by the two methods were statistically indistinguishable and since no other rational basis for selection of one or the other presented itself, the final solubility figure utilized (62.4%) was based on the mean of the pooled data from both methods. Several additional manometric measurements were made to test whether the addition of blood or sodium bicarbonate to solution B had any significant influence on the solubility of carbon dioxide. No differences were observed.

Comparative content data

Simultaneous determinations of the carbon dioxide content of blood using both the electrode system and the Van Slyke manometric technic are compared in figure 6. The heavy center line is the regression line for the data which consist of 82 different paired analyses performed over a period of 11 days on 41 separately treated blood samples. The equation for this line is as follows:

$$\text{Electrode (vol. \%)} = 0.881 + 0.982 \text{ Van Slyke (vol. \%)}$$

All the data points fell within a range of ± 3 vol. % of the regression line. The standard deviation of replicate samples run with the electrode was 0.76 vol. % and with the Van Slyke 0.12 vol. %.

The results with five plasma samples measured in the same manner as whole blood gave an average difference of less than 0.76 vol. %.

Carbon dioxide saturation curve

To further test the operational utility of the electrode system and the content technic, carbon dioxide saturation curves of human blood from the same individual were determined. Heparinized blood was equilibrated with several carbon dioxide tensions at 23° C. In the case of the reduced curve, nitrogen was used as the diluting gas. Nitrogen plus oxygen

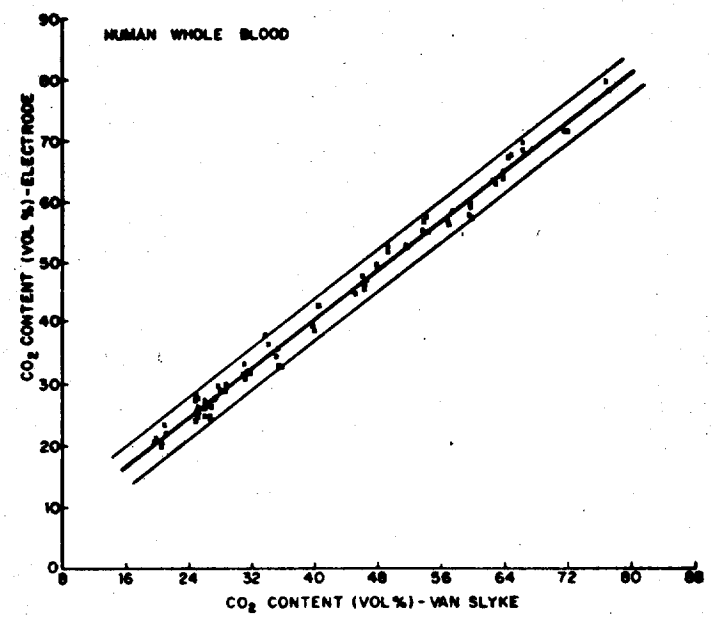

FIGURE 6

A comparison of the carbon dioxide content of blood determined simultaneously with the CO_2 electrode technic and Van Slyke manometric technic. The heavy line is the "least squares" regression line for the data.

sufficient to completely saturate the hemoglobin was used to obtain the oxygenated curve. The results (fig. 7) show the expected shape of the curve as well as the Haldane effect.

IV. DISCUSSION AND CONCLUSIONS

Analysis of the results obtained from the present study indicate that while the electrode technic for determining carbon dioxide content of blood is certainly convenient, its application should be attempted only where due allowance is made for the error which can be tolerated. The limiting precision of the carbon dioxide pressure detector (at a 99% confidence level) cannot realistically be set at better than approximately ± 2 mm. Hg under the conditions applying to the tests reported here. The limiting absolute accuracy is, of course, related to the accuracy of the micro-Scholander technic, which figure of merit is generally quoted as ± 0.2 mm. Hg. Since a 1 mm. Hg change in final carbon dioxide tension is equivalent to approximately 1 vol. % carbon dioxide content in the original blood sample at the dilution used in these tests, the range of values obtained in the comparisons with the Van Slyke (see fig. 6) is quite predictable on the basis of the limiting accuracies of the measuring technics used. The deviation of the least squares regression line shown in figure 6 from the origin and the lack of a perfect slope of 1.0 is probably accounted for by small, consistent errors in the micro-Scholander determinations of the calibrating gases as well as the small nonlinearity between the log P_{CO_2} and the cell voltage, particularly at the low end of the scale.

Data presented by Linden et al. (10), while appearing to be more uniform than those presented here, actually involve the use of

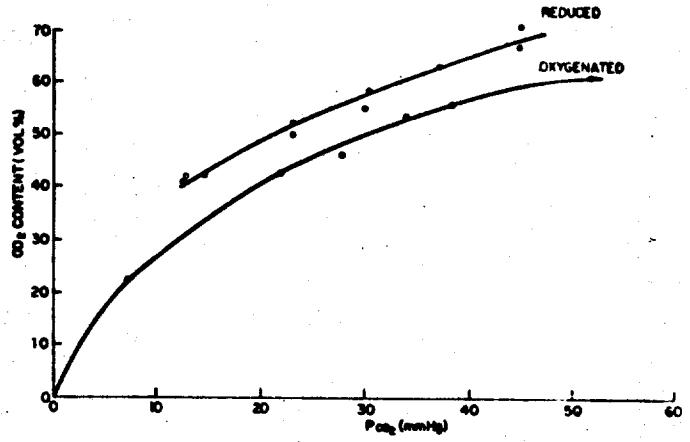

FIGURE 7

The relationship between CO_2 content and tension measured by the electrode technic using oxygenated and reduced whole blood samples.

statistically derived "points" in computing the regression line representing the comparison between the electrode and the Van Slyke technic. The precision of the electrode system used by Linden is stated to be "± 2.5% of the P_{CO_2} for a single determination (P = 0.05)," this being the same order of magnitude generally found with the system used in the present study. It should be noted, however, that Linden et al. calibrated their electrode before and after each experimental run, whereas our present results were obtained using a single daily calibration as a basis for our experimental computations of the carbon dioxide content of the blood samples. One might conclude from this either that the more elaborate calibration was unnecessary or that the electrode system used in the present study had greater stability than that used by Linden et al. At any rate, it appears reasonably certain that the practical application of the electrode content technic must involve some degree of compromise with accuracy as compared with the Van Slyke technic. If the nature of the application is such that the wider confidence limits applying to the electrode technic do not endanger conclusions based on the data, the convenience and time-saving obtained with the electrode method may dictate its selection over alternative methods.

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An electrochemical technic for measuring carbon dioxide content in whole blood has been devised and evaluated. This technic has been extended to measure the Bunsen solubility coefficient of carbon dioxide. The method requires a membrane-covered pH electrode. The electrode incorporates tris-(hydroxymethyl)aminomethane buffer in the isolated electrolyte and is covered with a Silastic membrane. These features provided good stability between readings which proved to be of advantage in carrying out the technic. Two-thirds of the full electrode response time was reached in 30 seconds. This electrode system permits rapid determinations of carbon dioxide content in blood samples of less than 1 ml. The measurement of carbon dioxide content is performed by hemolyzing and acidifying a blood sample in such a manner that the released carbon dioxide goes into physical solution. The increase of tension caused by this physically dissolved carbon dioxide is measured by exposing the sample to the previously calibrated electrode. Results obtained using this technic compare favorably with the Van Slyke manometric technic. While use of the technic apparently requires some compromise with accuracy (standard deviation of replicate samples = 0.76 vol. % compared with 0.12 vol. % for the Van Slyke), its convenience may outweigh this consideration in certain routine applications.

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A Concept of Triple Tolerance Limits Based on Chronic Carbon Dioxide Toxicity Studies

KARL E. SCHAEFER, M.D.

MOST OF THE physiological ranges of atmospheric components were, in the past, studied in acute experiments which lasted usually several hours but did not extend over days. Tolerance limits established on the basis of detected physiologic changes and impairment of performance in single or repeated exposures for eight hours have to be significantly reduced to be applicable to conditions of chronic exposure in the sealed space cabin or in the true submersible. Tamas[32] suggested as a rule of thumb that the maximal allowable concentrations (MAC values) of various agents published in *Industrial Health Reports* which are based on eight hours a day, five days a week exposure, might be lowered by a factor of 4 to be used as guides for prolonged continuous exposure. For example, the MAC value for carbon monoxide is 100 parts per million (p.p.m.) while the Navy proposed figure for nuclear-powered submarines is 25 to 30 p.p.m. In the older literature, 3 per cent carbon dioxide was considered a permissible threshold concentration; however, the experience in submarines during World War II[17,18] and subsequent experiments with prolonged exposure to 1.5 per cent carbon dioxide[2,7,16,19] established the necessity to keep the carbon dioxide concentration in the neighborhood of 1 per cent and preferably below 1 per cent carbon dioxide for conditions of continuous prolonged exposure. This serves to emphasize the existence of an inverse time-concentration relationship in which time can replace concentration.

In addition, a combination of changes in several factors can result in different effects dependent upon acute or chronic exposure. For example, it is well known that addition of carbon dioxide to low oxygen mixtures has, in experiments of short duration, a beneficial effect in alleviating symptoms of oxygen lack and preventing their occurrence.[5,9] This influence of carbon dioxide is based upon stimulation of respiration and circulation with a resulting improvement in oxygen supply. There is, however, evidence that these effects are greatly reduced or not present under conditions of chronic carbon dioxide exposure.[18]

As a consequence of the rapid development in space technology, the design engineer of life support systems in space capsules came up with requirements for a better quantitative description of man's characteristics and more reliable criteria of tolerance limits. This forces us to take a new look at what we call tolerance limits.

It appeared that the results of the extensive studies on chronic carbon dioxide toxicity carried out over the past ten years in the U. S. Naval Medical Research Laboratory, New London, Connecticut, would have some bearing on this problem and indicate a possible new approach.

Review of Experimental Findings.—Experiments in which human subjects were exposed to 3 per cent carbon dioxide in 21 per cent oxygen for periods of three to six days showed a depression of the respiratory response to 3 per cent and, more significantly, to 5 per cent carbon dioxide after several days.[3,22] A large number of physiologic changes were found to be associated with the two phases of uncompensated and compensated respiratory acidosis produced

From the U. S. Naval Medical Research Laboratory, New London, Connecticut.

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by inhalation of 3 per cent carbon dioxide such as alterations in respiratory exchange ratio,[22] decreased oxygen utilization during rest and exercise,[22] cardiovascular capacity and pulse

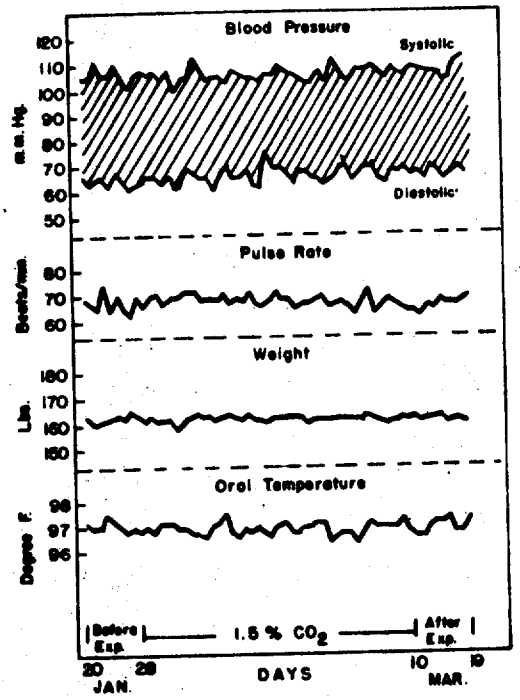

Fig. 1. Effect of prolonged exposure to 1.5 per cent carbon dioxide for forty-two days on systolic and diastolic blood pressure; pulse rate, body weight and oral temperature in twenty-three subjects.

rate,[23,24] response to heat and cold,[24] electrolyte exchange in blood and urine,[22] changes in central nervous system activity as indicated in chronaxia measurements[25] and EEG.[26] A significant impairment of performance was noted during prolonged exposure to 3 per cent carbon dioxide in 21 per cent oxygen.[17,25]

Field tests carried out on submarines during war patrols under conditions of exposure to 3 to 3.5 per cent carbon dioxide and 15 to 17 per cent oxygen also demonstrated significant physiologic changes and deterioration of performance.[17] Laboratory tests, in which subjects were exposed for seventy-two hours to 3 per cent carbon dioxide and 17 per cent oxygen also demonstrated a decrease in performance.[11] It has been pointed out that the lowered oxygen content does not play an important role in causing the described effects.[18]

In animal experiments in which guinea pigs, rats, mice and dogs were exposed to 3 per cent carbon dioxide in 21 per cent oxygen for prolonged periods biphasic reactions associated with periods of uncompensated and compensated respiratory acidosis were found in body weight, motor activity, blood sugar, alkali reserve and adrenal cortical activity.[6,27,28,31] Excitability of the central nervous system as tested with metrazol seizure activity showed a period of excitation followed by depression during prolonged exposure to 3 per cent carbon dioxide.[4] Histopathologic changes of liver, kidney, heart and striated muscle of guinea pigs exposed for periods up to twenty-one days to 3 per cent carbon dioxide were reported by Zinck.[33] Adrenal hyperplasia and involvement of lymphatic organs were noted in a subsequent study of guinea pigs exposed to 3 per cent carbon dioxide for prolonged periods.[29]

Thus these various effects of chronic exposure to 3 per cent carbon dioxide may be summarized by the statement that these exposures (the level of 3 per cent carbon dioxide) produced alterations in basic physiological functions and were accompanied by performance deterioration, none of which returned to normal during prolonged exposure in spite of developed acclimatization. Animal experiments revealed significant physiological effects and histopathological changes.

Studies of prolonged exposure to carbon dioxide concentration lower than 3 per cent were carried out in recent years at the U.S.N. Medical Research Laboratory in New London, Connecticut. Results of a large scale experiment (Operation Hideout) in which twenty-three subjects were confined in a submarine and exposed to 1.5 per cent carbon dioxide in 21 per cent oxygen over a period of forty-two days with a nine-day control period, prior to and also following exposure, showed that basic performance and basic physiological functions were not ap-

preciably affected at this lower level of carbon dioxide.[7]

As shown in Figure 1, weight, pulse rate, systolic and diastolic blood pressure and oral temperature, measured daily in twenty-three subjects at 8 A.M., did not exhibit significant alterations throughout the experiment.[3] Statistical evaluation of these data is described in Table I. The forty-two-day period of exposure to 1.5 per cent carbon dioxide has been divided into two parts corresponding to the phases of uncompensated respiratory acidosis (one to twenty-three days) and compensated respiratory acidosis (twenty-four to forty-two days). The mean values and standard deviations of these five parameters show a remarkable constancy throughout the experiment. Only the diastolic blood pressure shows a slight increase during the exposure to carbon dioxide and during the nine-day recovery period on air.

TABLE I. EFFECT OF PROLONGED EXPOSURE TO 1.5 PER CENT CO_2 ON SYSTOLIC AND DIASTOLIC BLOOD PRESSURE, PULSE RATE, BODYWEIGHT AND ORAL TEMPERATURE

		Control	Exposure to 1.5 Per Cent CO_2 for		9-Day Recovery On Air
			1-23 Days	24-42 Days	
Systolic blood pressure	Mean*	106.4	106.8	106.5	108.1
	S.D.	10.1	8.4	8.7	8.4
	N	23.0	23.0	23.0	23.0
Diastolic blood pressure	Mean	64.8	66.2	67.3	67.3
	S.D.	8.8	8.9	8.1	8.5
	N	23.0	23.0	23.0	23.0
Pulse rate	Mean	67.6	68.5	67.1	67.4
	S.D.	8.3	8.8	9.0	7.3
	N	23.0	23.0	23.0	23.0
Body-weight	Mean	162.0	162.0	162.2	162.0
	S.D.	19.4	24.2	21.7	20.8
	N	23.0	23.0	23.0	23.0
Oral temperature	Mean	97.10	96.90	96.94	97.04
	S.D.	0.89	0.73	0.66	0.46
	N	23.00	23.00	23.00	23.00

*Means of the mean values of data obtained from twenty-three subjects during each experimental period. Measurements were made daily.

Sensory and perceptual processes were not affected during prolonged exposure to 1.5 per cent carbon dioxide. Threshold measurements during adaptation to darkness using a biophotometer did not show significant changes throughout the experiment. Data on visual acuity, amplitude of visual accommodation and depth perception, as well as pitch discrimination, did not reveal any significant alterations during the exposure to 1.5 per cent carbon dioxide.

Studies of psychomotor efficiency as measured by a battery of tests such as Minnesota Manual Dexterity test, letter-cancelling test, McQuarrie test of mechanical ability, strength (maximum hand dynometer readings) and complex coordination test did not exhibit any impairment in psychomotor performance as a result of exposure to 1.5 per cent carbon dioxide.

No decrement was found in the perceptual span and immediate memory as well as in problem solving abilities during the experiment.[7] Eighteen subjects took the Thematic Apperception Test (TAT) and sentence completion test twice: (1) prior to the experiment and, (2) after spending thirty days in the submarine. At this time they had been exposed for twenty-one days to 1.5 per cent carbon dioxide. Results of the second test after confinement appeared to indicate increased uncooperativeness, apathy, heightened sexual needs and a desire to leave.[5]

Psychiatric studies conducted in "Operation Hideout" revealed only moderate increases in anxiety which appeared to be related to the situational factors involved. Subjects who were found to be unstable in a psychiatric assessment prior to the beginning of the experiment showed

more severe and prolonged subjective complaints.[14]

In contrast to the rather negative findings on basic physiologic functions and performance during prolonged exposure to 1.5 per cent carbon dioxide, were the results of studies on respiration,[19] acid base balance and electrolytes.[16] They demonstrated the existence of a phase of uncompensated respiratory acidosis lasting for twenty-three days and a phase of compensated respiratory acidosis from the twenty-fourth to the forty-second day of exposure to 1.5 per cent carbon dioxide. This is indicated in the fall and subsequent rise of blood and urinary pH, the latter changes being associated with alterations in urinary carbon dioxide excretion (Fig. 2). The carbon dioxide excretion of the lungs closely paralleled that of the urine. Furthermore, a significant depression of the respiratory response to 5 per cent carbon dioxide was found after thirty-five days of exposure to 1.5 per cent carbon dioxide (Fig. 2).

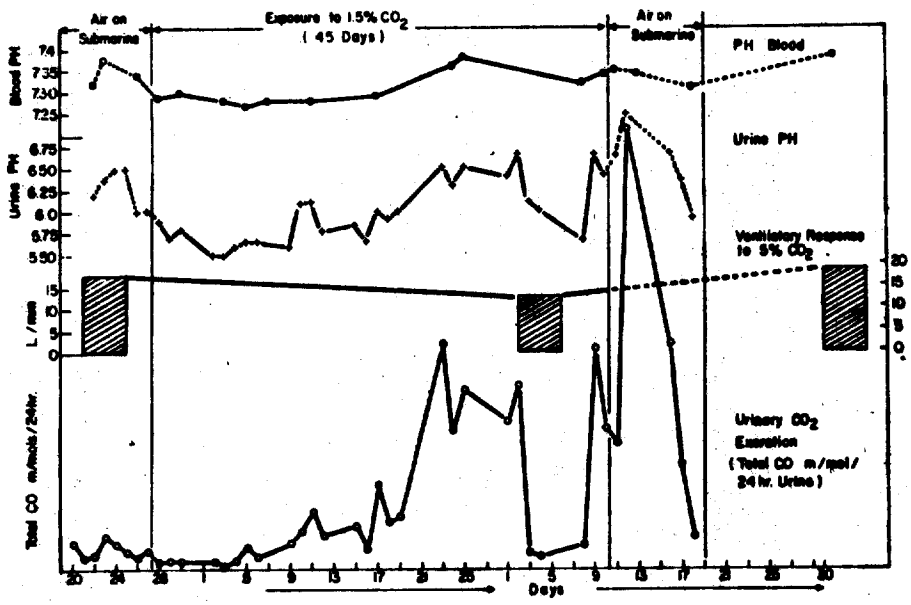

Fig. 2. Changes in acid base balance (venous pH, urinary pH, urinary carbon dioxide excretion) and ventilatory response to 5 per cent carbon dioxide during and after prolonged exposure to 1.5 per cent carbon dioxide for forty-two days (21 subjects).

During forty-two days' exposure to 1.5 per cent carbon dioxide as well as during the nine-day recovery on air period, sodium content of the red cells was increased and potassium content decreased in nearly equivalent amounts. The carbon dioxide content of the red cells was not only increased during the exposure period but remained elevated during the subsequent nine-day recovery period.

Data on plasma calcium and inorganic phosphorus obtained during this experiment are suggestive of an altered calcium phosphorus metabolism.[20] Plasma calcium in the venous blood paralleled the changes of the pH depicted in Figure 2 and showed a decrease during the first twenty-three days of exposure, a return to initial levels during the latter part of exposure, followed by a rise above control values during the eight to nine-day recovery period on air following exposure. After four weeks of recovery initial values were reached. Plasma inorganic phosphorus was elevated throughout the carbon

dioxide exposure period and remained at this increased level during the nine-day recovery period and even after four weeks of recovery.

Investigations of the cardiovascular capacity and the assessment of stress imposed on the organism during this prolonged exposure to 1.5 per cent carbon dioxide also demonstrated significant effects. Tests of the circulatory system with different degrees of work load, that is (1) postural change from supine to standing position, (2) exercise in the form of twenty-five hops on one foot and, (3) step-up tests, showed an increased pulse rate response under carbon dioxide and an increased time for pulse rate to return to initial levels. These findings were interpreted as a reduction of circulatory reserve under prolonged exposure to 1.5 per cent carbon dioxide which might become important under heavier workloads.[8]

An increased adrenal cortical activity was found during the forty-two days' exposure to 1.5 per cent carbon dioxide and the following ten-day recovery period on air as indicated by a decrease in the absolute number of circulating eosinophils and a significant increase in the 17-ketosteroid output in urine. It is noteworthy that, based on the indices of adrenal cortical response, the "physiological stress" was larger during the compensated phase of respiratory acidosis (twenty-four to forty-two days of exposure) and during the nine-day recovery period on air than during the early phase of uncompensated acidosis.[13] This is the more remarkable in view of the fact that the number of subjective complaints showed the opposite trend.[14] This would indicate that the psychological effects of confinement subsided during the twenty-four to forty-two day period of exposure and the nine-day recovery period which coincided with the greatest physiological stress. In the latter phases, the carbon dioxide tension (pCO_2) was elevated and the pH came back to normal while both pH and pCO_2 were changed during the early phase of uncompensated respiratory acidosis. The results suggest that the elevated pCO_2 level alone (independent of pH change) produces a significant "physiological stress" response. This interpretation is supported by evidence from experiments on rats and guinea pigs carried out simultaneously under the same conditions of exposure to 1.5 per cent carbon dioxide for forty-two days.[12] They also showed a significant increase in adrenal cortical activity during the same two periods in which the pH was not different from control levels and the pCO_2 elevated. The factor of confinement did not play a role in these animal experiments.

Growth curves were obtained in comparable groups of guinea pigs exposed continuously to 1.5 per cent, 3 per cent and 15 per cent carbon dioxide respectively for periods varying from fifty to ninety-three days. These tests showed a depression of growth under 1.5 per cent carbon dioxide which lasted approximately twenty-five days when the normal growth rate was resumed. A similar effect was observed under 3 per cent carbon dioxide for a period of thirty-eight days. Under 15 per cent carbon dioxide, transitory increases in weight were observed around the tenth and twentieth day which were followed by a slight but steady decline during the latter part of the fifty-day exposure period.[21]

Studies of the carbohydrate metabolism in rats and guinea pigs exposed for forty-two days to 1.5 per cent carbon dioxide,[12] showed a fall of liver glycogen and muscle glycogen while the blood sugar was maintained at a constant level.

It seems important to emphasize that these changes in carbohydrate metabolism were still present after twenty-five to twenty-eight days of exposure when a compensation of the respiratory acidosis was attained in animals as well as in men. One has to conclude therefore that under these conditions, the increased pCO_2 tension was the decisive factor rather than the change in pH. This interpretation is supported by recent findings of Hastings[10] who demonstrated that the net glycogen synthesis in liver slices is directly proportional to the logarithm of carbon dioxide tension at a constant pH of 7.40. One of the conclusions of Hastings has an important bearing on the problem of carbon dioxide tox-

icity, namely, that homeostasis of the carbon dioxide tension is just as important as that of pH. During adaptation to carbon dioxide the organism reaches a compensation of the respiratory acidosis (pH) but remains uncompensated as far as the carbon dioxide tension is concerned, since the latter stays at an elevated level as long as the exposure to increased carbon dioxide levels lasts.[20]

Subjects exposed to 1.5 per cent carbon dioxide for forty-two days showed, throughout the whole exposure period, a significantly increased alveolar pCO_2 level in spite of an increased ventilation.[19] The same is, of course, true for higher concentrations such as 3 per cent carbon dioxide and above.[22] In this connection it should be pointed out that the treatment of respiratory acidosis with trisbuffer (tris hydroxymethyl amino methane) as advocated by Nahas[18] is beneficial in eliminating the effects of a reduced pH but cannot alleviate the effects of increased carbon dioxide tension, since the latter is not reduced by trisbuffer.

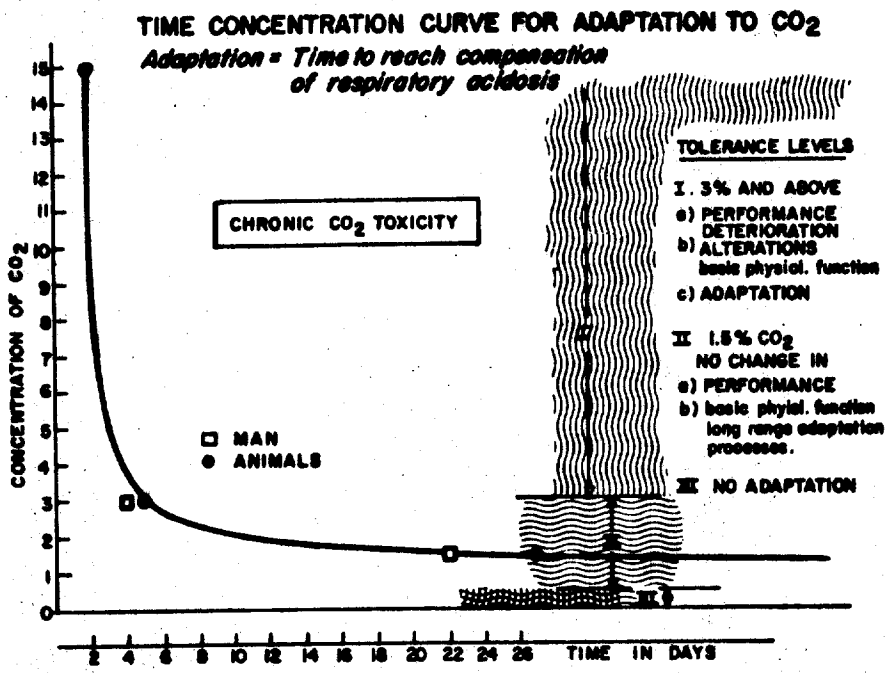

Fig. 3. Time concentration relationship in adaptation to increased carbon dioxide concentration based on experiments in humans and animals and tolerance limits for chronic carbon dioxide toxicity based on three different levels of activity.

Findings with human subjects living in 1.5 per cent carbon dioxide indicate that performance and basic physiologic functions are not affected and that, therefore, this level of carbon dioxide can be tolerated for prolonged periods. However, the long-lasting processes of acclimatization and de-acclimatization, the latter not being completed within twenty-eight days of recovery, suggest that some pathological residues might remain if insufficient time is allowed for recovery. Results of animal experiments and histopathologic findings appear to support this possibility.[21] For example, it was discovered that kidney calcification increased in guinea pigs exposed to 1.5 per cent carbon dioxide for periods

from forty to ninety-three days. These findings of an altered calcium metabolism appear to be in line with the changes in calcium and inorganic phosphorus content of blood plasma in men during exposure to 1.5 per cent carbon dioxide mentioned above.

CONCLUSIONS

In summarizing our data on chronic carbon dioxide toxicity, a time concentration curve for adaptation to carbon dioxide was established which is shown in Figure 3. Time for adaptation is defined as the time to reach a compensation of the respiratory acidosis induced by carbon dioxide. Measurements of pH, carbon dioxide, bicarbonate levels, and electrolytes in blood and urine were used for determination of the two phases in respiratory acidosis. It can be seen that less time is required to reach the compensation at higher carbon dioxide concentrations which is most probably due to a stronger stimulus on the kidney regulation.

Figure 3 also presents three levels of activities which may be used as tolerance limits: (1) The level producing performance deterioration, alterations in basic physiologic functions as expressed in changes of weight, blood pressure, pulse rate, metabolism and finally pathologic changes (3 per cent carbon dioxide and above). (2) The level at which basic performance and physiologic functions are not affected. Under these conditions, however, slow adaptive processes are observed in electrolyte exchange and acid-base balance regulations which might induce patho-physiologic states on greatly prolonged exposure (1.5 per cent carbon dioxide). (3) The level at which probably no significant physiologic psychological, or adaptive changes occur (0.5 to 0.8 per cent carbon dioxide).

The last level is not yet fully established. The main criterion for this level is that no adaptive processes occur. The role of adaptation to different environmental factors in relation to the functioning of the whole organism has been discussed from various aspects by Schaefer,[30] the main argument being that the organism pays for every adaptation. A gain, on one side, is achieved at the expense of a loss on another side.

It is hoped that the concept of triple-tolerance limits may provide stimulation for further refinement of the quantitative characteristics of man's requirements and reactions in normal and artificial atmospheres. The ranges outlined can give the design engineer a framework. He has to know what the human organism pays for compromises in the design. Determination of the ranges also may help to delineate relevant data from theoretical speculations which come up once in a while in regard to tolerance limits. For example, Bartlett[1] made the apparently serious suggestion to use carbon dioxide-rich atmospheres in confined spaces since man, after all, is accustomed to a high carbon dioxide level of 5.5 per cent in the alveolar air and shows a great tolerance to carbon dioxide under conditions of embryonic life. This statement was for obvious reasons based on a misunderstanding of the significance of carbon dioxide gradients between organism and environment, and unawareness of the established effects of chronic carbon dioxide exposure. It emphasized, however, the pressing need for a framework of tolerance limits.

SUMMARY

Results of studies on chronic carbon dioxide toxicity are summarized and a time-concentration curve for adaptation to carbon dioxide is presented which is based on the time to reach a compensation of the respiratory acidosis.

Experimental evidence demonstrating significant effects of elevated carbon dioxide tensions in blood independent of pH changes is reported. Based on these findings, it appears doubtful whether long-term adaptation to even slightly increased pCO_2 is possible without altering normal physiologic processes and producing histopathologic states.

A concept of triple tolerance limits for carbon dioxide toxicity is proposed for three different levels of activity including one at which no significant physiologic adaptive changes to carbon dioxide occur.

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CRYOGENIC FREEZING OF FOODSTUFFS

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FOREWORD

The Research Association is investigating the freezing and cold storage of fruits prior to jam manufacture. Although cryogenic freezing was not used in these investigations, its applications and potentialities were considered with a view to keeping abreast of current developments. The information obtained from the literature is now made available to Members in this Survey.

R. Spencer

Cryogenic Freezing of Foodstuffs

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INTRODUCTION

Freezing and frozen storage have been used for over a hundred years as a means of preserving food materials. Ice and ice-salt mixtures were the earliest materials used to freeze foodstuffs. They were acceptable for the fairly short term storage of robust materials, e.g. fish, but gave poor results with foodstuffs, the textural attributes of which were important, e.g. soft fruits.

Two of the major causes of the unsatisfactory results which were obtained using ice mixtures were that the temperatures available were too high and the rates of freezing were too low. The first of these difficulties was overcome by the introduction of mechanical refrigerators. However, whilst the desirability of rapid freezing has been known for many years[1], mechanisms for commercially obtaining these rates of cooling are more recent.

Theoretical considerations of heat transfer show that maximum rates of cooling are obtained by placing the material to be frozen in direct contact with a refrigerant at as low a temperature as is possible. Two methods of obtaining this end are currently used in food freezing processes. Particulate food materials may be passed through an upward blast of cold air at approximately −40 °C. Alternatively, food may be brought into contact with liquefied gases at temperatures as low as −200 °C: this is known as cryogenic freezing.

Cryogenic freezing is the faster of these two methods of freezing and has been under experimental investigation for a number of years. The German chemical industry developed a technique for freezing food with liquid nitrous oxide during the years 1939-45[2, 3] but this process is no longer of commercial interest. At that time one of the major disadvantages of commercial cryogenic freezing was the cost of the liquid gases. However, with the advent of new steel making processes and, in the U.S.A., the space exploration programme, which consume vast quantities of liquid oxygen, liquid nitrogen, which boils at −195 °C, has become available at an economic price to the frozen food producer. Commercial plant has also been developed which utilizes solid and liquid carbon dioxide, but this is used to a smaller extent than liquid nitrogen. In addition the Du Pont Co. Inc. has developed a process which uses freon as its cryogen.

Other cryogens, e.g. helium and propane, have been used experimentally, but they have been shown to be unsuitable for commercial use. If a refrigerant is to be used commercially in a freezing technique where it comes into direct contact with foods it must be non-toxic, must not give any undesirable flavours or colours to the food material, and if it cannot be easily recovered at the end of the freezing process, it must be cheap. The

ideal cryogen also has a high specific heat and a high latent heat of evaporation.

Although cryogenic freezing has been used on a number of products, it has been most rapidly introduced to freeze products which were difficult to freeze successfully by more conventional methods, e.g. breaded shrimps, sliced tomatoes, strawberries. However, it is continually being introduced to other products where marginal improvements in quality or convenience give the process economic advantages. As well as improving the quality of frozen food materials, cryogenic freezing offers the food processor increased flexibility. The equipment can be rapidly adjusted for different rates of throughput, different sizes of product, and can be closed down and restarted more rapidly than conventional freezing equipment.

EFFECT OF RATE OF FREEZING ON FOOD QUALITY

The specific arrangement of the constituents and phases in a food system is responsible for many of its organoleptic properties. Any alterations in the arrangement of the components will, therefore, lead to changes in the characteristics of the entire system. The rate of freezing may result in such alterations. Woodroof[1] showed that expanding ice crystals tended to tear cells apart and that water tended to migrate from one part of the system to another. Both these phenomena are affected by the rate of freezing. Because crystal nucleation increases with decreasing temperature at a faster rate than does crystal growth, high rates of cooling produce small crystals[4, 5]. In addition, rapid freezing leads to a shorter plateau period in a cooling curve, i.e. the time when ice and free water exist together, and thus there is less opportunity for water to migrate. Rogers[6] pointed out that the production of small ice crystals *in situ* allows for, on thawing, a more accurate and uniform re-formulation of the food product.

Experiments with food materials have shown that the rapid rates of freezing obtained by using liquid nitrogen lead to a better quality product. Wolford and Boyle[7] and Holdsworth[8] reported that rapidly frozen fruits and vegetables had, after thawing, a better texture and flavour and a lower drip loss. The former authors found that liquid nitrogen frozen strawberries were acceptable for 1 hour longer after thawing than were air blast frozen strawberries. Crawford, Finch and Daly[9] reported that tuna frozen with freon had a better flavour than when the fish were frozen in the conventional manner. Likewise, several authors[10-12] have found that, using colour, drip-loss and water holding capacity as parameters, cryogenically frozen chicken was better than blast frozen chicken. Bengtsson[13] reported that liquid nitrogen frozen egg white was much better than conventionally frozen controls, but that egg yolk was unaffected by the rate of freezing.

However, some authors maintain that the optimum rate of freezing foods is slower than that produced by liquid nitrogen. Evans[14] claimed that cryogenically frozen vegetables were indistinguishable from rapidly blast

frozen ones. Also Lee, Gortner and Whitcombe[15] were unable to distinguish between slow and rapid frozen fruit packed in syrup. The 1969 Annual Meeting of the American Society of Heating, Refrigerating and Air Conditioning Engineers was of the opinion that whilst there may be some advantages to be attained by cryogenic freezing of some products, these advantages are rapidly lost during storage[16].

Freezing a food material at too rapid a rate can cause shattering. If whole fish, for example, are immersed in liquid nitrogen for too long a shell of ice forms through the outer layer of cells, and when the centre of the fish reaches 0 °C, the 9 per cent expansion in volume which occurs on freezing may exert a pressure sufficient to fracture the outer shell of the fish[17]. Care must, therefore, be taken to remove the fish from the liquid nitrogen at a time when the shell is sufficiently plastic to allow for the expansion.

FREEZING WITH LIQUID NITROGEN

Three types of commercial system have been developed using liquid nitrogen as the refrigerant to freeze food materials: (*a*) food is immersed in the liquid; (*b*) food is sprayed with atomized liquid nitrogen; and (*c*) liquid nitrogen is allowed to evaporate and is then blown around the food.

Immersion Technique

The earliest commercial liquid nitrogen freezers employed this technique but it has largely been superseded by the spray techniques discussed below. Perforated baskets are filled with batches of the food to be frozen and are then dipped into an insulated tank of liquid nitrogen. After a brief period, which must be carefully controlled, the food is lifted out of the liquid and allowed to equilibrate for a short time in the cold gas over the tank. This system has the advantage of simplicity, which was very important before cryogenic engineers had developed the necessary technology to produce continuous systems.

The major disadvantages of this system are:

(i) The product is liable to shatter if it is left in the liquid nitrogen bath too long. Several authors[18-20] have reported that shattering occurs if the product is not removed from the bath within 2 seconds of the optimum time. In addition, it is necessary to carefully size-grade the product, otherwise small pieces may fracture before the large ones are adequately frozen.

(ii) The process is essentially a batch one and cannot be converted to a continuous flow system.

(iii) Gaseous nitrogen is evolved at the product-nitrogen interface and insulates the product from the refrigerant, thereby slowing the rate of cooling.

(iv) Cold nitrogen gas is allowed to escape before all of its cooling capacity has been utilized.

(v) Particles of food are liable to stick together in the basket.

Spray Technique

The spray technique overcomes the disadvantages listed above and is also more flexible. The unit consists of an insulated stainless steel tunnel through which runs a stainless steel mesh belt conveyor which can be driven at a range of speeds. Some units can, if required, be provided with wheels to make them portable within and without the factory.

Near the outlet of the tunnel, nozzles provide a uniform spray of liquid nitrogen on to the food being carried along by the belt. The liquid nitrogen is vaporized by the heat of the food and is then drawn back along the tunnel. A small proportion of the nitrogen vapour is allowed to pass through the exit of the tunnel in order to prevent the entry of moist air. Recirculation fans and dampers inside the tunnel provide means of controlling refrigerant flow, so that the maximum heat exchange efficiency can be obtained.

After being loaded on to the belt at the inlet of the tunnel the food comes into contact with relatively warm nitrogen vapour (approximately −10 °C). As it moves down the tunnel the food is progressively cooled by the decreasing temperature of the surrounding vapour. This pre-cooling rapidly prepares the food for ultra-fast freezing without surface cracking under the liquid nitrogen spray, where the temperature is approximately −195 °C. The food then passes through the equalizing zone of the tunnel where the hypercold exterior of the food rapidly absorbs the residual heat from within.

References [21-30] describe liquid nitrogen spray freezers used commercially to freeze a number of products. This system blends itself very well to automation relating throughput of food materials and desired end temperature to the flow of nitrogen[31]. Claims have been made that the spray technique using liquid nitrogen can utilize nearly 90 per cent of the available cold.

Cold Gas Blast

With this technique liquid nitrogen is completely evaporated before it is brought into contact with the food[13, 32]. The system can be adapted into any of the more conventional methods which use an air-blast, e.g. fluidized bed technique, the major difference being that the source of cold is a nitrogen evaporator rather than a mechanical compressor. This process shares the advantage of the spray technique in that it can readily be made continuous, but it suffers the disadvantage that the latent heat of vaporization is absorbed before the coolant is in contact with the food material.

FREEZING WITH OTHER CRYOGENS

Carbon Dioxide

Solid carbon dioxide was one of the earliest of the expendable ultra-cold substances widely used for the storage and transit of frozen foods[33]. It is not widely used for rapid freezing of food materials because of the difficulties associated with bringing two solids into intimate contact. Basically, a slightly inclined insulated drum is either rotated slowly or has an auger rotating within it while the food material and the powdered dry ice are fed in at the upper end[33, 34]. The food and dry ice are intimately blended by the tumbling and are to some extent fluidized by the gaseous carbon dioxide liberated. The main disadvantages of this system are that as the length of on-stream time increases, solid carbon dioxide is deposited on the inside walls of the tunnel. This increases the attrition of the particles of food and periodically the plant must be stopped in order to allow the removal of the accumulated dry ice and pieces of food. Also the system is unsuitable for food materials which are too fragile to withstand the tumbling action. In addition, solid cryogens are less flexible to use than liquids[17].

Freon

Freon, which has been pre-chilled to cryogenic temperatures, has a major efficiency advantage over liquid nitrogen in that it does not volatilize immediately it comes into contact with relatively warm food materials and thereby produce an insulating vapour layer. Thus it is technically possible to freeze materials faster with this refrigerant[36], but as freon is too expensive to be used as an expendable material until recently it has not been considered suitable for this purpose. However, the Du Pont Co. Inc. has developed a technique using freon as a recoverable cryogenic refrigerant[35-38].

The unpackaged food material is fed into an insulated tunnel where it is initially passed through water so that during freezing a layer of ice is formed on the surface which both improves the appearance of the product and prevents the absorption of the freon. The product is then passed through a shallow bath of liquid freon which produces an ice crust. It passes from the bath onto a moving wire mesh belt where it is sprayed with freon from overhead nozzles. Finally the foodstuff passes through an equilibration zone where the ultra cold outside equilibrates with the relatively warm centre. The excess liquid and the freon gas is then separated from the food and re-cooled before re-entering the circuit. As gaseous freon is four times as dense as air it is readily separable at the exit of the tunnel and losses are very small.

Several advantages over liquid nitrogen have been claimed for freon freezing. As has been previously mentioned, freon freezing can be faster, but at these rates of freezing, this is of doubtful commercial value. Freezing with freon has also been claimed to be much cheaper than freezing with liquid nitrogen[39], but this depends upon the local price of the nitrogen. In addition, food materials float on liquid freon and this phenomenon can be used to separate heavy foreign bodies from foods.

REFRIGERATED TRANSPORT WITH LIQUID NITROGEN

Liquid nitrogen has been used to cool the insulated cabinets of transport vehicles for nearly ten years[40-45]. The refrigerant is stored in an insulated vessel from where it flows, under the pressure created by the boil-off of the nitrogen, through a pneumatically operated control valve into a spray header. Refrigeration takes place in two stages: first the liquid flashes to a gas absorbing 47·75 cal/g of latent heat; then the gas, still at −195 °C, travels downwards through the load due to its high density, absorbing heat as it equilibrates with the load. In warming from −195 °C to −20 °C the gaseous nitrogen absorbs a further 44·5 cal/g.

A system for use in the food containers for air-liner meals has been developed where regular pulses of cold nitrogen gas are introduced into the container[40].

This system has a number of advantages over conventional refrigerated transport where small compressors are incorporated in the truck.

(i) The system will maintain any desired temperature down to −35 °C under any ambient temperatures likely to be met.

(ii) The rapid cooling effect of a liquid nitrogen spray can pull the temperature down to −20 °C within one minute of the closing of the door after a delivery.

(iii) The positive pressure generated by the expansion of the gas as it warms from −195 °C to approximately −35 °C prevents the entry of warm air into the container.

(iv) The inert gas envelope retards oxidative spoilage of the foodstuffs.

(v) There are fewer moving parts in the system to break down.

(vi) The system can be 99 per cent thermally efficient.

Some disadvantages of the system are[42]:

(i) Liquid nitrogen is lost by boil-off on the return trip when a mechanical compressor would not be running.

(ii) A full insulated tank is heavier than a compressor.

DEHYDRATION

When unpackaged food materials are frozen, water migrates from the warmest to the coldest surfaces. In a conventional air-blast freezer this is from the food material to the heat exchanger in the cold room. This leads to losses of up to 10 per cent from the product weight and a build-up of ice on the heat exchanger. When the same food material is frozen with liquid nitrogen a substantial proportion of this weight loss is avoided[8, 21, 22, 26, 47-52]. The major cause of this is that a thin shell of ice at a very low temperature is rapidly formed. The vapour pressure of this ice is much lower than that at the food surface in an air-blast freezer and the moisture loss is therefore, correspondingly reduced. Also as there is a positive pressure at the liquid nitrogen sprayer which is the coldest part of the system, water vapour is

unable to migrate to it. In addition, as the vapour pressure of water decreases with decreasing temperature, in a liquid nitrogen freezer, where the atmospheric temperature is very low, the water carrying capacity of the gas is also much lower.

Claims have been made that dehydration losses from peeled shrimps can be cut by 90 per cent[26]; smaller savings could be expected from other materials depending on the permeability of the surface layers of the product to water.

PRODUCTS FROZEN WITH CRYOGENIC LIQUIDS

Meat and Poultry

Information on the cryogenic freezing of red meat or meat products is scarce. Costello and Hendrickson[53] showed that the rate of temperature drop in beef (*biceps femoris* muscle) was substantially increased, but they did not claim that it led to an improvement in meat quality. Holdsworth[54] considered that liquid nitrogen is unsuited for this type of product because of the high pressures which are likely to develop within carcases; however, Gurvic *et al*[55] reported that it could be successfully used. When liquid nitrogen was used to freeze hamburgers[16, 21] a more tender product resulted, but the differences were small and were often lost after two weeks' storage.

Cryogenic freezing has led to an improvement in the quality of frozen chicken meat[10, 12, 34], and a lowering of the bacterial count[60]. Meat frozen in this way has been shown to have lower Kramer shear values[10], lower drip-loss[11, 12], a higher water-holding capacity[11], and a lighter colour[16]. A U.S. commercial installation[34] has cryogenically frozen a variety of Cornish game hen products including stuffed breasts, legs and gourmet items.

Seafoods

During air-blast freezing shrimps may lose 10 per cent of their weight by dehydration[26, 49]. As this is a relatively expensive food product, the savings obtained by reducing dehydration with liquid nitrogen freezing techniques have attracted a large number of shrimp freezing concerns. In addition, there have been many reports that the quality of the end product is improved[5] and claims have been made that it is indistinguishable from that of unfrozen shrimps[28].

The possibility of freezing other fish with liquid nitrogen has also been investigated. Catfish[25], tuna[3] and individual fish fillets[23] have all been successfully frozen with either liquid nitrogen or freon. However, Lorentzen[17] claimed that the advantages of liquid nitrogen freezing were overrated. Experimentally, frozen sprat and larger fish burst and the texture and appearance of the thawed products were markedly inferior to air-blast frozen fish. Careful size-grading and pre-cooling before the liquid nitrogen is applied can overcome some of these difficulties[23].

Liquid nitrogen is also much more flexible than conventional seafood freezing processes[56].

Fruits and Vegetables

A large range of fruit and vegetable products has been frozen with cryogenic liquids. Several fruits such as avocados, zucchini squash and tomatoes[16, 27] have not been successfully frozen by other techniques. Green beans have been a popular product for experimental work and the results indicate that cryogenic freezing leads to a more tender product[16, 19, 20]. However, Lee[69] reported that liquid nitrogen frozen peas were firmer than conventionally frozen ones. In experiments with cryogenically frozen peaches, cherries, strawberries and raspberries Wolford and Boyle[7] found that the quality of the end product, measured as the time required for 10 per cent of the original weight to be lost as drip, was markedly better after freezing with liquid nitrogen than after conventional freezing. There have been several brief reports that mushrooms can be successfully frozen with liquid nitrogen[57-59].

Bakery Products

The basic mechanism of bread staling is a polymorphic transition of the starch component of the crumb[60]. At temperatures above approximately 40 °C an amorphous form is thermodynamically stable[61], but below this temperature a crystalline form is the stable one. Transition of the amorphous to the crystalline form occurs at an increasing rate as the temperature is lowered below 40 °C. However, when the water component of the bread freezes the transition rate is markedly slowed down.

Liquid nitrogen freezers are an obvious means of reducing the time at which bread is in the temperature range of maximum staling[48, 62-64]. Liquid nitrogen also offers the advantages of improved flavour and texture retention associated with ultra-rapid freezing.

Early experiments using the immersion method of freezing with liquid nitrogen were unsuccessful in that the vigorous boiling of the nitrogen damaged the product surface, and the bread also cracked and peeled[62]. In addition, the method was inefficient in its use of liquid nitrogen. However, the introduction of continuous freezing with liquid nitrogen sprays has overcome these difficulties.

Another use of cryogenic liquids in bakery products is the injection of the liquid gases into cake mixtures to prevent excessive heating during mixing[65].

ECONOMIC ASSESSMENT OF CRYOGENIC FREEZING[22, 41, 43, 64, 66, 67]

The improvements in quality of frozen products obtained by freezing with cryogenic liquids may be sufficient, in themselves, to increase the sales and profitability of high price, high quality products. However, this would be insufficient to increase sales of low price goods where the advantages

may be lost by careless storage, for example in domestic refrigerators, unless the cost of cryogenic freezing is competitive with conventional freezing.

Freezing with liquid nitrogen is claimed to have five economic advantages over conventional freezing:

(1) The cost of the plant can be as low as half to a quarter of that of conventional freezing plants with similar capacity.

(2) The maintenance costs are in general lower as a result of having no compression equipment.

(3) The units are particularly flexible in that the throughput can be easily increased or decreased with only a small resulting drop in efficiency.

(4) The production rate is considerably faster because of the very short contact time required for freezing.

(5) Claims have been made that dehydration losses can be cut by up to 90 per cent and, therefore, the processor has more product to sell.

The economic factor which determines whether liquid nitrogen freezing is profitable is the consumption and price of liquid nitrogen. In particular the efficiency of the utilization of the cold of the nitrogen is an important factor. This is related to heat gained due to lagging inefficiencies, warm air infiltration, and from the fan; and the liquid nitrogen required for initial cooling of the equipment and that lost through boil-off.

Cryogenic systems which use recoverable cryogens which are re-cooled after use, e.g. freon, and therefore require a conventional compressor system, have not been in commercial use long enough for an accurate assessment of their economics to be made. However, claims have been made that they are substantially cheaper to run than liquid nitrogen systems.

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THE CARBON DIOXIDE BALANCE-SHEETS OF THE BODY: THEIR DETERMINATION IN NORMAL SUBJECTS AND IN CASES OF CONGENITAL HEART DISEASE*

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The hyperventilation induced by breathing a moderate percentage of carbon dioxide is not of rapid onset, and often a considerable time may elapse before respiration reaches a steady maximum level. During this interval, carbon dioxide gradually accumulates in the body, and a steady state may be pictured where accretion of this gas produces sufficient increase of ventilation to restore the normal metabolic output of carbon dioxide.

The time required to reach such a steady state is not known with certainty. Campbell, Douglas, Haldane & Hobson (1913) reported that even after 18 min some carbon dioxide was being dammed back within the body, for although carbon dioxide elimination was then equal to the resting output, there was an associated increase in metabolism which these authors put as high as 40–50%. Nielsen (1936) noted that whereas a steady state was reached fairly rapidly with low concentrations of carbon dioxide, with a 5·8% mixture ventilation continued to increase for 20–30 min, and with a 7·5% mixture at least 40 min elapsed before a steady level of ventilation was reached. On the other hand Loeschcke (1949), commenting on a 'hysteresis' in the response of the respiratory centre to experimental hypercapnia, claimed that this effect did not persist for more than 2 min, and concluded that loading and unloading of blood and tissue carbon dioxide was effected in a similar time. However, as Hesser (1949) pointed out, the saturation period for a given tissue varies with both the blood supply and the solubility coefficient of the tissue with respect to carbon dioxide; areas such as the respiratory centre may reach equilibrium in a matter of minutes, but the time required to complete the exchange of carbon dioxide with less vascular parts of the body is probably much longer.

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There seems only one previous attempt to measure the actual amount of carbon dioxide accretion during hypercapnia. The authors concerned—Adolph, Nance & Shiling (1929)—gave a 4·5% mixture of carbon dioxide to two normal subjects for 30 min; however, the estimated accretion varied widely between 941 and 4900 ml., and it was admitted that individual estimates of retention might show errors of up to 100%. It was thus thought of some interest to construct accurate balance sheets showing the exact intake and output of carbon dioxide at different stages during the course of experimental hypercapnia. From these balance sheets, the net accretion of carbon dioxide could be deduced, and the relationship between this accretion and the level of hyperventilation could be examined.

Initial experiments were carried out on nine normal subjects, but for comparison additional observations were made on twenty-two cases of congenital heart disease, the latter group having in varying degree abnormalities of circulatory and respiratory function.

METHODS

Measurement of carbon dioxide accretion

The total accretion of carbon dioxide during the period of hypercapnia was measured by the use of a number of Douglas bags. A cylinder gas mixture (5% carbon dioxide in air or oxygen) was passed at a steady rate through a reducing valve into a 200 l. bag serving as an inspiratory reservoir. On the expiratory side, gas was conducted along a short connecting tube (capacity about 100 ml.) to a three-way tap. From this tap, two hosepipes (each of about 270 ml. capacity) led to a pile of Douglas bags. The bags were connected in turn to the hosepipes, and then removed for sampling and measurement of total gas volume.

To calculate the net accretion of carbon dioxide, it was necessary to draw up balance sheets showing the various factors affecting carbon dioxide income and output as set out below:

CO_2 income	CO_2 output
(1) CO_2 uptake from inspiratory bag	(4) CO_2 output in expiratory bags
(2) Resting CO_2 output	(5) 'Apparent retention' of CO_2 caused by technical factors, including diffusion
(3) CO_2 output due to additional metabolic cost of hyperventilation	

$$\text{Net } CO_2 \text{ accretion} = (1 + 2 + 3) - (4 + 5)$$

(1) This was calculated quite simply from the cumulative expired gas volume (multiplied by the nitrogen ratio) and the carbon dioxide concentration of a gas sample collected from the inspiratory bag at the end of each experiment and analysed by the standard Haldane gas-analysis apparatus.

(2) This was determined by the conventional Douglas bag method immediately prior to the main balance-sheet experiments.

(3) The metabolic cost of hyperventilation during the early phases of hypercapnia may be calculated from the formula (23 BS + 1·20 EV) ml. STPD, where BS is the body surface in square metres, and EV is the observed total extra-ventilation in litres at standard temperature pressure, dry, STPD (Shephard, 1954, 1955*b*).

(4) Each bag was sampled and the volume measured immediately after filling, observing the precautions outlined elsewhere (Shephard, 1955*a*).

(5) There are a number of factors that lead to an 'apparent retention' of carbon dioxide. These include diffusion from the Douglas bags, changes in the carbon dioxide content of the dead-space

of the apparatus, and changes in the functional residual capacity of the lungs (Shephard, 1954). The amount involved may be as much as 60 ml., and it is essential to calculate these losses carefully in each experiment.

Alveolar and arterial samples

Alveolar samples were collected whilst the subjects were wearing a B.L.B. nasal mask. Gas was supplied at a rate of 9–10 l./min. The flow was interrupted at the peak of a normal inspiration, and an alveolar sample was collected immediately by the Haldane–Priestley technique (which emphasizes speed of delivery).

Arterial samples were collected anaerobically in syringes lubricated with silicone fluid and containing a trace of heparin. The pH was measured at room temperature by means of a Stadie cell (Stadie, O'Brien & Laug, 1930), and the pH at body temperature was calculated by the coefficients of Rosenthal (1948). The whole blood carbon dioxide content was measured by the Van Slyke manometric apparatus, and the corresponding carbon dioxide tension was estimated by means of nomograms (Van Slyke & Sendroy, 1928; Van Slyke, Wu & McLean, 1923).

Subjects and experimental conditions

The normal subjects were healthy adults—medical staff and technicians—all of whom had some experience in the use of respiratory apparatus. Details of these subjects are shown in the Appendix, Table 1.

The congenital heart patients were selected on the basis of the degree of co-operation shown during the initial diagnostic investigations. In most cases it was possible to carry out two complete tests, one during the morning and the other during the afternoon session. Details of age, sex and diagnosis are given in the Appendix, Table 2.

Measurements were made after a preliminary period of 15 min rest in the sitting position. No experiment was commenced sooner than 1 hr after a meal. Observations on normal subjects showed that conditions obtained in this way were approximately 12% above the true basal level.

RESULTS

Observations on normal subjects

Two subjects (R.J.S. and S.S.) were studied in detail, and more limited observations were made on the remaining seven normal cases.

Accretion of carbon dioxide. In order to allow a comparison between the different subjects, the total accretion of carbon dioxide has been expressed as the ratio to the resting output of this gas over a period of 1 min. The path of accretion has been defined within fairly close limits in the two principal subjects (Fig. 1), and it can be seen that a maximum value has not been reached even at the end of 30 min.

There are significant differences both in the average rate of accretion for the two subjects and also in the day to day rate of accretion in the same subject. These differences are most marked in R.J.S., successive experiments showing a greater and more steady accretion of carbon dioxide. Observations on the remaining seven normal subjects give further evidence of wide individual differences in the pattern of accretion, the ratio at the end of 30 min ranging from 4·0 in B.M. to 13·1 in J.B. Comparison of the ventilatory equivalent and the alveolar carbon dioxide concentration for different members of the group suggests that there may have been some differences in the efficiency of respira

tion and in the carbon dioxide combining power of the blood, but the main cause of the variation in response seems a difference in approach to the experiments. Four subjects who illustrate this point are discussed below.

Subject R.J.S. viewed the experiments with personal interest. There was a tendency to analyse symptoms, and during the earlier observations a measure of anxiety lest the technician should fail to carry out the necessary manipulations correctly. These factors probably contributed largely to the rather variable response in early experiments with this subject, and the changing pattern of response as the subject became familiar with the experiment.

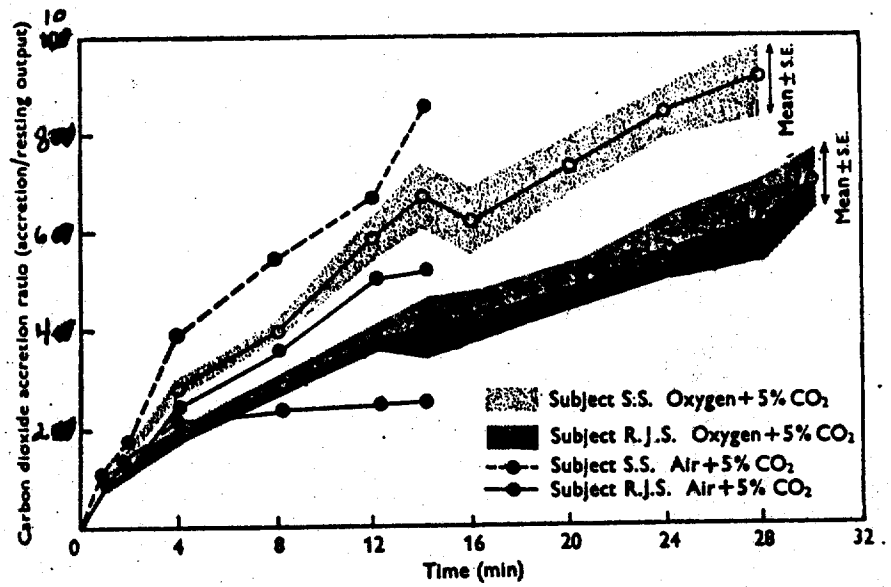

Fig. 1. Carbon dioxide accretion during breathing of 5% mixtures of carbon dioxide. Two normal subjects.

Subject S.S. approached the experiments with indifference and at times even boredom. For her, the periods of carbon dioxide inhalation were part of the daily routine as a wage-earner, and her thoughts were usually far from the physiology of breathing whilst acting as subject. This is in keeping with the larger and more steady accretion of carbon dioxide observed in her case.

Subject J.E. was an experienced respiratory subject who was not closely involved in the research project, and it is probable that he achieved a satisfactory degree of objectivity in his approach to the experiments. In his case there was a large and steady accretion of carbon dioxide.

Subject P.S. was the least experienced of the normal subjects, and being a Polish refugee with a rather poor command of the English language may have approached the experiments with some anxiety. In most experiments he showed a rather small accretion of carbon dioxide.

The experiments with an air/carbon dioxide mixture show an accretion of carbon dioxide similar to the oxygen/carbon dioxide experiments (Fig. 1), and it was therefore considered satisfactory to use the cheaper oxygen/carbon dioxide mixture in subsequent observations on clinical material.

Although it is not possible to attain a limiting accretion of carbon dioxide within a reasonable period of time, the rate of accretion is fairly steady after

the first few minutes. It thus appears that equilibrium has been reached between the arterial blood and the tissues of the respiratory centre even though the level of hyperventilation is not yet sufficient to prevent further accretion of carbon dioxide, and the ratio of increase of ventilation to accretion of carbon dioxide at this stage should give an indication of the sensitivity to carbon dioxide mixtures.

Changes in ventilation. Before attempting to correlate the level of extra-ventilation with carbon dioxide accretion, it is appropriate to consider briefly the pattern of hyperventilation, with particular reference to the problem of habituation.

The change in response with repetition of the experiment is of considerable importance, for with the clinical material it is necessary to draw conclusions from observations limited to 1 or at most 2 days. The changes have been analysed in S.S. and R.J.S. by plotting the ventilatory minute volume ratio for a given period of hyperventilation against the date on which the experiment was carried out. In subject R.J.S., regressions calculated in this way are highly significant for the first 4 min of hyperventilation, but in subject S.S. the regressions are barely significant except at 8 min (Fig. 2). These differences can probably be correlated with the differences in personality and approach to the experiments previously noted.

A number of the early experiments with R.J.S. showed a peak of respiration at 4–6 min, as described by Campbell *et al.* (1913). However, with successive experiments, this feature became less obvious, and ventilation tended to increase smoothly towards a maximum. The early peak is probably a further manifestation of voluntary over-ventilation, and as the subject becomes more accustomed to the experiment, this feature may be expected to disappear. From the practical point of view, it would seem that voluntary over-ventilation exerts its maximal effect in the first few minutes, and if this period is discounted, there is little difference between the first and subsequent experiments.

The average pattern of hyperventilation has been compared in the different subjects by expressing the ventilatory minute volume as a ratio to the resting value. The minute volume increases rapidly for the first 4 min and then continues to climb slowly, so that in some subjects a maximum value has not been reached even at 30 min (Fig. 3). The differences between the ventilatory ratio in different subjects are rather large to explain in terms of a chance variation and support the existence of genuine individual differences in the response to carbon dioxide.

Extra-ventilation/accretion ratio. Different subjects have been compared in terms of the simple ratio of response (extra-ventilation EV) to stimulus (accretion A). Typical curves for a period of experimental hypercapnia (5% CO_2) are illustrated in Fig. 4. For the 1st min the EV/A ratio is low, since there

is a relatively large accretion of carbon dioxide in the lungs and arterial blood, with little change in the respiratory minute volume. As the carbon dioxide reaches the respiratory centre, there is a rapid increase of ventilation, and the rate of accretion falls. This is shown by an abrupt rise of the EV/A ratio during

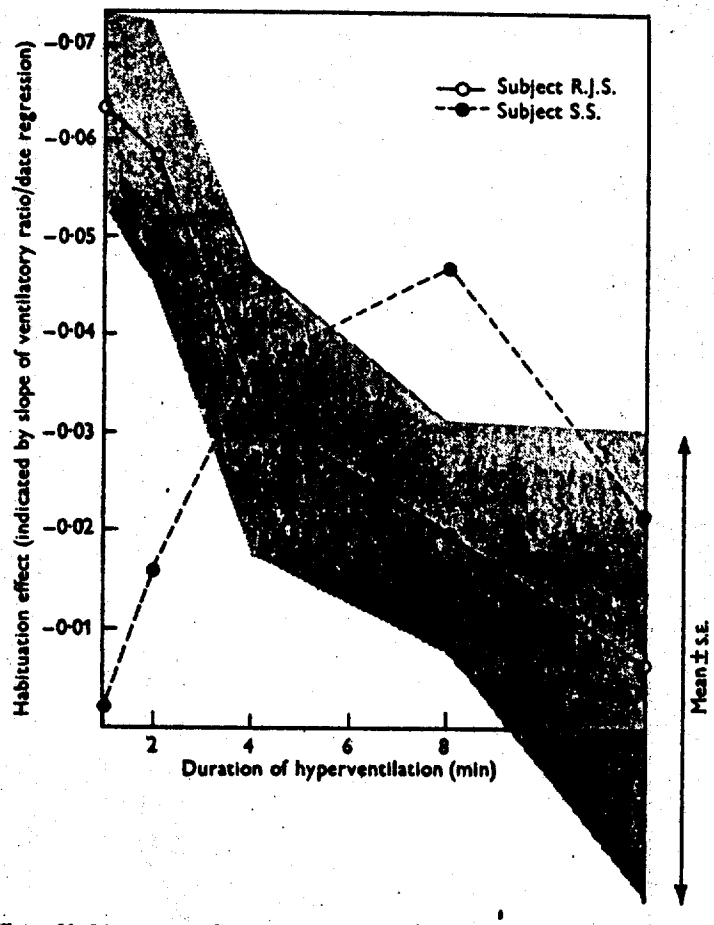

Fig. 2. Effect of habituation on hyperventilation produced by breathing 5% carbon dioxide for varying periods of time. Two normal subjects. At each time interval, a linear regression line relating ventilatory minute volume to the date of the experiment has been fitted by the method of least squares. The slope of these regressions is here taken as an index of habituation, and is plotted against the period of hyperventilation.

the 2nd min. The rate of increase in ventilation declines as soon as equilibrium is established between the arterial blood and the tissues of the respiratory centre, but the accretion of carbon dioxide continues steadily as this gas is distributed amongst other tissues with a poorer circulation. This phase is associated with a rapid fall in the EV/A ratio. From 8 min onward, the fall

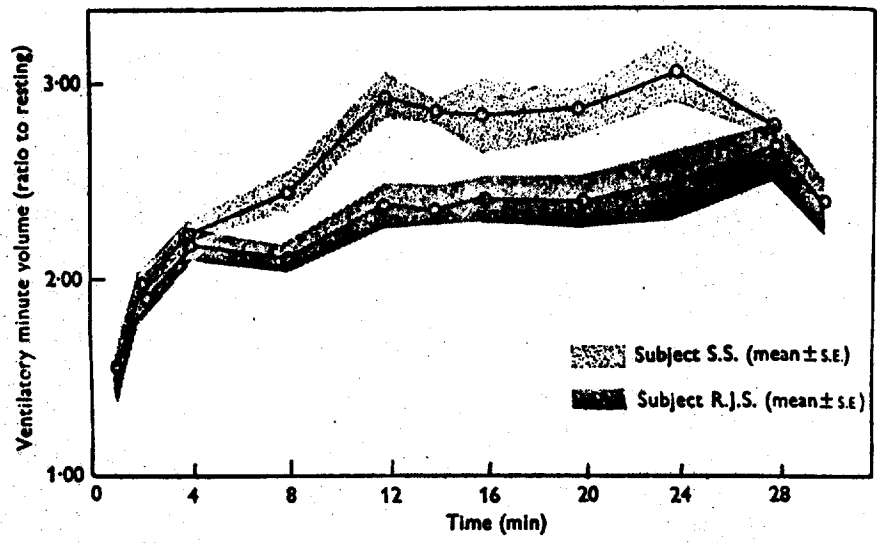

Fig. 3. Hyperventilation produced by 5% mixture of carbon dioxide in oxygen. Two normal subjects.

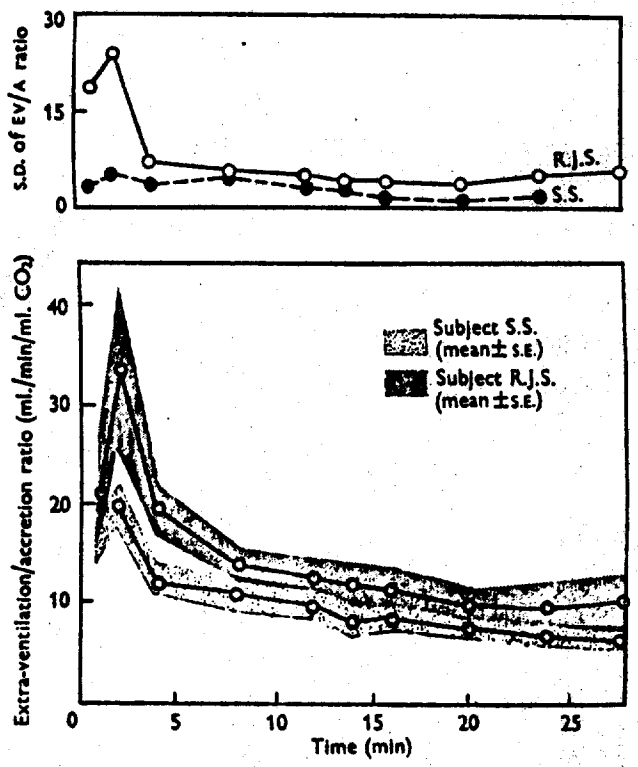

Fig. 4. Extra-ventilation/accretion ratios for two normal subjects breathing mixtures [illegible] carbon dioxide in oxygen. The standard deviation of a single estimation of the EV/A [illegible] [illegible] subject in the upper graph.

becomes more gradual, and it may be assumed that the EV/A ratio approaches a limiting value as equilibrium with the remaining tissues is achieved. After 30 min hyperventilation, the ratio has reached a fairly constant value in most subjects, although even at this stage the level of extra-ventilation is still not sufficient to prevent further accretion of carbon dioxide.

Changes in the EV/A ratio with repetition of the experiment are much as would be expected from the changes in ventilation previously described. In R.J.S. there is a rather large habituation effect for the first 4 min of successive periods of hypercapnia, but there is no consistent change at the longer time intervals. This is reflected in a much smaller standard deviation of the EV/A ratio for longer periods of hyperventilation, an optimum standard deviation being observed at 12–20 min. Even in this range, the error of single observations is considerable, and although observations on the remaining seven normal individuals help in establishing the range within which normal values may be expected to fall, it is not possible to comment on the small differences that were observed between these individuals. It is also obvious that little information can be gained from isolated observations on clinical cases, and to obtain satisfactory evidence of possible deviations of the EV/A ratio it is necessary to compound observations drawn from a group of similar cases.

Observations on cases of congenital heart disease

Having considered the pattern of carbon dioxide accretion in normal individuals, it is possible to examine the path of accretion in cases of congenital heart disease. From investigations of resting respiration in such patients (Shephard, 1955*d*), it did not seem likely that any gross alteration in sensitivity of the respiratory centre would be discovered. However, so far as is known, the response of congenital heart cases to carbon dioxide has never been investigated previously, and it was thought that cases of this type would provide a convenient test of the possibility of applying the balance-sheet principle to clinical subjects.

Accretion of carbon dioxide. The course of accretion is very similar in all four categories of congenital heart disease (Fig. 5), although the actual amount of this accretion seems rather greater than the average for normal subjects. This is rather surprising, particularly in view of a tendency to a diminished alkali reserve in these cases (Shephard, 1955*d*); the explanation seems a slower development of hyperventilation, and the reasons for this are discussed below.

Pattern of hyperventilation. Hyperventilation tends to develop less rapidly than normal in all forms of congenital heart disease (Fig. 6), although in patients with central cyanosis the difference is not very great. Davison, Armitage & Arnott (1953) have reported a similar slow development of hyperventilation on exercising patients with central cyanosis; they attribute this to a

depression of the respiratory centre produced by an increase of the venous-arterial shunt. However, this mechanism cannot operate in acyanotic and peripheral cyanotic patients, and in the present series the slow response to carbon dioxide is *less* marked in the central cyanotic group. One factor is that the clinical subjects did not know the odour of carbon dioxide, so that emotional disturbances introduced by this gas may have been less than in the normal subjects until the patient noticed that he was hyperventilating.

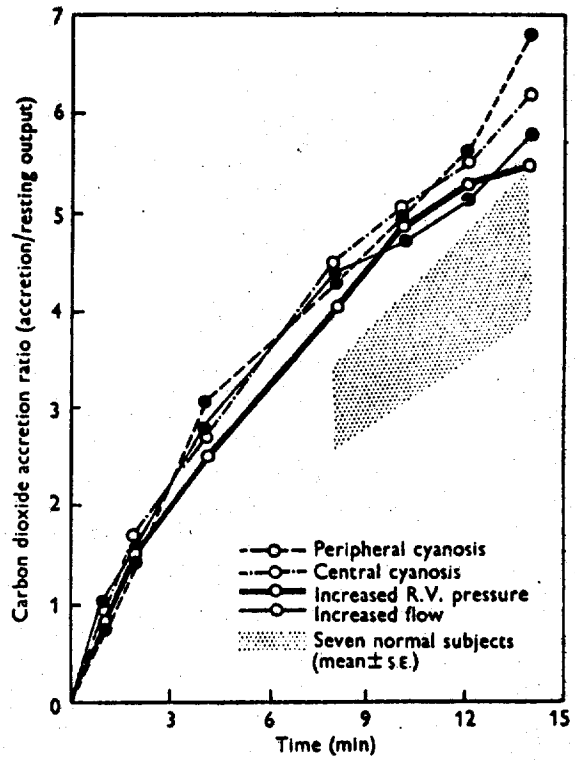

Fig. 5. The course of carbon dioxide accretion during hypercapnia (5% CO_2 in O_2) in four different types of congenital heart disease. The number of cases in each group is shown in the Appendix (Table 2).

It is also possible that the mechanical problems of hyperventilation may be somewhat different in a patient who already shows some hyperventilation at rest.

Comparing the different categories of congenital heart disease, it is interesting to note a relatively greater minute volume in the early part of the central cyanotic curve, and it may be tentatively suggested that this is due to some diminution of alkaline reserve in this group. In the later stages of the hyperventilatory response, the patients with an increased pulmonary flow tend to show a greater minute volume than normal, and here an explanation can be

offered on mechanical lines. The pattern of respiration is shallow in this group on account of the increased viscous resistance of the lungs (Shephard, 1955*d*), and a larger total ventilation may therefore be necessary to achieve an elimination of carbon dioxide equal to that found in other forms of congenital heart disease.

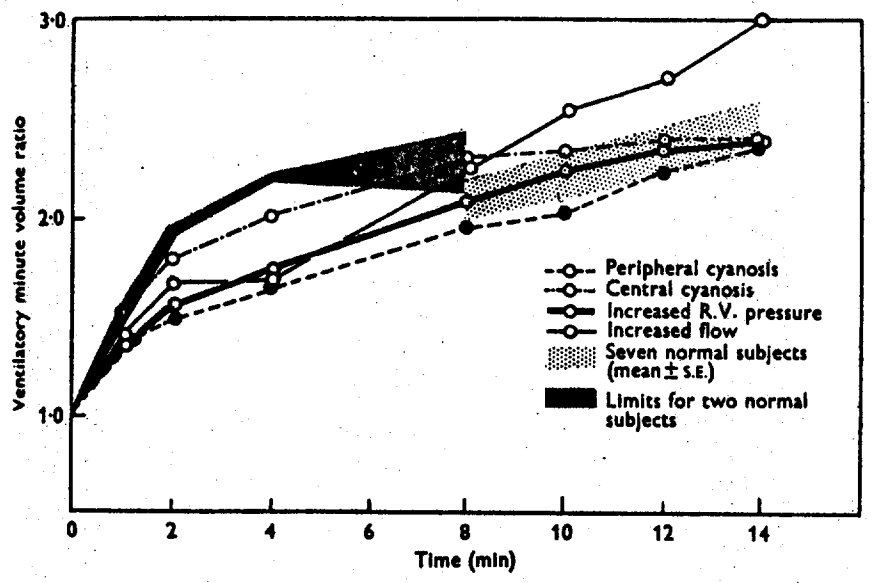

Fig. 6. The hyperventilation produced by 5% carbon dioxide in oxygen in four different types of congenital heart disease. The number of subjects in each group is shown in the Appendix, Table 2.

Extra-ventilation/accretion ratio. Comparing the different forms of congenital heart disease (Fig. 7), it will be noted that in the central cyanotic group the EV/A ratio appears to decline more slowly. However, this may represent merely a chance variation of response. It cannot be attributed to a difference in sensitivity of the respiratory centre, since the EV/A ratio is later very similar to that observed in other forms of congenital heart disease. For the same reason, it is difficult to explain in terms of an altered alkaline reserve, unless other factors such as a poor physique or central depression can be invoked to explain the secondary diminution of ventilation.

In the later stages of the experiments, where the subjects are nearer a steady state, it can be seen that the relationship between stimulus and response is close to normal. Since there is no more than a slight reduction of alkaline reserve in most cases, it would appear that the sensitivity of the respiratory centre is within normal limits. Davison *et al.* (1953) reached similar conclusions by application of the chemical ventilation equation (Gray, 1946) to their data for resting respiration.

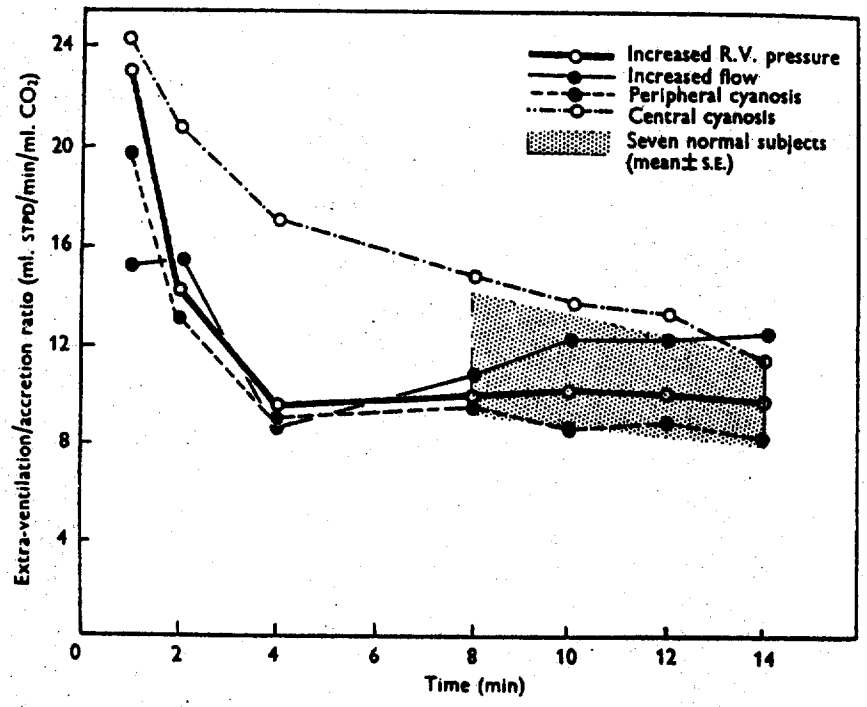

Fig. 7. The extra-ventilation/accretion ratio during breathing of 5% mixture of carbon dioxide in oxygen. Four different types of congenital heart disease. Number of cases in each group shown in the Appendix, Table 2.

Distribution of retained carbon dioxide

It is finally of some interest to consider the distribution of accumulated carbon dioxide, and in particular to examine the relative importance of the different systems that intervene to shield the respiratory centre from the direct impact of this gross stimulus.

The pulmonary gas volume. The first system intervening between the inspired air and the respiratory centre is the pulmonary gas volume. To follow changes in this system, it is necessary to examine the functional residual volume and the carbon dioxide concentration of alveolar gas.

The functional residual volume changes but little during the breathing of moderate concentrations of carbon dioxide (Shephard, 1955*b*), and resting values of sufficient accuracy for the present purpose were obtained by the simple dilution method of Peters & Van Slyke (1932). Changes in carbon dioxide concentration were followed by the collection of alveolar samples after periods of carbon dioxide inhalation ranging from ½ to 5 min. Individual readings were recorded as deviations from a previously determined resting

value, and these results have been plotted as deviations from the average resting value for a given subject (Fig. 8).

There is a slight difference between the response to air/carbon dioxide and oxygen/carbon dioxide mixtures, particularly in subject R.J.S. These changes are in keeping with the expected effect of oxygen on respiration, but the number of observations is rather small to permit further comment on this point. With both types of gas mixture, there is a considerable increase of

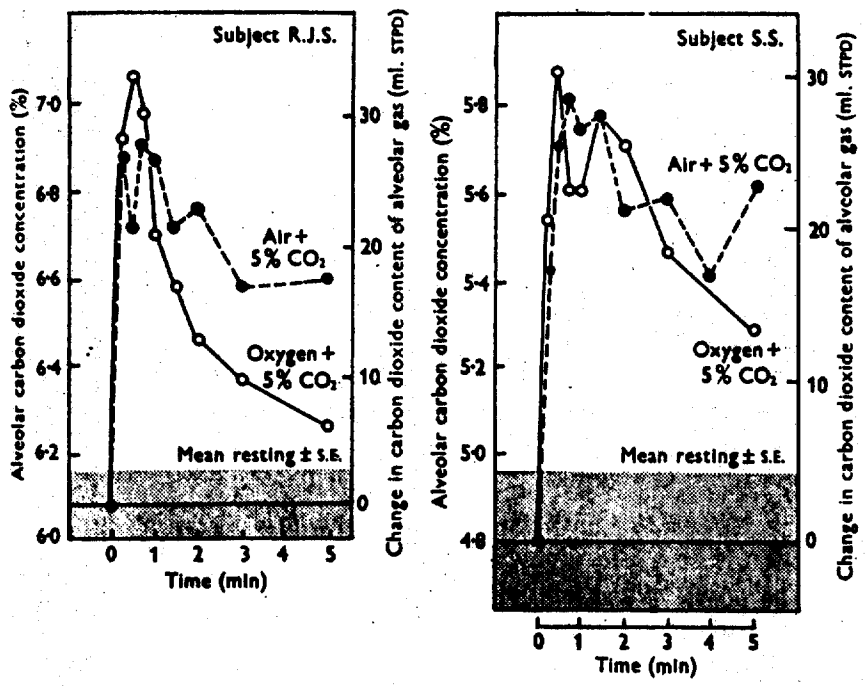

Fig. 8. Changes n the carbon dioxide content of alveolar gas during the breathing of 5% mixtures of carbon dioxide. Two normal subjects.

carbon dioxide concentration in the first minute, amounting on average to about 1%. This peak tends to restore the partial pressure gradient from alveoli to inspired air, allowing some loss of carbon dioxide by diffusion, and it also produces a rise in the carbon dioxide tension of pulmonary venous blood. The latter initiates an increase of pulmonary ventilation, so that in the steady state an adequate elimination of carbon dioxide can be achieved with a smaller partial pressure gradient from alveolar gas to inspired air. Thus the alveolar carbon dioxide concentration declines steadily, and at the end of 5 min is no more than 0·4% above the resting level.

There are corresponding variations in the accretion of carbon dioxide in the pulmonary gas volume. If V is the average functional residual volume in

litres, the peak accumulation of carbon dioxide during the first minute amounts to 0·01 V l., and the steady value is 0·004 V l. or less. In a normal adult, with a functional residual volume of 3 l., this implies an initial retention of some 30 ml. of carbon dioxide, the amount declining rapidly to about 12 ml.

The lung tissues. Dubois, Fenn & Britt (1953) have drawn attention to the considerable capacity of washed lung tissues for carbon dioxide. These tissues attain almost spontaneous equilibrium with alveolar gas, and form the next system protecting the body against a sudden rise of inspired carbon dioxide tension.

From the figures of Dubois *et al.*, it would seem that in a person of average build, the lung tissues absorb about 20 ml. of carbon dioxide for each 1% change in alveolar carbon dioxide concentration. Thus the amount involved under the present experimental conditions is initially of the order of 20 ml., but declines to around 8 ml. as a steady alveolar carbon dioxide concentration is reached.

The blood. It is generally assumed that the carbon dioxide tension in the blood leaving the lungs corresponds closely with the tension observed in alveolar gas. This has been confirmed at cardiac catheterization in a number of patients by a direct measurement of changes in the composition of pulmonary venous blood during the first minute of experimental hypercapnia. Each patient showed a rapid rise of carbon dioxide tension of similar magnitude to that previously noted in alveolar gas. There was an associated small but consistent fall in pH (0·04–0·06 units), and an increase of carbon dioxide content (2·2–2·3 ml./100 ml. serum). The buffering capacity of the blood in these patients was close to normal, and it therefore seems reasonable to apply these findings to the balance-sheet experiments as a whole. On this basis, a person with a blood volume of 5 l. would show an initial retention of 110–115 ml. of carbon dioxide in the blood, and assuming that the pulmonary venous tension continues to mirror the alveolar tension, this retention would decrease fairly rapidly to about 45 ml.

The body tissues. The systems described above account for a carbon dioxide retention of 160 ml., declining rapidly to 60–70 ml., and it is obvious that after the first minute the major proportion of the retained carbon dioxide is distributed amongst the remaining body tissues. Adolph *et al.* (1929) estimated that the tissues as a whole had a carbon dioxide capacity 6 to 25 times that of the blood, and the present figures are towards the upper limit of their estimate. The volume of the remaining tissue fluids is approximately 10 times the volume of the blood, so that the present data would suggest that the tissues have a rather greater capacity for carbon dioxide than has the blood.

The time required to complete saturation of these tissue buffers is relatively long. The degree of equilibrium that has been achieved at any given time depends partly on the general circulation rate, and partly on the relative

efficiency of the circulation to different parts of the body. Of the different forms of congenital heart disease that have been studied, severe pulmonary stenosis (peripheral cyanotic group) would seem the most likely to show abnormalities of the circulation. However, even in this condition there is no evidence of a decrease in the general circulation rate (Swan & Wood, 1953), although it is possible that an increased proportion of the systemic flow is directed to the head (Shephard, 1955*e*). It is clear from Fig. 7 that in the cases studied, changes of circulation rate were not sufficient to influence the equilibrium time.

DISCUSSION

The original objective of the balance-sheet experiments was to reach a steady state where the increase of ventilation caused by accretion of carbon dioxide within the body was sufficient to restore the normal carbon dioxide output. However, it is clear that this position is not reached even after a 30 min period of hypercapnia. It might be thought that secondary changes of acid/base balance would limit further extension of the experiment, but in fact there is no secondary decrease of ventilation during the first 18 hr of exposure to carbon dioxide (Nielsen, 1936; Schäfer, 1949). A more important practical difficulty is the period of hyperventilation that relatively inexperienced clinical subjects will tolerate. Some of the patients, particularly the younger children, were very tolerant, but others needed reassurance in the latter part of the experiment, and 15 min probably represents the upper limit of time for which the co-operation of the average subject may be retained.

If patients are followed for this period of time, most achieve a fairly stable extra-ventilation/accretion ratio. This ratio varies in any one subject from time to time, and there are also considerable differences between the average response of different normal subjects. Thus to use the ratio to examine respiratory function in congenital heart disease, it is necessary to compound observations on a group of similar cases. On this basis, it has been possible to show that in each of four types of congenital heart disease the ratio is within normal limits, implying, in the absence of a significant change in alkaline reserve, that the sensitivity of the respiratory centre is normal in these forms of congenital heart disease.

Until a limiting accretion of carbon dioxide has been reached, the relationship between pH and accretion lacks precision. However, it is of some interest to calculate an approximate value for the pH change at the stage where a steady $\Delta V/A$ ratio has been reached. At the end of 30 min, the average accretion of carbon dioxide is around 1500 ml., and all but 12 ml. of this gas is distributed through some 50 l. of body fluid, so that the change in carbon dioxide concentration should be of the order of 2–4 m-equiv/l. Assuming, further, that the buffering properties of the tissues correspond with those of blood, this should produce a rise in carbon dioxide tension of some 5–10 mm

Hg, and a fall in pH of 0·02–0·04 units. Direct potentiometric estimates of the change in pH show an immediate fall of 0·04–0·05 units, but there is reason to believe that the final change of pH is nearer 0·02 units. An indirect estimate of the pH change may also be obtained from the increase of ventilation. In most experiments, this amounted to about 200%, which according to the figures of Campbell *et al.* (1913) would correspond with a change in pH of 0·024 unit. Thus pH values calculated by the balance-sheet method are of the same order as those obtained by other techniques.

SUMMARY

1. A method for the determination of total carbon dioxide accretion during the course of experimental hypercapnia is described. This depends on the accurate assessment of carbon dioxide income and output by means of a series of Douglas bags.

2. At the end of a 30 min period of breathing 5% carbon dioxide, normal subjects show a total carbon dioxide retention of 1000–2000 ml. At this stage, the ratio of extra-ventilation to carbon dioxide accretion has reached a steady value in most subjects, although a maximum of carbon dioxide accretion has not yet been achieved.

3. The course of carbon dioxide accretion varies considerably in some subjects. This effect is probably due to the influence of higher centres; it is most marked during the first few minutes of each experiment, and tends to disappear with training.

4. The pattern of carbon dioxide accretion is similar in each of four types of congenital heart disease, and the steady ratio of extra-ventilation to carbon dioxide accretion is within normal limits, suggesting that these patients have a normal sensitivity to carbon dioxide.

5. Small amounts of retained carbon dioxide are to be found in the pulmonary gas volume, the lung tissues, and the blood, but the major proportion is distributed to the remaining tissues.

6. The change of pH may be calculated from the amount of carbon dioxide retained in the body. This value agrees satisfactorily with direct potentiometric measurements, and indirect estimates based on the observed change of ventilation.

My thanks are due to Miss Scott for technical assistance, and to the Department of Medica Illustration for their preparation of the figures.

APPENDIX

TABLE 1. Details of normal subjects taking part in balance-sheet tests

Subject	Age (yr.)	Sex	Height (ft. in.)	Weight (lb.)	Body surface (m^2)	Resting ventilation ($l./m^2/min$)	Alveolar (CO_2 %)	Ventilatory equivalent ($l./100$ ml. O_2)
R.S.	24	M.	6 0	157	1·91	4·31	6·08	2·74
S.S.	19	F.	5 3	136	1·63	3·82	4·81	2·53
J.E.	21	M.	5 10	154	1·87	4·88	5·54	2·73
P.S.	31	M.	5 10	142	1·80	3·74	—	2·78
J.B.	35	M.	5 7	128	1·67	3·01	6·85	1·81
H.P.	32	F.	5 0	116	1·48	4·23	4·91	2·63
P.F.	23	F.	5 4	146	1·71	3·88	5·16	2·29
B.M.	50	M.	5 10	175	1·98	4·55	5·66	3·14
F.M.	60	M.	5 8	173	1·90	5·23	5·79	2·76

TABLE 2. Details of cases of congenital heart disease taking part in balance-sheet tests

Obs. No.	Session	Sex	Age (yr)	Diagnosis
	Peripheral cyanotic group			
3	a.m.	M.	21	Pulmonary stenosis
4	p.m.			
	Increased right ventricular pressure group			
7	a.m.	F.	21	Pulmonary stenosis
8	p.m.			
14	a.m.	F.	10	Pulmonary stenosis
15	p.m.			
16	a.m.	M.	18	Pulmonary stenosis
17	p.m.			
18	a.m.	M.	8	Pulmonary stenosis
19	p.m.			
24	a.m.	F.	9	Fallot's tetralogy after valvotomy
25	p.m.			
26	a.m.	M.	19	Pulmonary stenosis
27	p.m.			
28	a.m.	M.	21	Pulmonary stenosis
29	p.m.			
30	a.m.	F.	19	Pulmonary stenosis
35	a.m.	M.	10	Aortic stenosis
36	p.m.			
37	a.m.	M.	8	? Normal heart
	Central cyanotic group			
11	a.m.	F.	12	Atrial septal defect + ?
12	p.m.			
13	a.m.	F.	11	Fallot's tetralogy
20	a.m.	F.	48	Fallot's tetralogy
21	p.m.			
23	p.m.	F.	9	Cyanosis, ? cause
31	a.m.	F.	9	Fallot's tetralogy
32	p.m.			
33	a.m.	F.	25	Atrial septal defect + ?
34	p.m.			
	Increased pulmonary flow group			
1	a.m.	F.	44	Atrial septal defect
2	p.m.			
5	a.m.	M.	14	Ventricular septal defect
6	p.m.			
9	a.m.	F.	22	Ventricular septal defect
10	p.m.			
38	a.m.	F.	45	Patent ductus arteriosus
39	p.m.			
40	a.m.	M.	8	? Patent ductus arteriosus
41	p.m.			

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Gas Embolism

Roentgenologic Considerations, Including the Experimental Use of Carbon Dioxide as an Intracardiac Contrast Material[1]

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THE USE OF gas injection for contrast in clinical diagnostic roentgenology is increasing. The danger of venous or arterial gas embolism is always inherent in such diagnostic procedures as well as in therapeutic procedures in which entry of air into the circulation may occur.

The hazards of gas embolism are particularly present in the direct translumbar injection of air or oxygen into the perirenal region, and enthusiasm for the procedure is therefore limited in many clinics. Recently, injection of air for visualization of retroperitoneal structures has been commonly carried out by the safer presacral route.

Deliberate introduction of air or oxygen by the presacral route into the mediastinum is now being widely advocated for diagnostic purposes. Pneumoperitoneum and pneumothorax have long been employed in diagnostic roentgenology; insufflation of the urinary bladder and many of the larger joints has also been performed. Angiocardiography involves a potential hazard because of possible accidental venous air embolism.

The accidental occurrence of air embolism is well known in other clinical situations, particularly in surgical procedures involving the veins of the neck or the dural sinuses, performed with the patient in a sitting position, in uterine curettage, and during delivery of patients with placenta previa. Serious sequelae following accidental entrance of air into intravenous apparatus are rare.

The greatest practical hazard probably lies in the practice of obstetrics and gynecology, since the uterine sinuses are the veins into which air may most readily enter. A number of deaths from air embolism have been reported, for example, as a result of vaginal insufflation for *Trichomonas* infestation either during pregnancy or in non-pregnant women near the time of menstruation (1).

This problem is currently of additional interest because of the possibility of gas embolism in connection with explosive decompression at high altitudes, in aviation accidents, as for instance rupture of a sealed pressurized cabin (2, 3).

Differentiation of Pulmonary (Venous) and Arterial Air Embolism: In pulmonary (venous) air embolism, the air enters one of the systemic veins and produces its effect by an air "trap" in the outflow tract of the right ventricle and main pulmonary artery and, when the patient is supine, by blockade of the small peripheral pulmonary arteries. This requires fairly large amounts of air.

In arterial embolism, the air enters the pulmonary venous channels and is carried thence to the superiorly located systemic arteries. In this fashion, very small quantities of air may produce serious blockade of one or more of the coronary or cerebral vessels.

The two forms of embolism present clinical pictures entirely different from each other. In venous embolism, the air in the right ventricle produces a loud, churning sound which may be audible without the stethoscope. This has been termed a "millwheel" murmur. If the patient is in such a position that an air trap develops in the right ventricle, the venous pressure rises and cyanosis ensues; the systemic blood pressure falls, the pulse becomes rapid and thready,

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and syncope develops as a result of cerebral ischemia.

In arterial embolism, when the head is higher than the level of the aortic arch, air enters the cerebral circulation and causes such neurological manifestations as aphasia, blindness, and hemiplegia. Air may be observed in the retinal vessels on ophthalmoscopic examination, and a marbled appearance of the skin may indicate its presence in superficial, cutaneous vessels. Myocardial infarction has been demonstrated electrocardiographically, and blockade of the coronary arteries by air has been shown in postmortem examinations.

Cases have been reported in which both types of embolism were observed in the same individual; air entering the systemic veins produced manifestations of both pulmonary and arterial air embolism. In 2 such cases (4, 5) an interatrial septal defect was found at autopsy. Arterial air embolism caused by entrance of air into the systemic veins is referred to as "paradoxical air embolism."

Experimental Studies on Mechanisms in Venous Air Embolism: Experimental studies in dogs, including biplane roentgenography (6, 7), have demonstrated that the fundamental causes of serious circulatory disturbances in venous air embolism are both mechanical and physiological. In right decubitus or the supine position, an air trap develops in the outflow tract of the right ventricle and acts as an effective circulatory block. The pressure in the pulmonary artery beyond this trap rises, indicating a further block in the peripheral pulmonary circulation.

The immediate, primary rise in pulmonary arterial pressure is followed by a decline and then a secondary rise. Neurogenic pulmonary arterial spasm from mechanical stimulation by air bubbles, local anoxia beyond the air block, or further delivery of air increments from the ventricle may account for this secondary increase.

The systemic blood pressure falls immediately following intravenous air injection, with accompanying bradycardia and biphasic respiratory disturbances. This fall in systemic blood pressure is explained by decreased inflow into the left heart because of mechanical blockade in the pulmonary vessels. Pressoreceptors in the heart and lungs, described by Aviado (8), and veratridine reflexes from the lesser circulation, studied by von Bezold (9), may account for the hypotension and alternating apnea and hyperpnea which are observed. The low systemic pressure also contributes to the hyperpnea.

Localized right ventricular ischemia occurs. The coronary pressure gradient is markedly reduced, since coronary arterial inflow pressure is low and the pressure at the coronary venous outflow into the right heart is elevated. At the same time, right ventricular work is increased.

Body Position in Relation to Venous Air Embolism: The mortality of venous air embolism in dogs was dramatically reduced by placing the animal in left lateral decubitus (6, 7). This position provides for continuity of blood flow from the cavae to the right ventricular outflow tract, since in this position the pulmonary artery is dependent. Gas, because of its buoyancy, rises away from the outflow tract.

Roentgenographic evidence of the air block occurring in the pulmonary artery as a result of accidental air embolism during an angiocardiographic procedure has been obtained (7). Injection of the contrast material was carried out through a cervical vein, since adequate peripheral veins in the arms were not available. The patient was in the sitting position. When the saline-containing syringe was disconnected to permit attachment of the syringe of radiopaque contrast solution, a sucking sound was audible. The injection of the contrast material was completed uneventfully and subsequently the patient was able to walk across the room without difficulty. After lying down for a brief period, he became nauseated, sat up, then collapsed, and was unresponsive for about two minutes, during which

Fig. 1. Roentgenogram of dog's heart following intravenous air injection. Note tricuspid ring crossing air obliquely above fluid level.

he exhibited mild generalized convulsive movements, stertorous breathing, and marked bradycardia. Complete recovery ensued rapidly, and it was thought that the reaction was attributable to the iodine-containing contrast material. When the films were processed, however, a large air bubble was disclosed in the pulmonary artery and its left main branch; this had obviously caused a marked diminution in the blood flow to the left lung while the patient was in the upright position and was responsible for the transient symptoms subsequently observed.

A somewhat similar accident has been reported in the French literature (10). In this instance, a gas pressure apparatus employed for the injection of the radiopaque material for angiocardiography was incorrectly connected so that a quantity of oxygen was rapidly injected intravenously. The authors of the report attribute the recovery of the patient to the fact that the head was immediately lowered; in this position, the continuity of blood flow through the right ventricle into the pulmonary artery is maintained.

Safety of Carbon Dioxide as an Intracardiac Contrast Medium Demonstrated Experimentally in Dogs by Cinefluorography: In experimental air embolism studies, roentgenograms frequently afforded excellent demonstrations of the internal anatomy of the dog's heart. In the supine position, with the x-ray beam horizontal, the tricuspid ring and pulmonary valve were shown (Fig. 1). This type of angiocardiography seemed to have possible clinical application, provided a safe gas could be found. Carbon dioxide was selected for testing, since it is approximately twenty times as soluble in serum as air or oxygen. It has been shown by experiments on anesthetized dogs that carbon dioxide rapidly injected intravenously is well tolerated in large doses, and repeated injections in the same animal have produced no evident ill effects (11). Injection of air in similar quantities (7.5 c.c. per kilo) resulted in 100 per cent mortality in animals in the right decubitus position. Numerous comparative studies have been performed. Carbon dioxide was found to cause only a brief drop in systemic arterial pressure and a rise in pulmonary artery pressure. Air and oxygen are almost equally hazardous and, when injected intravenously, produce prolonged blood pressure and respiratory effects in anesthetized animals, frequently with fatal termination.

When carbon dioxide (7.5 c.c. per kg.) is given intravenously, the average change in whole blood carbon dioxide content is a 5 to 10 volume per cent increase; the change in oxygen is a 1 to 2 volume per cent decrease. Both these effects are of extremely brief duration, reaching a maximum within fifteen to thirty seconds and disappearing in one or two minutes. Under these circumstances the blood *p*H actually changes hardly at all.

Direct injection of carbon dioxide into the left ventricle or root of the aorta has also been carried out frequently, in the anesthetized dog, without apparent ill effects, in contrast to the regularly fatal outcome when air or oxygen is similarly employed (Fig. 2). Moore and Braselton (12, 13) had previously reported that carbon dioxide was well tolerated on injection into the intrapulmonary veins in

cats, while air injection under similar conditions invariably caused a typical coronary death. In our experience, a residual bubble has been consistently observed following the prompt disappearance of the bulk of the carbon dioxide injected into the left ventricular cavity. Analysis of samples of this bubble obtained *via* catheter show that it is chiefly oxygen which has diffused from the arterial blood into the injected carbon dioxide. This bubble, a potential source of an arterial gas embolus, must be considered in the application of left heart carbon dioxide injection to clinical problems.

Cinefluorography was carried out with the Philips image amplifier employing low x-ray intensity. Sixteen-millimeter motion picture film was used, with exposures at the rate of 64 frames per second (14, 15). These studies have demonstrated the possibilities of carbon dioxide for visualization of the valve structures of the heart and valvular lesions, as well as the investigation of cardiac dynamics. Admittedly, the procedure is unphysiologic as far as cardiac mechanics are concerned, since the valve structures are incompetent for gas, permitting its flow back and forth through the valves. However, visualization of the intracardiac structures by this means is free of the difficulties of layering, irregular streaming, and dilution which impair the value of water-soluble opaque contrast substances for angiocardiography (16).

Fig. 2. Injection of carbon dioxide into left ventricle of dog *via* aortic catheter. A. Roentgenogram immediately after injection. The aortic valve cusp is indicated by the arrow. B. Three seconds after injection. Much of the gas is already dissipated.

The results of these experiments suggest also that development of a safe technic for selective carbon dioxide angiocardiography in the human subject may well be possible; for example, visualization of a localized area such as the pulmonary conus, the pulmonary valve, and pulmonary artery. Rapid intravenous injection of carbon dioxide (7.5 c.c./kg.) in 2 infants was well tolerated in the supine position, with only transient apnea. In one the injection was repeated within a few minutes with no difficulty. Radiographs showed the right heart chambers to be briefly outlined by gas (Fig. 3).

These studies have provided dramatic demonstration of the clinical advantages of employing for diagnostic studies a

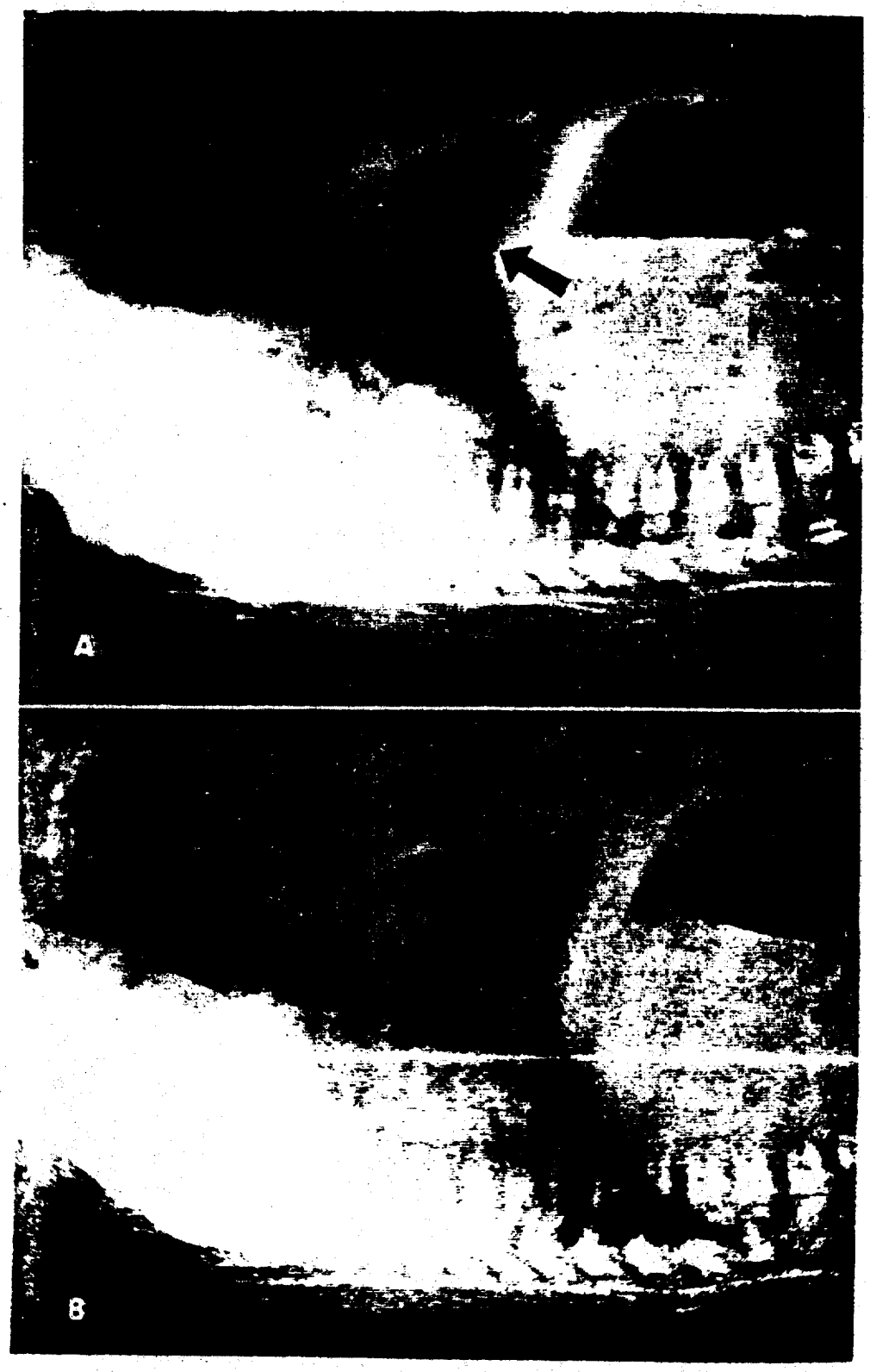

Fig. 3. Roentgenograms of infant after intravenous injection of carbon dioxide (7.5 c.c./kg.). A. Right atrium and right ventricle filled with gas immediately after injection. Tricuspid ring (arrow). B. Ten seconds after injection. Fluid level and small residual bubble in right ventricle.

rapidly soluble gas such as carbon dioxide wherever possible.

Practical Clinical Considerations: Rubin (17) in 1919 introduced the use of carbon dioxide for pneumoperitoneum by tubal insufflation, emphasizing, in a subsequent report, the advantages of rapid absorption of this gas from the peritoneal cavity. No mention was made, however, of the safety of the procedure in case of accidental introduction of gas into the systemic veins.

Teschendorf (18) recognized the importance of a readily soluble gas for use in diagnostic roentgenology and in 1951 reported his long experience with nitrous oxide. He emphasized the advantages of this gas in eliminating the hazard of gas embolism. Nitrous oxide and carbon dioxide have similar solubility coefficients in serum at body temperature, each being approximately twenty times as soluble as air or oxygen.

The results of clinical and experimental studies suggest the following recommendations for clinical practice:

1. A readily soluble gas should be used wherever possible for diagnostic or therapeutic insufflation. Carbon dioxide would seem to be preferable, although nitrous oxide may be equally satisfactory.
2. When air embolism is suspected, the patient should be immediately placed in left decubitus with the head dependent.
3. Artificial respiration should be instituted at once.
4. If other measures fail, aspiration of air from the right atrium may be attempted.

SUMMARY

The injection of gas as a contrast material in clinical diagnostic roentgenology involves hazards of venous and arterial gas embolism due to the entry of air into the circulation. The adverse physiologic effects of venous air embolism result from air trap in the outflow tract of the right ventricle and pulmonary artery and from blockade of the small peripheral pulmonary vessels. In an emergency, elimination of the air trap by placing the patient in the left decubitus position may be life-saving. In arterial embolism, the air enters the pulmonary venous channels and is carried to the superiorly located systemic arteries. In this fashion, very small amounts of air may produce serious blockade of one or more of the coronary or cerebral vessels.

The possibilities of carbon dioxide as a contrast medium for angiocardiography were demonstrated in cinefluorographic studies. Rapid intravenous injection of large amounts of this gas (7.5 c.c./kg.) in dogs produced only minor, transient physiologic alterations. Two infants tolerated similar doses well.

This study indicates that use of a soluble gas, such as carbon dioxide, for insufflation in diagnostic roentgenology should eliminate the hazard of gas embolism.

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ADDENDUM

Since this paper was submitted, an important pertinent contribution has been made by C. L. Ransom, R. R. Landis, and R. McClelland (Air Embolism and Cardiac Arrest Following Presacral Air Injection, Mid-Atlantic Section, American Urological Association, Hot Springs, Va., April 19–21, 1956). Three hundred urologists, replying to a questionnaire, reported 53 fatalities with injection of air or oxygen (21 presacral and 32 flank injections) in a total of 8,000 patients injected.

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SUMARIO

Embolia Gaseosa. Algunas Consideraciones Diagnósticas Comprendiendo el Empleo Experimental de Bióxido de Carbono como Substancia Intracardíaca de Contraste

La inyección de gas como substancia de contraste entraña riesgos de embolia gaseosa venosa y arterial debida a la entrada de aire en la circulación. Los adversos efectos fisiológicos de la aeroembolia venosa dimanan del atrapamiento de aire en el trayecto de salida del ventrículo derecho y la arteria pulmonar y del bloqueo de los pequeños vasos pulmonares periféricos. En un apuro, la eliminación del aire atrapado colocando al enfermo en la posición de decúbito izquierdo puede salvar la vida. En la embolia arterial, el aire penetra en los conductos venosos del pulmón y es conducido a las arterias situadas más arriba en el organismo. En esa forma, pequeñísimas cantidades de aire pueden producir intensa oclusión de uno o más de los vasos coronarios o cerebrales.

Estudios experimentales en perros demostraron que las causas fundamentales de los trastornos circulatorios en la aeroembolia venosa son tanto mecánicos como fisiológicos. También se demostró experimentalmente que la rápida inyección intravenosa de grandes cantidades (7.5 c.c./kg.) de bióxido de carbono no producían más que pequeñas, y pasajeras alteraciones fisiológicas. Dos lactantes toleraron bien dosis semejantes. Las posibilidades de este gas como medio de contraste para la angiocardiografía quedaron demostradas en estudios cinefluorográficos.

Indica este estudio que el uso de un gas soluble, tal como el bióxido de carbono, para insuflación en la roentgenología de diagnóstico debe eliminar el riesgo de la embolia gaseosa.

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THE CENTRAL NERVOUS SYSTEM CHANGES RESULTING FROM INCREASED CONCENTRATIONS OF CARBON DIOXIDE*

by

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The increasing use of carbon dioxide as a biological form of psychiatric treatment (1) has raised the question of the probable effect of this treatment on the tissues of the central nervous system. Many investigators have reported on the pathologic-physiologic effects of increased concentrations of carbon dioxide in man and animals but few have investigated the morphologic changes.

In an effort to study these effects the albino rat was utilized, the animals being observed in an air-tight chamber in which the carbon dioxide-air mixture could be controlled and analyzed. Both acute and chronic experiments were conducted, and the tissues were subsequently subjected to histopathologic examination.

Acute Experiment: In this phase of the work twelve healthy adult albino rats were placed into a toxic atmosphere of carbon dioxide and kept there until they died. The concentration of oxygen varied from 18 to 21 per cent throughout the experiment and the toxic concentration of carbon dioxide was increased to 20 per cent within two hours, after which it was raised to 38 per cent and finally to 43.2 per cent at the close of the experiment. The rats survived periods of time which varied from 2½ to 19⅓ hours under such conditions.

The activity of the rats greatly diminished as the carbon dioxide concentration rose above 15 per cent. They lay quietly on the floor of the animal chamber with their necks and bodies extended and their eyes widely open. Their respirations were deep and slow as compared with the rapid respirations at the beginning of the experiment. The animals were perfused through the aorta immediately after respirations ceased. Nissl, Bodian, Weil, Cajal, Oil red O and Hortega preparations were used in the study of the morphologic alterations.

MICROSCOPIC OBSERVATION

Central Nervous System: In thionin (Nissl) preparations the greatest changes were seen in the optic thalamus, brain stem and spinal cord. The cytoarchitecture of the cerebral cortex was well preserved but many of the nerve cells were slightly swollen and they exhibited peripheral chromatolysis (fig. 1b). In the other regions of the central nervous system the degree of cell change varied from animal to animal and from locus to locus. The large nerve cells showed the most severe changes — they were swollen and vacuolated, and exhibited shrinkage of the

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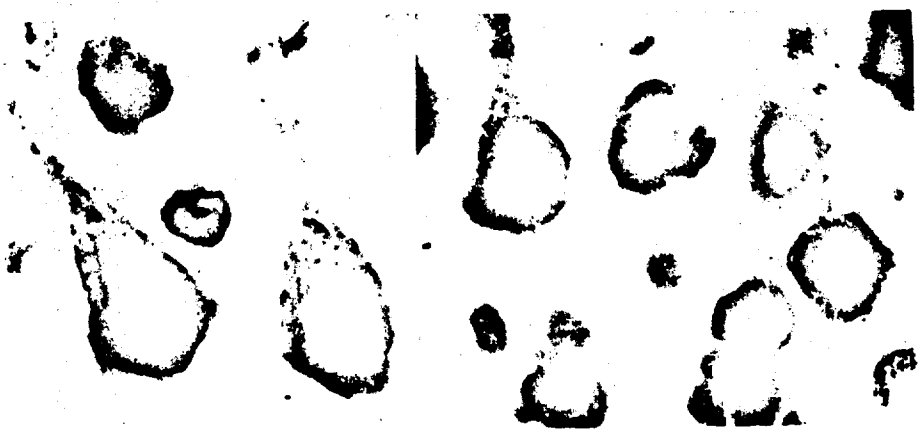
nucleus. Chromatolysis was marked and the cytoplasm stained deeply. In the loci most severely affected the oligodendroglia appeared swollen when compared with the control animals, and the nuclei of the neuroglia stained more deeply. The



(a) (b)

FIG. 1. High power (oil) photomicrograph, showing pyramidal cells from the cerebral cortex of albino rats. Thionin stain. *(a)* Control rat. *(b)* Acute rat; nerve cells slightly swollen; chromatolysis greater at the periphery; nucleus and nucleolus swollen.

cytoplasm of the mitral cells of the olfactory bulb often contained large round vacuoles, whereas the nerve cells in the dorsal and lateral nuclei of the thalamus were ballooned and contained many small vacuoles. The midbrain showed the greatest changes in both the large and small nerve cells. Chromatolysis, vacuolation, satellitosis and neuronophagia were prominent changes, shown in Figure 2 (from the region of the red nucleus). In all levels of the spinal cord the motor nerve cells showed severe cell changes, many of the changes being more marked than in affected areas in the brain. They are shown in Figure 3. On the whole the changes were most severe in the animals that lived in the toxic atmosphere of carbon dioxide for the longest period of time. The blood vessels were dilated and engorged, but otherwise were unchanged. Bodian, Weil and Oil Red O preparations showed no significant pathological alterations.

Viscera: Significant changes were observed in the lungs, liver, kidneys and myocardium. The lungs were congested, the alveolar epithelium was swollen and in places completely obliterated the alveolar spaces. Other alveoli were filled with edema fluid, and occasional hemorrhages were observed. The liver showed extreme congestion particularly in the central portion of the lobules, the central veins and sinusoids being filled with blood. In some animals the liver cells about the periphery of the lobules were vacuolated and necrotic, these changes being present to a high degree. In a few of the animals the liver cells about the central veins were

badly stained and slightly vacuolated. The kidneys uniformly showed congestion and cloudy swelling of the cells of the convoluted tubules. The myocardium showed only slight changes, some of the muscle fibers being granular in appearance.

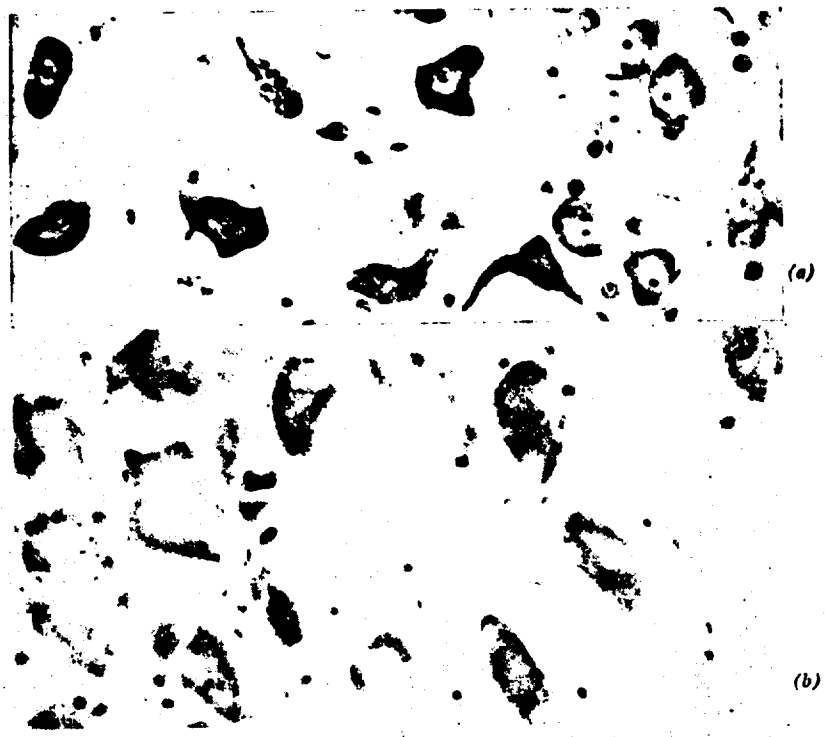


FIG. 2. High power photomicrograph, thionin stain, showing sections from red nucleus of a rat. *(a)* Control rat, showing the two types of nerve cells in the red nucleus. *(b)* Acute rat, showing marked vacuolation of cytoplasm, chromatolysis, swelling, and disintegration of nerve cells.

CHRONIC EXPERIMENT

In this phase of the work twenty-six healthy adult albino rats were placed in the gas chamber and kept there from 10 to 166 days. Seventeen lived from 10 to 107 days, and died during the experiment. The nine rats that survived up to 166 days were sacrificed at that time. The concentration of oxygen varied from 18 to 23 per cent and the toxic concentration of carbon dioxide was maintained at from 6 to 10 per cent. Occasionally the carbon dioxide content fell to that of room air when the chamber was opened for cleaning, and at other times the concentration rose to as high as 17 per cent, but only for short periods of time. At no time did the gas chamber hold more than ten rats—replacements being made as they died.

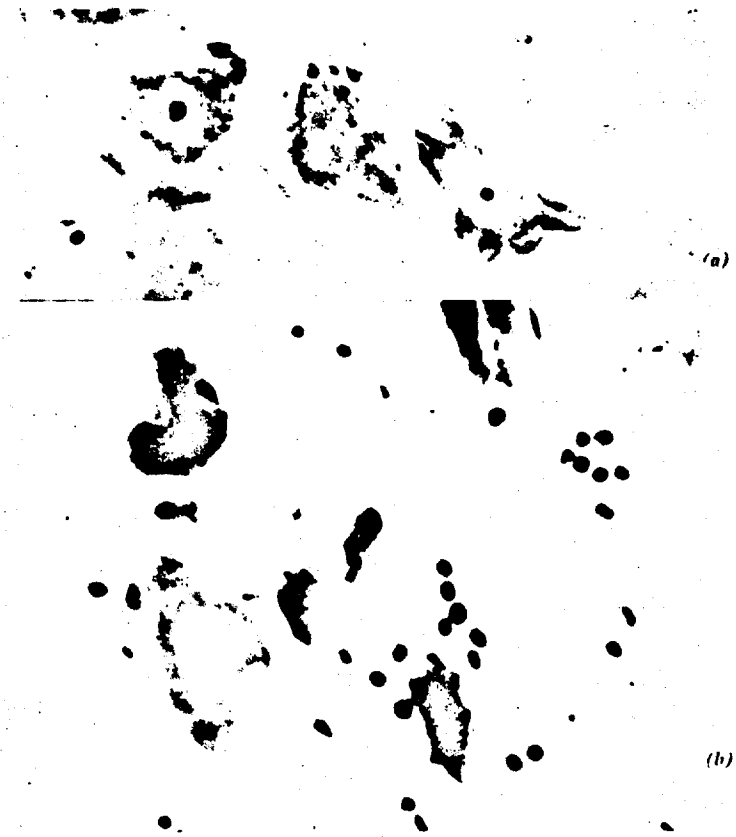


FIG. 3. High power photomicrographs showing anterior horn cells from the spinal cord of rats. Thionin stain. *(a)* Control rat. *(b)* Acute rat, showing vacuolation, shrinkage and disintegration of nerve cells, increase in neuroglia cells, satellitosis and neuronophagia.

In contrast to the acute experiment, the animals which acclimatized remained active and ate well. Some of them were sluggish, ate poorly, lost weight and died after a period varying from 10 to 107 days. The hair of all of the animals in the chronic experiment developed a brownish discoloration. These animals were perfused as in the acute experiment and the same histopathologic techniques were employed for study.

MICROSCOPIC OBSERVATION

Central Nervous System: Although there were variations in the findings from animal to animal the sites of the most severe changes corresponded to the areas

affected in the acute experiment. As in the acute experiment the relative immunity of the cerebral cortex stood out in sharp contrast with the alterations in the thalamus, brain stem and spinal cord. The histopathologic alterations were most striking in the nerve cells. In thionin preparations the nerve cells were swollen, many exhibited chromatolysis and the cytoplasm stained more deeply than in the control animals. Only occasionally could one observe vacuolated cells as in the acute experiment. In the cerebral cortex there was mild chromatolysis and the nucleus stained more deeply. In the thalamus chromatolysis was more marked and nucleus, nucleolus and cell body were swollen. In the brain stem and spinal cord the changes were similar to those in the thalamus but many more cells were affected. The changes in the cells of the red nucleus and anterior horns of the

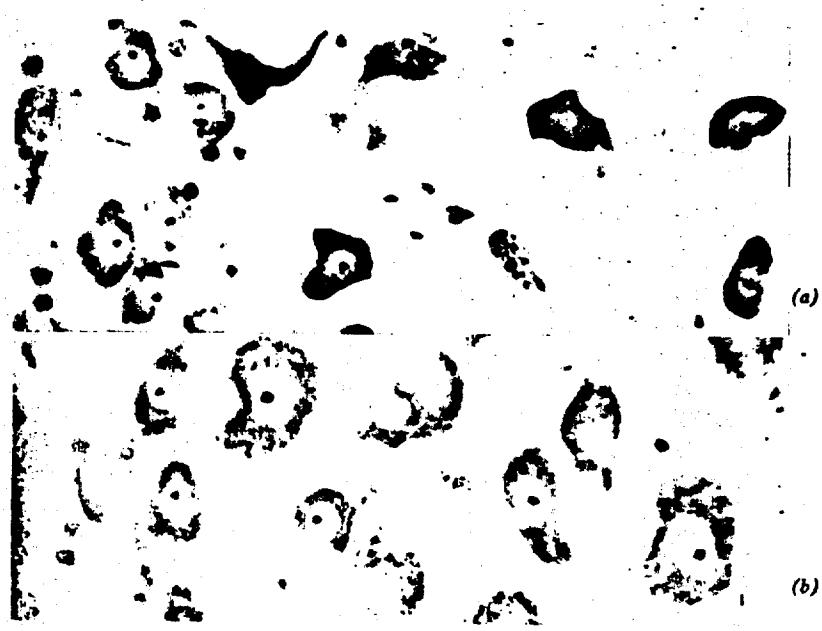


FIG. 4. High power photomicrograph, thionin stain, showing sections from the red nucleus of a rat. *(a)* Control rat. *(b)* Chronic rat, showing swollen nerve cells and chromatolysis. The cells are so swollen that the two types of cells shown in the control cannot be recognized.

spinal cord are shown in Figures 4 and 5. Myelin sheath preparations showed no change in the myeloarchitecture, and no free fat was observed in the Oil Red O preparations. The neuroglia appeared increased in number and their nuclei stained more deeply in specimens stained with thionin. The animals that did not survive the chronic experiment but died from infection and poor nutrition while in the

gas chamber showed more severe changes than the animals that survived. On the whole the lesions were in the same sites but the severity of the histopathological changes were "in between" that found in the acute and the surviving animals.

FIG. 5. High power photomicrographs showing anterior horn cells from spinal cord of rats, thionin stain. *(a)* Control rat. *(b)* Chronic rat, showing swelling of cells and nucleolus and chromatolysis.

Viscera: As in the acute experiment the significant changes were in general in the lungs, liver, kidneys and myocardium, similar in nature to the changes in the acute animals, but more mild. In the lungs congestion and emphysema were the commonest findings. Chronic bronchitis and pneumonia were also frequently observed, and not present in the acute animals. There was very little congestion in the livers. The commonest finding was slight cell change, greater at the periphery of the lobules in half of the specimens. In the kidneys and myocardium the changes were confined to a few swollen and vacuolated cells.

SUMMARY

From the foregoing descriptions of the histopathologic alterations one may summarize the essential findings as follows: In the acute experiment as well as in the chronic the greatest alterations were found in the thalamus, brain stem and spinal cord. All gradations of acute nerve cell changes were observed — from swelling, chromatolysis, and vacuolation of the cell body to its complete disintegration. In the animals dying in the course of the chronic experiment, subacute changes were observed in the nature of swelling of the nerve cells and nuclei, shrinkage of the nucleolus, partial chromatolysis, and increased basophilic staining of the cytoplasm. In the animals that survived the chronic experiment the changes were minimal. The alterations in the neuroglia in all animals were increase in number and the staining reaction, while phagocytosis and neuronophagia varied according to the severity of nerve cell changes in any locus. The blood vessel changes were dilatation and congestion in the acute animals.

DISCUSSION

In order to understand the histopathologic changes in the central nervous system associated with increased concentrations of carbon dioxide in the inspired air some of the physiologic changes which have been reported should be considered. Moderate concentrations and high concentrations of carbon dioxide have to some extent an opposite effect. The one improves the use of available oxygen and the other impedes. The one increases electrical activity of the brain and the other decreases it (2). Some investigators (3, 4, 5, 6, 7, 8) have shown that moderate concentrations of carbon dioxide increase pulmonary ventilation and blood supply to the brain. Gibbs and associates (9) have shown that man is able to carry on normal cerebral activity for a short time in the presence of reduced oxygen tension in the inspired air as low as 2 per cent if the carbon dioxide tensions of the body are maintained by sufficiently increasing the carbon dioxide tension in the inspired air. This is said to be accomplished by hyperventilation, increased blood flow through the brain and decreased peripheral circulation through constriction of blood vessels, and disassociation of oxyhemoglobin. But when the carbon dioxide tension in the inspired air rises much above 15 per cent the cellular activity is impaired. The oxygen consumption (10), pulmonary ventilation (11), and electrical activity (2) are slowed. Zuntz (11) showed that ventilation was decreased under such conditions, as was observed in this experiment. Barbour and Seevers (10) reported oxygen consumption in rats decreases rapidly when the carbon dioxide concentration rises above 20 per cent. Craig (12) states that cortical cells are anesthetized when carbon dioxide concentration is this great, and according to Miller (13) cellular anoxia exists with this percentage of carbon dioxide even though the arterial blood is saturated with oxygen above normal. He claims that carbon dioxide depresses the oxidation process in the cells and thus produces the same effect as hypoxia. Carbon dioxide as Jacobs (14) has shown acts directly on the cells. Craig (12) also has shown that oxygen consumption by

the brain cells is reduced in the presence of excessive carbon dioxide, and that cells in the medulla oblongata are affected more than those of the cerebral cortex. On the basis of these physiologic changes it appears that the histopathologic changes observed in the acute experiment were due to cellular anoxia of the asphyxia type, the failure of cellular oxidation.

The true asphyxia experiments as reported by Thorner and Lewy (15) and by Yant and associates (16) there were blood vessel changes, hemorrhage, and plugging of the vessels which did not occur in the carbon dioxide experiments, thus contributing an anemia factor. Such changes as shrinkage of the cells and large acellular areas not seen in the present experiment may have been due to the anemia. Similar changes have been described in anemia experiments (17, 18). Varying types of cell changes in anemia experiments have been reported according to Gildea and Cobb (17) who made a survey of the literature, but the most common changes were nonspecific as observed in the present observations and in the asphyxia experiments, (15, 16)—chromotolysis and swelling or shrinkage of the nerve cells. It appears that the present experiment shows an uncomplicated histopathologic alteration resulting from cellular anoxia since there was abundant blood supply to the brain.

In the chronic experiment where the carbon dioxide was below the physiologic toxic level (10) there were mild alterations in the nerve cells. Under such conditions it has been shown physiologically (10, 13) that oxygen consumption is normal, but that an acidosis persists. The cell changes may not be due to anoxia but to toxic metabolites such as lactic acid, and potassium.

It is of interest that in both the acute and chronic experiments there was a tendency for the severity of the cell changes to increase with the duration of the exposure, and for the greatest damage to be located in the thalamus, the brain stem and the spinal cord. This seems to indicate that the longer the cell lived or continued to be active the greater was the damage to its structure. The nerve cells of the cerebral cortex being the most susceptible to oxygen want (19) are anesthetized early when the carbon dioxide rises to 20 per cent (12). Hence they need less oxygen, and are protected from the anoxic changes. Gibbs and associates (2) have shown that excessive carbon dioxide depresses electrical potentials of the cells of the cerebral cortex, and Meduna (20) has explained certain motor phenomena in psychoneurotic patients subjected to 30 per cent carbon dioxide and 70 per cent oxygen as being due to suppression of the cortical cells by carbon dioxide which seems to be in agreement with the theory that the cells exhibiting greatest structural damage had maintained greatest physiologic activity. Hence the cell damage may be considered to be the physical result of an adaptive physiologic mechanism. The damaging underlying physiologic activity was the imbibition of fluid and lysis of chromatin. This seems to be nature's effort to dilute the toxins which results in swelling and vacuolation of the cells and increases as imbibing of fluid continues, a process which is active in any toxic condition, thus accounting for the nonspecific type of cell changes.

The animals in the acute experiment apparently lived until electric activity of the cells of the medullary respiratory center (21) was suppressed by the anoxia. Since the changes were greater in the thalamus, the brain stem and the spinal cord it appears to be the evidence of a greater cell resistence in these areas to anoxia, rather than the reverse which might at first glance be considered because the cells showed the greatest damage.

The changes in the cortical cells were apparently not advanced enough to be irreversible. The cells were protected through being inactivated. Whether or not the changes at lower level are reversible is a matter of conjecture, but since there were no severe blood vessel changes, no hemorrhage, and only slight glial stimulation, it seems that such would be the case. However, it appears that many of the severely damaged cells — as in the anterior horns of the spinal cord or in certain places in the brain stem — would be lost. For this reason it seems well to consider the possible remote or delayed effect of repeated damage to these cells as contributory to conditions resulting from damage to motor nerve cells as observed in amyotrophic lateral sclerosis, progressive spinal muscular atrophy, and also family periodic paralysis where there is a known potassium disturbance, one of the toxic metabolic substances resulting from excessive carbon dioxide. It is felt that such possible sequelae should be considered when carbon dioxide is to be used repeatedly in excessive amounts for therapeutic purposes, in psychiatric patients, for instances.

CONCLUSION

1. Carbon dioxide in increased concentrations in the inspired air with normal oxygen tension produces nonspecific histopathologic changes in the nerve cells resulting from cellular anoxia.

2. The cell changes are not complicated by anemia caused by blood vessel changes as in anoxic anemia, but present a pure form of organic change in nerve cells from anoxia.

3. Cells which are anesthetized early as in the cerebral cortex are protected from severe histopathologic changes.

4. Prolonged physiologic and electric activity of the cell in the presence of cellular anoxia results in increased organic change in the cell architecture somewhat proportional to the duration of this activity.

5. The cells of the primitive levels of the brain being less sensitive to oxygen want sustain the greatest structural damage, and thus account for the apparent cumulative effect of the excess carbon dioxide.

6. The nerve cell damage in albino rats acclimatizing to carbon dioxide after prolonged exposure to moderate amounts with normal oxygen tension in the inspired air is due to the toxic cellular metabolites rather than to anoxia.

7. The cell changes in the animals exposed chronically to concentrations of carbon dioxide rarely exceeding 10 per cent are reversible, and the cell changes in the animals exposed acutely to highly toxic concentrations are also reversible, except possibly those in the most damaged areas.

8. Because of the increase in the severity of the histopathologic changes with

the duration of the exposure, the severe damage to large motor cells may become irreversible, or lead to their destruction, caution in the repeated therapeutic use of carbon dioxide is warranted.

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Neurophysiological Effects of Carbon Dioxide*

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A systematic neurophysiological study of the profound effects of CO_2 on the central nervous system has not yet been made. However, detailed analyses are available on the effects of CO_2 on peripheral nerve fibers and autonomic ganglion in vitro.

It has been known for a long time that even minute concentrations of CO_2 modify the functional properties of nerve fibers (Waller, 1897), but only recently has it become evident that the presence of a certain concentration of CO_2 in the surrounding atmosphere is essential in maintaining nerve fibers in conditions closely resembling those that are found in vivo (Lorente de Nó, 1947a). As a matter of fact, careful analysis of in vitro preparations, particularly mammalian nerve fibers, requires that such preparations be dissected in an atmosphere containing 5% CO_2 in air or oxygen. In this respect nerve fibers are more susceptible to a temporary lack of CO_2 than to brief periods of anoxia.

EFFECT OF CO_2 ON MEMBRANE POTENTIAL

The basic effect of CO_2, discovered by Lorente de Nó in 1940 and published later (Lorente de Nó, 1947a), is to increase the membrane potential and specifically its slow electrotonic component. According to Lorente de Nó (1946) the total membrane potential is composed of two major fractions, *L* and *Q*. The *L* fraction is the labile and nonpropagated slow electrotonus associated with excitability changes, e.g., the negative and positive after potentials. The *Q* fraction is correlated with excitation and the conducted spike potential as well as the fast electrotonus. This specific increase of the *L* fraction caused by the action of CO_2 is in nerve *A* fibers roughly proportional to the logarithm of the concentration of the gas. In an atmosphere containing 5% CO_2 in oxy-

* Supported by the Hall-Mercer Hospital, Pennsylvania Hospital Division.

gen the L fraction is approximately 5 to 10% of the total membrane potential or 2-5 mv when recorded with surface electrodes. However, when sensory and motor nerve A fibers are studied separately, the CO_2 effect on the slow electronic potentials show quantitative and temporal differences between afferent and efferent fibers (Lundberg, 1951) and similar observations have been made in reference to extensor and flexor motor nerve fibers. A systematic study of the CO_2 effects on nerve fibers of smaller diameter is not available. However, the experimental material clearly indicates that, as an effect of CO_2 the ratio between slow electrotonic potential and spike potential (L/Q ratio) is much larger in C than in A fibers (cf. Lorente de Nó and Laporte, 1950a; Gasser, 1958). In this L/Q ratio the L fraction is particularly conspicuous in the pre-synaptic and dendritic arborizations of the superior cervical ganglion and its ganglion cells (Lorente de Nó and Laporte, 1950a; Eccles, 1935).

Biochemical differences between neurons (Lorente de Nó, 1949; Gasser, 1950) might account for some quantitative differences in the effect of CO_2. However, the basic fact remains that a moderately increased CO_2 tension leads to an increase of the slow electrotonic component of the membrane potential and that this effect is more pronounced in small nerve fibers. Since the L/Q ratio is higher in the cell body and the peripheral arborizations than in the axon, it is conceivable that the CO_2 effect is in some way related to the structure of the membrane.

CONSEQUENCES OF THE INCREASED L FRACTION PRODUCED BY CO_2

An increase in the CO_2 tension is accompanied by an increase in the threshold of stimulation and directly related to the increased slow electrotonic potential, as during a positive after potential. The greater this slow electrotonic component (L fraction) is, the lower the excitability of the neuron to externally applied cathodal pulses and the lower the intrinsic and trans-synaptic excitability. However, if the slow electrotonus is decreased by cathodal polarization or removal of the CO_2, the excitability increases rapidly. The analysis of the action potential has revealed that in the presence of CO_2 the temporarily decreased slow electrotonus during the negative after potential is accompanied by a decreased threshold of stimulation which is equivalent to that observed in CO_2-free atmosphere (Lorente de Nó, 1947a,b). Similarly, the intrinsic excitation, which occurs in the neuron during the propagation of a nerve impulse, is facilitated by a decrease in the slow electrotonic component and thus the speed of conduction is increased in the absence of CO_2. An increased slow electrotonus produced by CO_2 increases the duration of the action potential in view of the after potentials.

Thus, the three commonly noted effects of CO_2: (1) increased threshold of stimulation; (2) decreased speed of nerve impulse conduction; (3) increased

duration and height of the action potential are consequences of the increased slow electrotonic component of the membrane potential. In addition, CO_2 increases the ability of nerve fibers to endure prolonged repetitive stimulation and delays significantly the onset of anoxic depolarization. This is explained by the metabolic reaction $L \rightleftharpoons Q$ where the transformation of L to Q is the dominant feature during periods of recovery from loss of the Q fraction (spike potential) (Lorente de Nó, 1947a,b).

FEATURES OF THE CO_2 EFFECTS ON EXCITABILITY

As has been said before, the slow electrotonus of *A* fibers corresponds to the CO_2 concentration and is correlated with a given degree of excitability. However, during a period of change in the CO_2 tension the excitability has been found to vary more than the value of the electrotonus would indicate at a given moment. This "discrepancy" between excitability and electrotonic potential depends on the rate by which the CO_2 tension is being altered (Therman, 1952). The greater the rate of change of CO_2, the greater is the rate of excitability change exceeding that of the altered electrotonus. Thus, lowering of the CO_2 tension leads to an excessively increased excitability with a temporary peak which is followed by a decreased excitability occurring when the nerve reaches equilibrium with the lowered CO_2 tension. It is not uncommon under these experimental conditions to find freshly excised nerves responding with "spontaneous" discharges during periods of rapid lowering of the CO_2 tension. On the other hand, a gradually increased CO_2 tension shows the reverse picture with a temporary maximum of reduced excitability. Fluctuating the CO_2 tension between 4 and 5% about one or two times a minute in frog fibers leads to a sustained increase of excitability significantly greater than that obtained under steady state conditions in 4% CO_2. The simplest explanation for this would be a difference in the rate of uptake and release of CO_2 from the nerve fibers (cf. Fenn, 1928; Gerard, 1932) with the release of CO_2 being slower than the uptake. Under these conditions a differentiation of functionally different nerve fibers can be obtained when the common sciatic nerve trunk is stimulated. In a nerve-muscle preparation, with only the nerve in 5% CO_2, extensor (gastrocnemius) contractions are more easily obtained with short, strong pulses (1 msec) and flexor contractions (tibial group) with long, weak pulses above 10 msec in duration (Therman, 1949; 1950).

In reference to nerve-muscle preparations in general, it is a commonly observed fact that nervous stimulation of the muscle is rapidly impaired when the surrounding medium of the muscle contains CO_2. Recent studies by Gesell and co-workers (cf. Steinberger, 1957) have confirmed this and found that CO_2 reduces the endplate potential while pure O_2 restores it. Whether this is a consequence of a change in the electrolyte balance of the muscle or whether this is a unique action of CO_2 directly on the endplate is not clear.

ANODAL POLARIZATION AND CO_2

Davis, Pascual, and Rice (1928) demonstrated similarities between anodal polarization and the commonly known effects of CO_2. The close correlation between the *L* fraction of the membrane potential and anelectrotonus has been studied in detail by Lorente de Nó (1947a). The principal difference between these phenomena is that the anelectrotonus is derived from externally applied currents and that its value, determined by the current strength, can be much larger than the *L* fraction which is limited to inherent metabolic factors affected by CO_2. The close relationship between these two modes of increasing the membrane potential is emphasized by the fact that in the presence of CO_2 only minimal anodal currents are needed to produce a conduction block and that, similarly to CO_2, anodal polarization increases the ratio of slow and fast electrotonus. Furthermore, the *L* fraction and the anelectrotonus both enhance the recovery of the membrane potential after conduction of impulses through similar displacement and transport of electrically charged particles across the membane. The only difference is that the *L* fraction produced by CO_2 requires inherent metabolic mechanisms while the anodal current is the external source of energy.

EFFECTS OF CO_2 ON SYMPATHETIC GANGLION

The primary effect of CO_2 on the superior cervical ganglion of the turtle is an increase of the slow electrotonic potential (*L* fraction). The differences between nerve trunks, dendrites, synapses and pre-synaptic arborizations make the analysis more complex. Furthermore, in their extensive study of this preparation, Lorente de Nó and Laporte (1950a) have referred to the importance of the nutritional condition of the animal, a fact supported by our experience. Ganglion preparations excised from animals kept on a protein-rich diet exhibit powerful trans-synaptic excitations facilitated by the presence of 3–5% CO_2 without the tendency to create a trans-synaptic block. Pre- and post-synaptic *B* and *C* fibers react to the introduction of CO_2 with an increase in the value of the *L* fraction much the same way as *A* fibers and with similar functional consequences. Although it is generally recognized that the recovery of *B* fibers, after the passage of an impulse, does not exhibit a temporary increase of excitability (cf. Gasser, 1939; Grundfest, 1939; Lorente de Nó and Laporte, 1950b), it has been our experience that, when carefully prepared, super-normality can be observed in the pre- and post-ganglionic trunks in the presence of 5% CO_2 as well as in trans-synaptic excitation (Lloyd, 1939). The slowed speed of conduction caused by CO_2 is relatively greater in *B* than in *C* fibers (Laporte and Lorente de Nó, 1950a,b). In reference to their axons *B* ganglion cells have a significantly greater *L* fraction in the presence of CO_2 and similarly the *L* fraction is greater in the dendritic and pre-synaptic arborizations than in the

axons. As a matter of fact, it is conceivable that under certain conditions, particularly with increased CO_2 tension (5–10% instead of 3–5%), the L/Q ratio might attain an increasing value in the distal arborizations. This would produce a condition equivalent to conduction with decrement of the spike potential with ultimate block or inexcitability. The partial or complete transmission block (Heinbecker, 1930; Laporte and Lorente de Nó, 1950c) observed in the presence of 5–10% CO_2 as well as the long synaptic delays (Therman *et al.*, 1940) might be due to the increased L/Q ratio. To what extent this increased L/Q ratio, which CO_2 produces, applies to the more recent studies on relative inexcitability of cortical dendrites (Clare and Bishop, 1955; Purpura and Grundfest, 1956; Grundfest, 1957) remains to be seen. That conduction with decrement actually does occur in nerve fibers, in spite of the generally accepted opposite viewpoint, has recently been demonstrated by Lorente de Nó (1958). The analysis of trans-synaptic excitation and its modification in the ganglion by agents like CO_2 has shown all the elementary phenomena that are known to appear during reflex activity of the spinal cord (Lorente de Nó and Laporte, 1950c). However, in the CNS there are the additional complicating factors like the activity of internuncial neurons (Forbes, 1922).

NATURE OF THE CO_2 EFFECT

It has been proposed that the CO_2 effect depends on a change of the internal *p*H of the nerve fiber (cf. Adrian, 1920; Fenn, 1928; Lehmann, 1937). This would be in agreement with present concepts of acid-base equilibria since CO_2 is a buffering agent. However, whether the internal *p*H is changed by CO_2 is not known, but lowering the external *p*H in the absence of CO_2 does not reproduce some of the essential aspects of CO_2 effects. Thus, it must be concluded that CO_2, besides being a buffering agent, has its own specific properties (cf. Lorente de Nó, 1947a). In view of the fact that nerve fibers have an oxidative reserve (cf. Gerard, 1932) and that the beneficial effect of CO_2 on the functional ability of the nerve can be obtained under anoxic conditions but only in the presence of this metabolic reserve, it must be concluded that CO_2 produces its action by participating in reactions derived from oxidative metabolism. Lorente de Nó (1947a) has postulated that CO_2 as a metabolic end product displaces the point of equilibrium of reversible metabolic reactions by increasing the concentration of oxidized substances. It is of interest to note that CO_2 fixation occurs in animal tissue (cf. Werkman and Wood, 1942; Evans, 1944) and that in nervous tissue the total CO_2 fixed from air is proportional to its concentration (Buchanan, 1951). It is well known that many substances containing an amino group react reversibly with CO_2 and form unstable carbamino compounds. Among substances giving such a reaction are NH_3, aliphatic amines, and amino acids (cf. West, 1956). The condensation which occurs between NH_3 and CO_2 forming carbamate has certain similarities to the

reaction between water and CO_2. The phosphorylated compound, carbamyl phosphate, has been found to be an intermediary of biological importance in a variety of enzyme systems (Jones *et al.*, 1955). Thus, CO_2 enters into biochemical equilibrium reactions and by virtue of its concentration it shifts the point of equilibrium according to the law of mass action as this law applies to enzymatic systems, that is to say, living systems. To what extent this type of metabolic reaction occurs in the nervous system remains to be seen.

EFFECT OF CO_2 ON THE CENTRAL NERVOUS SYSTEM

Although the gross effects of even minor pCO_2 changes in the central nervous system (CNS) are accompanied by distinct psychological and physiological manifestations, a systematic and detailed neurophysiological study of these has not yet been undertaken. It is a difficult task to study the effect of CO_2 in the CNS with its complex interneuronal patterns and the role of different parts of neurons in a volume conductor. However, there is every reason to believe that the principal effect of CO_2 in the CNS is the same as in simpler structures, namely, an increase of the L fraction and thus of the threshold of excitability. A further complication arises from the fact that due to fluctuations in blood flow and blood pCO_2, the CO_2 concentration in nervous tissue is not stable and thus excitability, with the slow electrotonic potentials, would be likely to show minor fluctuations. It is also to be expected that different structures of the CNS will exhibit functionally different reactions to CO_2 depending primarily on neuroanatomical differences. All these difficulties are already apparent from the debate on the effect of CO_2 on respiration which has continued since Legallois (1812) initiated the concept of a respiratory center. It is not the purpose of this article to review either the studies on localized neurophysiological action of CO_2 on respiration (cf. Gesell, 1939; Pitts, 1946) or the extensive literature on carbon dioxide in brain physiology. It will suffice to point out that analogies exist between features in the CNS and simpler structures like nerve fibers and sympathetic ganglia.

In the spinal cord an increased CO_2 tension produces an increased synaptic delay in a monosynaptic reflex arc (Brooks and Eccles, 1947). Such central inhibitory effects of CO_2 have been studied by several investigators (cf. Stein & Pollock, 1949; Carey, Schaefer, and Delgado, 1955). There is general agreement that a moderately increased CO_2 concentration to approximately 20% decreases cortical excitability in much the same way as it does in the sympathetic ganglion. In the hypothalamus, Gellhorn (1957) has found differences in the sensitivity to CO_2 between the posterior and anterior parts. Other studies have dealt with differential effects of CO_2 on subcortical structures (Pollock and Gyarfas, 1952; Dunlop, 1957a,b). In reference to cortex and hypothalamic-cortical interrelationship it seems that CO_2 suppresses cortical activation from hypothalamic overactivity (cf. Gellhorn, 1957).

Differential effects of exposure to CO_2 on carbonic anhydrase activity and electrolyte exchange in hypothalamic and cortical regions were observed (Schaefer and Barton, 1956).

In moderate concentrations CO_2 acts as an anticonvulsant (Toman and Goodman, 1948; Woodbury *et al.*, 1958) and prevents epileptic seizures (Lennox and Cobb, 1928). On the other hand, any rapid lowering of the CO_2 tension, whether from a high or normal level, will markedly increase the excitability and the tendency for convulsive seizures as is well known from the effect of hyperventilation in epileptics. In concentrations above 20%, CO_2 can produce convulsions as well as rapid loss of consciousness (cf. Meduna, 1950).

The development of carbon dioxide withdrawal seizures in rats was found to be related to: (1) a drop in blood CO_2 tension exceeding 100 mm Hg and resultant decreases in brain tissue CO_2 content; (2) a fall in plasma calcium; and (3) changes in the extra and intracellular H_2O and Na content (cf. Carey and Schaefer, 1959).

In reference to electroencephalographic studies, both with Bremer's "encephale isole" preparation (Bremer and Thomas, 1936) as well as in humans (Lennox, Gibbs, and Gibbs, 1936), there is a vast literature (cf. Hill and Paar, 1950) on the changes of the cortical potentials and their rhythmic activity produced by changes in the CO_2 tension. It is generally understood that recordings of electrical potentials from the surface of the cortex are primarily manifestations of fluctuations in the membrane potentials of the apical dendrites (Li and Jasper, 1953; Clare and Bishop, 1955; Purpura and Grundfest, 1956). In view of this it is perhaps relevant to point out the analogy in the sympathetic ganglion where CO_2 produces marked changes in the slow electrotonic component of the membrane potential (L fraction) of the dendrites and presynaptic arborizations. However, a systematic study of the CO_2 effect on cortical dendrites as well as on other structures of the CNS has to be carried out before more comprehensive correlations can be made.

COMMENTS

Detailed neurophysiological studies on the direct effects of carbon dioxide exist only in reference to isolated parts of the nervous system, peripheral nerve fibers and sympathetic ganglia, maintained in vitro. The basic effect of CO_2 is to increase the slow electrotonic component of the total membrane potential by which the following changes are produced: increased threshold of stimulation, increased duration and height of the action potential, and decreased speed of nerve impulse conduction. Since the slow electrotonic component is correlated with excitability, fluctuations of the CO_2 tension lead to specific excitability changes which manifest themselves in a sustained lowering of the threshold of stimulation. A rapid decrease of the CO_2 tension tends to produce "spontaneous" discharges of nerve impulses. There is a difference in the degree

of the CO_2 effect on different nerve fibers and different parts of a neuron. The presence of CO_2 is essential for the proper maintenance of nerve fibers and ganglia since the slow electrotonic component is needed in the recovery processes. The CO_2 effect is directly dependent on the presence of an oxidative reserve and only indirectly on the presence of oxygen. The effect of CO_2 cannot be reproduced by alteration of the external *p*H. It is suggested that the CO_2 effect is related to the condensation of CO_2 and certain amino groups forming unstable carbamino compounds which participate in reversible biochemical equilibrium reactions.

The neurophysiological effects of CO_2 on the CNS have not been systematically studied, but available evidence suggests that they are essentially similar to those found in simpler structures.

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MASS CARBON DIOXIDE INTOXICATION

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Introduction

Carbon dioxide gas (CO_2) is a colorless gas that is heavier than air. It has already caused many cases of intoxication in industry. These cases occurred predominantly in closed spaces (silos, vaults, ships' holds), in which CO_2 had accumulated as a result of fermentation processes. If the harmful effect of CO_2 on the human body is examined more closely, it is necessary to pay attention to the following factors:

- 1. The stimulating action of CO_2 on important brain centers and cerebral cortex;
- 2. The paralyzing effect of CO_2 on brain centers and cerebral cortex;
- 3. The irritating action on skin and mucous membranes.

The fact should not be omitted that many older investigators shared the belief that CO_2 is not toxic per se but causes dangers only in its role as an oxygen displacer.

CO_2 and Metabolism

CO_2 plays an important role in many vital functions. CO_2 and H_2O are produced in the cell by combustion. Respiration serves to remove the CO_2 and supply oxygen. The gas is transported via the blood. A small portion of the CO_2 is dissolved in the blood; the greatest part is chemically bound.

Sodium bicarbonate is produced with Na_2CO_3. Another fraction is bound to protein, chiefly to hemoglobin. The total carbon dioxide content at the

alveolar carbon dioxide pressure of 40 mmHg in the arterial blood is approximately 50 vol.%, 60 vol.% in venous blood. The 10% difference is given off to the venous blood from the tissues. The H ion concentration of arterial and venous blood is practically the same so that the ratio between the free CO_2 and bicarbonate must be the same. The blood behaves as a buffer solution. The ratio of CO_2 to $NaHCO_3$ is 1:20; it is held as constant as possible. The CO_2 coming from the tissue is bound to alkali. The total quantity of substances present in the blood capable of binding CO_2 is called the alkali reserve.

As a result of the release of oxygen to the tissue, the 70-times stronger acid of the oxyhemoglobin passes over into the weaker acid of the hemoglobin. A greater fraction of the alkali bound to the oxyhemoglobin is released and stands in opposition to the CO_2. The opposite reaction takes place in the lung. Through the absorption of oxygen, oxyhemoglobin is produced which binds alkali again. The CO_2 is exhaled. This exhalation takes place in accordance with the laws of gas diffusion. The gas flows from places with higher to places with lower gas pressure.

The normal ambient air consists of 0.03% CO_2 and 21% O_2; the remainder is nitrogen. The exhaled air consists of 4-5% CO_2 and 16% O_2. The composition of the alveolar air is, besides nitrogen, 14.5 vol.% O_2 and 5.5 vol.% CO_2. The alveolar O_2 pressure is 120 mmHg, and the CO_2 pressure is 40 mmHg.

Carbon dioxide forms the adequate stimulus for the respiratory center by nervous regulation. Carbon dioxide is regarded as the respiratory hormone. (The stimulating effect of CO_2 on respiration has recently led to the addition of CO_2 to medical oxygen.) O_2 lacks a stimulating effect.

Every shift in the CO_2 and $NaHCO_3$ ratio toward the acid side leads to increased respiration. Franke has demonstrated by numerous experiments the elevation of CO_2 in the blood as the cause of the stimulation of the respiratory center. An elevated CO_2 pressure also has an effect on the blood circulation; it causes vasodilatation. According to Eppinger, carbon dioxide has a stimulating effect on the intestine.

The body responds to an increase in the carbon dioxide in the inhaled air with hyperventilation. If the carbon dioxide concentration of the ambient air approaches that of the alveolar carbon dioxide content of 5.5%, then the CO_2 can no longer be flushed out of the blood. The CO_2 pressure in the blood increases. This is first countered by hyperventilation; and finally it is no longer possible to maintain the respiration rate, and an increase in the CO_2 pressure of the blood is unavoidable. Paralysis of the respiratory center occurs, and death ensues with symptoms of cerebral stimulation and paralysis. The hyperventilation, in the extreme the respiration of Kuszmaul, can also arise at the other end, when other acids such as lactic acid and keto acids occur in the organism in large quantities. These bind too much alkali so that now sufficient alkali is no longer available for the CO_2 produced during the combustion.

As a result of the hyperventilation, the CO_2 pressure in the blood is reduced as far as possible, and in this way the state of acidosis can be compensated longer. During muscular work, lactic acid and phosphoric acid as well as a large amount of CO_2 are produced as a result of intensified oxidation processes.

The CO_2 stimulates the respiratory center as well as the vasomotor and heart-vagus center, and likewise the motor centers. It acts on the

heart and vessel tonus and on muscle tonus. The effect is always biphasic. The stimulation phase is followed by the paralysis phase.

Prausznitz and Schultzik investigated the toxic effect of CO_2 in different concentrations in animals. Naturally, nothing was said of an O_2 deficit in these cases. They carried out the tests with dogs, cats, rabbits, and rats. Up to 10% CO_2, they saw nothing abnormal. With increasing concentrations, the respiratory frequency and depth increased. At 25-30% CO_2, the animals fell asleep, consciousness had apparently vanished, corneal and light reflexes were extinguished. At 50% CO_2, in most cases there was a brief excitation stage, the animals collapsing after a few seconds. At this time they displayed frequent tonic and clonic convulsions; there was very strong opisthotonus in cats and rabbits. In other animal tests, frequently briefly after the respiratory stoppage, there was a temporary relaxation of the extremities and tonic contractions of the stretching musculature in the manner of decerebration rigidity.

The Cheyne-Stokes respiratory type was observed very frequently; in dogs, a considerable saliva flow was registered. They often had a bloody foam on the mouth, which was regarded as a sign of damage to the respiratory tract, also loss of urine and defecation. The pulse was often accelerated and irregular. If the animals were restored to fresh air, then they often vomited but recovered soon. The rate of recovery depended less on the percentage CO_2 in the air than on being accustomed and on the length of the test. The high tolerance of the animals to the gas was striking when one considers that humans lose consciousness at 10% CO_2, while the animals still did not display any symptoms at this concentration. Even at a concentration of 60-70% CO_2, the rabbits remained

alive for a few minutes. Only the behavior of cats and mice was noteworthy at 10% CO_2, they sat together in a quite friendly manner and entirely forgot their natural enmity.

According to Starling, increasing the CO_2 tension in the blood causes an enlargement of the heart volume during diastole and to a lesser degree during systole. The stroke volume increases with increasing CO_2 pressure from 2% to 8%. At higher CO_2 pressure of approximately 12-20% CO_2, the systole is influenced more strongly in the sense above, resulting in reduction of the stroke volume. Eppinger recorded in animal tests, in addition to blood pressure elevation, simultaneously a pulse retardation. In animals which at the beginning of the tests already had a low heartbeat frequency (vagus-tonus), a long-lasting extrasystole was frequently observed. It was known that the bradycardia produced by the inhalation of CO_2 was dependent on the action of the heart-vagus center. When the vagus was cut, no changes occurred.

Since the bradycardia always set in within a few seconds, it is assumed that the cause must be sought not only in the stimulation of the vagus nerve by the increase of the CO_2 content of the blood but also especially in a reflexive action (probable trigeminus vagus reflex of the nasal mucosa).

CO_2 intoxication, according to Eppinger, causes a strong expansion of the heart chambers, chiefly on the right, and the progressing intoxication causes disturbances of the stimulation conduction as well as a lengthening from auricle to ventricle. An anatomical examination revealed swollen heart valves and occasionally hemorrhages under the endocardium. The unopened heart was contracted (reduced heart tonus). American

researchers found in animal tests that even small quantities of CO_2 caused dilation of the blood vessels.

The rhythmic activity of the vascular muscles was halted under the influence of CO_2 if the vasomotor centers were isolated. If the vessels were connected to the vasomotor center, then vasocontraction occurs. The result of the vascular contraction was a blood pressure elevation. However, if the CO_2 concentration remained above 30%, then the stimulating effect was lost, and the blood pressure decreased below normal levels until total heart stoppage was reached. Elevated CO_2 pressure in the blood causes contriction of veins. The venous contriction was eliminated after the cutting of the nervous connections.

In every case of CO_2 intoxication, one must wonder whether a pure CO_2 intoxication is involved or not. One must distinguish between cases in which in addition to the elevated CO_2 content, the O_2 content of the air is normal, and those cases in which the O_2 content is strongly lowered. If the O_2 content of the air drops from a normal 21% to 14%, then the respiration is already considerably impaired. A content of 11% causes suffocation.

The individually different sensitivity plays a great role in CO_2 intoxication. It is higher in cases of physical stress, heart and lung diseases, and pregnancy. A certain adaptation to CO_2 can take place. Of decisive importance for the symptoms occurring in a case of CO_2 intoxication is the concentration of the CO_2 and the duration of its inhalation. Opinions are divided concerning the harmful concentration. Paul found a content of 1% CO_2 to be harmless. Ordinarily, a concentration of 2% is regarded as innocuous.

Tests on American submarines revealed respiratory disturbances already at 2% CO_2, and most authors observe a distinct effect on the average only above 3%. The increase in respiratory efficiency is essentially based more on the deepening of the individual breaths rather than on an increase in respiratory rate. At 5-6% CO_2, the respiratory value is doubled. Prausnitz found a frequency increase from a normal 18 to 28.3 at this concentration. With increasing CO_2 concentration in the air, the dyspnea increases rapidly. Strong persons can tolerate a concentration of 8% CO_2 for a long time, although they are quickly fatigued by the stepped-up respiration. Weak persons collapse already at a concentration of 6% CO_2. The duration of inhalation is decisive.

Schultzik recorded manifest dyspnea after remaining 15 minutes in a room containing 5% CO_2. At 7%, CO_2 dyspnea already occurred after 12 minutes, and at 8%, it occurred immediately. Lower concentrations evoke the same effects after longer inhalation as higher concentrations in a shorter time. The dyspnea cannot be tolerated longer than 15 to 20 minutes at 8% CO_2. Remarkable is the fact that the stimulating effect on the respiratory center still persists in spite of the loss of consciousness. Only at 30% CO_2 does the critical point occur between the stimulating and paralyzing action on the respiratory center. At this concentration, paralysis of the respiratory center occurs with great probability. According to some, the latter occurs already at 20% CO_2 in a few seconds, according to others only after some time.

It is not simple to establish precisely at which concentration of CO_2 death finally occurs. From the foregoing, however, it appears that animals possess a much greater tolerance to CO_2 than humans.

Symptomatology

The symptomatology of CO_2 intoxication is as follows:

Initial Symptoms:

0.1% is regarded as the outer limit of harmlessness of the CO_2 content of the air. Above 2% CO_2, the first symptoms of headache. Within increasing concentration, there is a stronger brief feeling of pressure in the head, usually coupled with a buzzing in the ears and visions of stars. At 3%, intensified respiration occurs. Many persons, chiefly mine workers, who have had experience with the effect of CO_2-rich air note as the first symptom at approximately 2% CO_2 a stimulation of the skin and mucous membranes, conjunctivitis, an irritating taste on the tongue or a mild stinging in the nose. Generally symptoms appear from the direction of the skin and mucous membranes only after 4-5% CO_2.

The exposed persons note on tender parts of the skin such as the scrotum and the inside of the thigh a stimulation, and in the mouth and cheek a dry feeling and an irritation of the conjunctiva. Frequently there are disturbances of sensation as well as a reduction of pain and the sense of touch.

Upon inhalation of CO_2 concentrations above 10%, a brief excitation stage appears immediately (paralysis of the cerebral hemispheres, unrest, narrowing of consciousness, contraction of the pupils, sleep, and possibly vomiting), anesthesia and narcosis. During the tolerance stage (spinal cord paralysis) we see the disappearance of the reflexes and of the slow regular pulse. During the collapse stage (medulla paralysis) we see dilated pupils, irregular respiration, weak pulse, cyanosis and subsequently death through paralysis of the respiratory and vasomotor center.

The complete intoxication picture is as follows: from 5% CO_2 on, we see increasing depth of respiration and to a lesser degree increased respiratory frequency.

At 8% CO_2, distinct dyspnea arises, which is tolerated for about 15-20 minutes; 9.5% CO_2 is the upper limit that is tolerated. There is an increase in the blood pressure, a further elevation of the pulse rate, reduced stroke volume, an increase of the flowing blood volume, activation of resting blood depots, and an elevation of venous tone.

After 10% CO_2, anesthesia and ataxia occur, which pass over into narcosis.

At 20% CO_2, narcosis occurs directly. In the outer air, the CO_2 disappears rapidly from the blood. Before awakening, frequently states of recovery appear, motor unrest, tongue biting, incontinence, and vomiting are observed. The patients often behave as if they were in an alcoholic fit or suffered from epilepsy. A strong euphoria also appears and an absolute lack of any subjective feelings of illness. When fresh air is suddenly admitted, occasionally seizures of epileptiform character are seen.

The paralysis of the respiratory and vasomotor center begins with approximately 30% CO_2. The reflexes vanish, a collapse of the blood circulation occurs with reduction of heart and vessel tone, and a drop of the blood pressure to shock values.

The after-effects consists of headache, dullness, vertigo, convulsions in the limbs, thirst, and a slight temperature elevation. Frequently an albuminuria and a glucosuria persist for several days. Lung changes can also appear, which form the early stage of lung edema.

Frequent rhythm disturbances and dilatation are found in the heart. In heart patients, we also occasionally see auricular fibrillation.

The therapy consists of placing the patient in the fresh air and administering pure O_2. In cases of lost consciousness, artificial respiration must be applied as quickly as possible, since here only a few minutes decide between life and death. Protection against chilling is also important. Intracardiac injection of epinephrine 1 on 1000 and cardiac massage may be applied. Artificial respiration must be continued on the patient until recovery or until rigor mortis sets in. During states of recovery, the patients must be sedated so that the O_2 consumption is not increased by motor unrest, which could counteract the recovery. Transportation must always be accomplished with the patient prone and never without accompaniment.

Case History

Below is a description of an accident where the aforestated is applicable.

On Wednesday, 5/4/61, about ± 11:30 a.m. a very serious accident occurred near a wharf, which costs the lives of seven men. The accident occurred on board an oil tanker. During the extensive investigation by the Labor Inspection and Police, it was found that an installer employed by a firm dealing in fire extinguishing equipment, during activities in the deck house cabin where the carbon dioxide gas fire extinguishing equipment was installed on the ship, made a mistaken action. There is a fire extinguishing installation aboard this ship which is designed to extinguish rapidly any large fires occurring in the engine room. This fire extinguisher consists of several hundred steel bottles installed in a special room that are filled with liquefied carbon dioxide CO_2. Two of these bottles formed the so-called starting battery. Near an access door

to the engine room is a control panel where the mechanism is installed for putting the starting battery into operation. When this operating panel is open, the warning sirens in the engine room are automatically set off so that any personnel located there can vacate this room in time.

The actuation is supposed to take place by drawing a steel cable located in the operating panel and running to the starting battery, which opens the rapid cut-off valves of the starting bottles. The out-flowing carbon dioxide then opens the cut-off valves of all the other bottles so that in a very short time the carbon dioxide is flowing via a central pipeline to the discharge opening into the engine room. It must be regularly checked whether all bottles are entirely filled with carbon dioxide. During repair operations, for which reason the ship was in dock, these check-ups were carried out by two workers of a firm specializing in fire extinguishing installations. They were engaged in the CO_2 room with deactivating the installation by uncoupling at the starting battery the steel cable with which the cut-offs of the starting bottles are opened.

During the uncoupling of one of the two starting bottles, the installer exerted too much pressure on the cable connecting the heads of two bottles. As a result, the cut-off of the second bottle was opened and the gas flowed out of this bottle under a pressure of 60 atmospheres into the pipeline. This resulted in the activation of the entire installation, and the process could no longer be stopped. The engine room was immediately filled with part of the contents of the CO_2 bottles. Since CO_2 gas is heavier than air, it sank to the floor of the engine room. The gas was mixed with an odor-producing substance resembling soap, since it is by nature odorless.

The installer had realized immediately the serious consequences of his

act and ran to the engine room to warn the workers present there. The personnel of the wharf immediately threw a compressed air hose into the engine room chamber, and one has probably to thank this for the fact that this accident did not cost the lives of many more men.

After the danger had passed, an investigation was made of the contents of the CO_2 bottles installed in the CO_2 chamber. A total of $\pm$ 1473.5 m^3 of gaseous CO_2 had been released.

The bottles 1 through 23 were each filled with 33 kg of CO_2; the bottles 24 through 112 were empty; starting bottle I was filled with $\pm$ 33 kg of CO_2, and starting bottle II was filled with $\pm$ 23 kg of CO_2.

Since before the accident took place, bottles 24 through 112 and starting bottles II should each have contained 33 kg of CO_2, then a total of $89 \times 33 + 10 = 2947$ kg of liquid CO_2 should have been discharged into the engine room, which agrees with $\sim$ 1473.5 m^3 of gaseous CO_2. With respect to the entire engine room capacity (5950 m^3), this amounts to $1473.5/59.5 = 24.76\%$. Since CO_2 is heavier than air, it thus preferably seeks lower-lying spaces; thus, in the lower part of the engine room the concentration should have been even higher.

A specification of the Maritime Inspection applies for Dutch ships, according to which there must be a cut-off between CO_2 chamber and engine room so that this cut-off can be tightly closed during repair operations so that no direct connection exists any longer between CO_2 chamber and engine room. However, this specification does not apply to foreign ships. It seems desirable in those cases when no cut-off is present in the CO_2 line between these two chambers to require that in such cases work cannot be done simultaneously in CO_2 room and engine room.

30 persons were involved. Four of these were overcome immediately, three others died during transportation to or in the hospital shortly after admission. One of the ship's crew, chiefly Italians, died; six crew members were admitted to hospitals. The victims brought out of the engine room through the top by rescuers with oxygen masks were almost all unconscious; they were very pale and cyanotic. Most of them recovered consciousness at the wharf after administration of O_2. Upon admission, a number of the patients were agitated and had to be sedated. Some displayed changes in the EKG shortly after admission which later rapidly vanished.

As a result of the fact that many persons were working in the lower part of the engine room, because of the long distance that they had to cover in order to get out, they became aware of the dangerous situation but could not reach the exit before losing consciousness. Either there were many of these cases or they were injured by others. Many had black eyes, graze wounds, and minor internal bruises. Sofar as I know, no fractures occurred.

Shortly after arrival at the hospital, 11 persons were sent home. A total of 21 were admitted. On 10/4/61, six patients, and on 12/4/61, four patients were still present in the hospital. All had been discharged by 28/4/61.

Symptoms upon Admission to Hospital

Upon admission to the hospital, the following symptoms were found:

Deviations	No. of patients
loss of consciousness	5
retrograde amnesia	1
motor unrest	3
drowsiness	3
low pressure	8
low body temperature	3

Deviations	No. of patients
headache	3
vomiting	3
conjunctivitis	2
peripheral vascular collapse	2
rapid pulse	1
slow pulse	1
strongly disturbed EKG	3
auricular fibrillation	1
cyanotic tongue	2
blood deviations: leucocytosis	7
urine deviations: albuminuria	7
acetone (traces)	2
reduction (strongly positive)	1
reduction (trace)	1
sediment deviations	3
paraesthesia	2
blood sputum on first day	2

One patient had initial glucosuria of 2%, this percentage dropping in the course of a few days, although the symptoms had not completely disappeared at the time of discharge.

Two young men of ± 25 years had serious EKG disturbances, to wit negative T waves in several leads, indicating damage to the myocardium. They were treated with bed rest for three weeks. After this rest treatment was completed they were discharged; the EKG continued to display deviations so that some permanent damage to the myocardium might well be assumed. They should remain under specialist control.

One 64 year-old man also had serious EKG deviations, indicating damage to the myocardium as a result of O_2 deficit. He was a chronic lung patient. He was treated with bed rest and anticoagulant therapy. He was advised not to work any longer in view of his age.

It is a pity that an EKG was not made on all of the victims; presumably it would have revealed more deviations and fewer persons would have been sent home directly.

Autopsy Report

An autopsy was performed on one of the "soundest" corpses. It was that of a 38 year-old man.

Findings:

A. Numerous punctiform hemorrhages in the connective tissue of the eyelids and in the white of the eyes; punctiform hemorrhages on heart and lungs; the same in the mucous membrane of the renal pelvis; lungs heavy, rich in blood, and congested; liver, kidneys rich in blood; soft brain tissue rich in blood, body covered with purplish red and blue spots; blood in the heart dark red.

B. A hemorrhage in the mucous membrane of the lower lip and under the mucous membrane of the tongue.

C. No other pathological changes of significance.

The changes summarized under A above indicate suffocation, which was the cause of death. No cause for this suffocation death could be demonstrated.

In cases of fatal carbon dioxide intoxication, symptoms of suffocation are encountered but no definite specific changes. The deviations revealed by autopsy are thus not in conflict with carbon dioxide intoxication although by themselves are no proof of it. A chemical study in cases of carbon dioxide intoxication does not provide useful results.

The lesions of the lower lip and tongue (B) may have been caused by muscular spasms (as a result of suffocation).

The first aid in this area was extremely well organized. In the absence of the plant physician, the first aid was performed under the leadership of the temporary plant physician. Police, fire department,

G. G. B., the Labor Inspection, and a number of doctors arrived at the scene of the catastrophe very rapidly. Ambulances were escorted by the police and all important crossroads were held open.

Discussion

Van Raalte: Cannot permanent T wave changes in the electrocardiagram also be caused by a scar after bleeding in the myocardium? Then one can no longer speak of permanent damage of the myocardium. This makes the prognosis appear entirely different.

Vogelenzang: Indeed.

Van Raalte: In a case of serious CO_2 intoxication, especially frequently on a dock, the best and quickest manner of proper therapy is the administration of oxygen under overpressure and preferably in a pressure chamber.

Vogelenzang: No diving bell was present.

Kaan: Is anything presently known on the carbon dioxide content of poorly ventilated office rooms? We have very many persons who are present all day in whom many headache complaints arise at the end of the day.

Vogelenzang: The CO_2 accumulation in poorly ventilated rooms can probably run to a few per cent, but this does not explain the headaches.

In response to the question of to what extent CO_2 intoxication is aggravated by asphyxiation, above all in persons located in the lower part of the ship where the carbon dioxide had taken on such a concentration that it displaced the oxygen, Vogelenzang answers that this is difficult to decide. Animal experiments have shown that CO_2 has a toxic effect but it also acts by displacing O_2.

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Massale vergiftiging door koolzuurgas

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Inleiding

Koolzuurgas (CO_2) is een kleurloos gas, zwaarder dan lucht. Het heeft in de industrie reeds vele vergiftigingen veroorzaakt. Deze zijn voornamelijk voorgekomen in besloten ruimten (silo's, kelders, scheepsruimen) waarin zich door gistingsprocessen CO_2 had opgehoopt. Wanneer men de schadelijke invloed van CO_2 op het menselijk lichaam nader beschouwt, is het noodzakelijk aandacht te schenken aan het volgende:

– 1. de stimulerende werking van CO_2 op belangrijke hersencentra en hersenschors;

– 2. de verlammende invloed van CO_2 op hersencentra en hersenschors;

– 3. de prikkende werking op huid en slijmvliezen.

Niet onvermeld mag blijven, dat vele oudere onderzoekers de mening waren toegedaan, dat CO_2 op zichzelf niet toxisch zou zijn, doch slechts in zijn rol als zuurstofverdringer gevaren opleverde.

CO_2 en stofwisseling

CO_2 speelt bij vele levensfuncties een belangrijke rol. In de cel ontstaat bij verbranding CO_2 en H_2O. De ademhaling dient voor de uitscheiding van CO_2 en voor de toevoer van zuurstof. Het gas-transport gaat via het bloed. Een gering deel van de CO_2 is in het bloed opgelost, het grootste deel is chemisch gebonden.

Met NA_2CO_3 ontstaat natriumbicarbonaat. Een ander deel is aan eiwit gebonden, vooral aan haemoglobine. Het totale koolzuurgehalte bij de alveolaire koolzuurspanning van 40 mm kwik bedraagt in arterieel bloed ongeveer 50 vol.%, in veneus bloed 60 vol.%. Het verschil van 10 % wordt uit het weefsel aan het veneuze bloed afgegeven. De H-ionen-concentratie van arterieel en veneus bloed is praktisch gelijk, zodat de verhouding tussen het vrije CO_2 en het bicarbonaat gelijk moet zijn. Het bloed gedraagt zich als een bufferoplossing. De verhouding van CO_2 tot $NaHCO_3$ is als 1 : 20; zij wordt zoveel mogelijk constant gehouden. De uit het weefsel komende CO_2 wordt aan alkali gebonden. De totale hoeveelheid der in het bloed aanwezige stoffen, die CO_2 kunnen binden, duidt men aan als alkali-reserve.

Door de zuurstofafgifte aan het weefsel gaat het 70 keer sterkere zuur van het oxyhaemoglobine over in het zwakkere zuur van het haemoglobine. Een groter gedeelte van het aan oxyhaemoglobine gebonden alkali komt vrij en staat tegenover het CO_2. In de long vindt de omgekeerde reactie plaats. Door opneming van zuurstof ontstaat oxyhaemoglobine, dat weer alkali bindt. De CO_2 wordt uitgeademd. Deze uitademing vindt plaats volgens de wetten der gasdiffusie. Het gas stroomt van plaatsen met hogere naar plaatsen met lagere gasdruk.

De normale buitenlucht bestaat uit 0,03 % CO_2 en 21 % O_2, de rest is stikstof. De uitademingslucht bestaat uit 4-5 % CO_2 en 16 % O_2. De samenstelling van de alveolaire lucht is, behalve stikstof, 14,5 vol.% O_2 en 5,5 vol.% CO_2. De alveolaire O_2-spanning bedraagt 120 mm kwik en de CO_2-spanning bedraagt 40 mm kwikdruk.

Voor het ademcentrum vormt naast nerveuze regulatie de koolzuur de adequate prikkel. Men beschouwt koolzuur als het hormoon der ademhaling. (De stimulerende invloed van CO_2 op de ademhaling heeft destijds ertoe geleid om CO_2 toe te voegen aan medicinale zuurstof). Ook O_2-gebrek zou stimulerend werken. Iedere verschuiving in de verhouding CO_2 en $NaHCO_3$ naar de zure kant leidt tot versterkte ademhaling. Franke heeft door talrijke proeven de CO_2-stijging in het bloed als oorzaak der prikkeling van het ademcentrum aangewezen. Ook op de bloedsomloop heeft een gestegen CO_2-spanning een werking; het geeft vasodilatatie. Volgens Eppinger werkt koolzuur prikkelend op de darm.

Op een vermeerdering van de koolzuur in de inademingslucht antwoordt het lichaam met een hyperventilatie. Nadert de koolzuurconcentratie van de buitenlucht die van het alveolaire koolzuurgehalte van 5,5 %, dan kan de CO_2 uit het bloed niet meer uitgewassen worden. De CO_2-spanning in het bloed stijgt. Deze wordt eerst tegengegaan door hyperventilatie, tenslotte is het niet meer mogelijk, de ademhalingsfrequentie verder op te voeren en een stijging van de CO_2-spanning van het bloed is onvermijdelijk. Er treedt verlamming van het ademcentrum op en onder cerebrale prik-

kelings- en verlammingsverschijnselen treedt de dood in. De hyperventilatie, in het extreme, de ademhaling van Kuszmaul kan aan de andere kant ook ontstaan, wanneer in het organisme andere zuren zoals bijvoorbeeld melkzuur en ketozuren in grote hoeveelheden voorkomen. Deze binden teveel alkali, zodat nu voor de bij de verbranding ontstaande CO_2 niet voldoende alkali meer ter beschikking staat.

Door de hyperventilatie wordt de CO_2-spanning in het bloed zoveel mogelijk verlaagd en op deze wijze kan de toestand van acidose langer gecompenseerd blijven. Bij spierarbeid ontstaat melkzuur en fosforzuur en bovendien veel CO_2 tengevolge van gestegen oxydatieprocessen.

De CO_2 prikkelt het ademcentrum alsmede het vasomotoren- en hart-vagus-centrum, eveneens de motorische centra. Het werkt op de hart- en vaattonus en op de spiertonus. De werking is steeds bifasisch. Na de prikkelende komt de verlammende fase.

Prausznitz en Schultzik gingen de toxische werking van CO_2 in verschillende concentraties na bij dieren. Uiteraard was er in deze gevallen geen sprake van O_2-gebrek. Zij deden proeven met honden, katten, konijnen en ratten. Tot 10 % CO_2 zagen zij niets abnormaals. Bij toenemende concentraties nam de ademfrequentie en ademdiepte toe. Bij 25-30 % CO_2 sliepen de dieren in, het bewustzijn was schijnbaar verdwenen, cornea en lichtreflex opgeheven. Bij 50 % CO_2 ontstond meestal een kort excitatie-stadium, de dieren vielen na enige seconden om. Daarbij vertoonden zij dikwijls tonische en clonische krampen; bij katten en konijnen bestond zeer sterke opisthotonus. In andere dierproeven werden dikwijls kort na de ademstilstand een voorbijgaande verslapping der extremiteiten en tonische contracties van de strek-muskulatuur op de wijze van de onthersenings-stijfheid gezien.

Het ademtype van Cheyne-Stokes werd zeer dikwijls waargenomen; bij honden werd aanzienlijke speekselvloed geconstateeerd. Dikwijls hadden zij bloederig schuim op de bek, wat als een teken van beschadiging van de luchtwegen werd beschouwd, alsmede afgaan van urine en ontlasting. De pols was dikwijls versneld en onregelmatig. Werden de dieren weer in de frisse lucht gebracht dan braakten zij dikwijls, doch zij herstelden zich snel. De snelheid van herstel hing minder af van het percentage CO_2 in de lucht dan van de gewenning aan en de lengte van de proefnemingen. Opvallend was de hoge tolerantie van de dieren tegen het gas als men bedenkt, dat de mens bij 10 % CO_2 het bewustzijn verliest, terwijl de dieren bij deze concentratie nog geen verschijnselen vertoonden. Konijnen waren nog bij een concentratie van 60-70 % CO_2 enige minuten in leven te houden. Vermeldenswaard was bij 10 % CO_2 slechts het gedrag van katten en muizen, die geheel vreedzaam samen zaten en hun natuurlijke vijandschap geheel vergaten.

Volgens Starling veroorzaakt toeneming van de CO_2-spanning in het bloed een vergroting van het hartvolume tijdens de diastole en in geringere mate tijdens de systole. Het slagvolume neemt toe met het stijgen der CO_2-spanning van 2 % tot 8 %. Bij hogere CO_2-spanning van ongeveer 12-20 % CO_2 wordt de systole meer in voornoemde zin beïnvloed, wardoor het slagvolume vermindert. Eppinger stelde in dierproeven naast bloeddrukstijging gelijktijdig een polsverlangzaming vast. Bij dieren, die bij het begin der proeven reeds een geringe hartslagfrequentie hadden (vagus-tonus) zag men dikwijls een langdurige extra-systole. Bewezen werd, dat de door inademen van CO_2 ontstaande bradycardie afhankelijk was van de werking op het hart-vagus-centrum. Bij doorsnijding van de n.vagus ontstonden geen veranderingen.

Daar de bradycardie steeds binnen enkele seconden inzette, wordt aangenomen, dat de oorzaak niet slechts in de prikkeling van de n.vagus door de stijging van het CO_2-gehalte in het bloed, doch ook in het bijzonder in een reflexwerking (waarschijnlijk trigeminus-vagus-reflex van het neuslijmvlies uit) gezocht moest worden.

CO_2-intoxicatie veroorzaakt volgens Eppinger een sterke verwijding der hartholten, vooral rechts en de voortschrijdende vergiftiging geeft stoornissen van de prikkelgeleiding, zoals een verlenging van boezem naar kamer. Een anatomische bevinding toonde gezwollen hartkleppen en soms bloedingen onder het endocard. Het niet geopend hart was samengevallen (verminderde hart-tonus) Amerikaanse onderzoekers vonden in dierproeven dat reeds geringe hoeveelheden CO_2 verwijding van bloedvaten gaven.

De rhythmische werkzaamheid van de vaatspieren stopte onder inwerking van CO_2, indien de vasomotorische centra werden geïsoleerd. Stonden de vaten met het vasomotorencentrum in verbin-

ing, dan ontstond vaatvernauwing. De uiting van vaatcontractie was een bloeddrukstijging. Bleef de CO_2-concentratie echter boven 30 %, dan viel de prikkelende werking uit en de bloeddruk zonk onder normaal, totdat volledige hartstilstand was bereikt. Verhoogde CO_2-spannig in het bloed geeft constrictie van venen. De venen-constrictie bleef na doorsnijding van de nerveuze verbindingen uit.

Bij iedere CO_2-intoxicatie moet men zich de vraag stellen, of er sprake is van een zuivere CO_2-vergiftiging, ja dan neen. Men moet onderscheid maken tussen die gevallen, waarin naast het verhoogde CO_2-gehalte, het O_2-gehalte van de lucht normaal is, en die gevallen, waarin dit O_2-gehalte sterk verminderd is. Daalt het O_2-gehalte van de lucht van normaal 21 % tot 14 %, dan ontstaat reeds een aanzienlijke bemoeilijking der ademhaling. Een gehalte van 11 % doet verstikking ontstaan.

De individueel verschillende gevoeligheid speelt bij de CO_2-vergiftiging een grote rol. Zij is hoger bij lichamelijke inspanning, hart- en longziekten en zwangerschap. Er kan een zekere gewenning aan CO_2 plaats vinden. Doorslaggevend voor de optredende verschijnselen bij een CO_2-vergiftiging is de concentratie van de CO_2 en de tijdsduur van haar inademing. Over de schadelijke concentratie zijn de meningen verdeeld. Paul vindt een gehalte van 1 % CO_2 nog onschadelijk. Meestal wordt een concentratie van 2 % nog als onschuldig beschouwd.

Bij proefnemingen op Amerikaanse onderzeeboten werden reeds bij 2 % CO_2 stoornissen in de ademhaling merkbaar, in doorsnede wordt door de meeste auteurs eerst vanaf 3 % een duidelijke werking waargenomen. De verhoging van de werkzaamheid der ademhaling berust in wezen meer op een verdieping der afzonderlijke ademteugen dan op toeneming der ademhalingsfrequentie. Bij 5-6 % CO_2 verdubbelt zich de ademgrootte. Prausnitz vond bij deze concentratie een frequentie stijging van normaal 18 tot 28,3. Met toenemende CO_2-concentratie in de lucht neemt de ademnood snel toe. Krachtige mensen kunnen een concentratie van 8 % CO_2 een tijd lang verdragen, zij raken echter door de opgevoerde ademhaling zeer vermoeid. Zwakke mensen collaberen reeds bij een concentratie van 6 % CO_2. Beslissend is de tijdsduur van de inademing.

Schultzik stelde duidelijk dyspnoe vast na 15 minuten oponthoud in een 5 % CO_2 bevattende ruimte. Bij 7 % CO_2 trad reeds na 12 minuten dyspnoe op en bij 8 % ontstond dit direct. Lagere concentraties roepen na langere inademing dezelfde werkingen op als hogere concentraties in kortere tijd. De dyspnoe is bij 8 % CO_2 niet langer dan 15 tot 20 minuten te verdragen. Vermeldenswaard is, dat de prikkelende werking op het ademcentrum nog blijft bestaan ondanks de verlamming van het bewustzijn. Eerst bij 30 % CO_2 ligt het critische punt tussen de prikkelende en de verlammende werking op het ademcentrum. Bij deze concentratie volgt met grote waarschijnlijkheid verlamming van het ademcentrum. Volgens sommigen vindt dit laatste echter reeds bij 20 % CO_2 plaats in enige seconden, volgens anderen eerst na enige tijd.

Het is niet eenvoudig precies vast te stellen, bij welke concentratie CO_2 uiteindelijk de dood intreedt. Uit het voorgaande blijkt echter wel, dat dieren een veel grotere tolerantie tegen CO_2 bezitten dan de mens.

Symptomatologie

De symptomatologie van de CO_2-intoxicatie is als volgt:

Initiaal symptomen:

Als uiterste grens voor de onschadelijkheid van het CO_2-gehalte in de lucht geldt *0,1 %*. Boven 2 % CO_2 bestaan de eerste verschijnselen uit hoofdpijnen. Bij stijgende concentratie ontstaat een sterker drukgevoel in het hoofd, meestal gepaard gaande met oorsuizingen en sterretjes zien. Bij 3 % vindt een versterkte ademhaling plaats. Vele mensen, vooral mijnwerkers, die ervaringen over de werking van CO_2-rijke lucht hebben, merken als eerste symptoom bij ongeveer 2 % CO_2 een prikkeling van de huid en slijmvliezen op, conjunctivitis, prikkelende smaak op de tong of lichte steken in de neus. In het algemeen treden van de kant van de huid en slijmvliezen eerst van 4-5 % CO_2 af verschijnselen op.

De geëxponeerde mensen merken aan tere huidplaatsen, zoals scrotum en binnenzijde van de dij een prikkeling op, verder in de *mond en kaak* een droog gevoel en een prikkeling van de *conjunctiva*. Dikwijls komt het tot sensibiliteitsstoornissen zoals vermindering van pijn en tastgevoel.

Bij inademing van CO_2-concentraties boven 10 % ontstaat direct na een kort excitatiestadium (verlamming der grote hersenen, onrust, bewustzijnsvernauwing, verkleining der pu-

pillen, slaap en eventueel braken), verdoving en narcose. Tijdens het tolerantiestadium (ruggemergsverlamming) zien wij het verdwijnen der reflexen en der langzame regelmatige pols. Tijdens het collaps-stadium (medulla-verlamming) zien wij wijde pupillen, onregelmatige ademhaling, kleine pols, cyanose en vervolgens dood door verlamming van adem- en vasomotorencentrum.

Het volledige vergiftigingsbeeld is als volgt: Van 5 % CO_2 af zien wij toenemende diepte der ademhaling en in iets geringere mate verhoging der ademfrequentie.

Bij 8 % CO_2 ontstaat duidelijke dyspnoe die ongeveer 15-20 minuten verdragen wordt; 9,5 % CO_2 is de hoogste grens die verdragen wordt. Er treedt een stijging van de bloeddruk op, verder verhoogde polsfrequentie, vermeerderd slagvolume, vermeerdering van het stromende bloedvolume, in werking treden van rustende bloed-depots en een verhoging van de venustonus.

Van 10 % CO_2 af ontstaat een verdoving en treedt ataxie op, die in narcose overgaat.

Bij 20 % CO_2 treedt direct een narcose op. In de buitenlucht verdwijnt de CO_2 snel weer uit het bloed. Vóór het ontwaken treden dikwijls opwindingstoestanden op, men neemt motorische onrust, tongbeet, incontinentie en braken waar. De patiënten gedragen zich dikwijls, alsof zij in een alcoholische roes verkeren of aan epilepsie lijden. Er bestaat ook een sterke euphorie en een absoluut gemis aan enig ziekte-inzicht. Bij plotselinge toevoer van frisse lucht zag men nogal eens krampen van epileptiform karakter optreden.

Met ongeveer 30 % CO_2 begint de verlamming van het adem- en vasomotorencentrum. De reflexen verdwijnen, er treedt een collaps van de bloedcirculatie op, vermindering van hart- en vaattonus, daling van de bloeddruk tot shockwaarden.

De na-verschijnselen bestaan uit hoofdpijnen, suffheid, duizeligheid, trekkingen in de ledematen, dorst en lichte temperatuurstijging. Vaak bestaat gedurende enige dagen een albuminurie en een glucosurie. Ook kunnen longveranderingen optreden, die het voorstadium vormen van longoedeem.

Aan het hart vindt men vaak rhythmestoringen en dilatatie. Bij hartpatiënten zien wij nogal eens boezem-fibrilleren. De therapie bestaat uit de patiënt in frisse lucht plaatsen en toedienen van zuivere O_2. Bij bewustelozen moet zo snel mogelijk kunstmatige ademhaling worden toegepast, aangezien hier steeds weinige minuten beslissen over leven en dood. Belangrijk is verder bescherming tegen afkoeling. Eventueel kan intracardiale inspuiting van adrenaline 1 op 1000 en hartmassage worden toegepast. De kunstmatige ademhaling moet bij de patiënt voortgezet worden tot zijn herleving of totdat lijkstijfheid intreedt. Bij opwindingstoestanden moeten de patiënten gesedeerd worden, opdat door de motorische onrust het O_2-verbruik niet verhoogd wordt wat het herstel zou kunnen tegengaan. Een transport moet steeds liggend en nimmer zonder begeleider plaats vinden.

Casuïstiek

Er volgt een beschrijving van een ongeval, waarop het voorafgaande betrekking heeft.

Op woensdag 5.4.'61 om ± 11.30 uur vond op het terrein van een scheepswerf een zeer ernstig ongeluk plaats, hetwelk aan 7 mensen het leven heeft gekost. Het ongeluk gebeurde aan boord van een olietanker. Bij het uitgebreide onderzoek door Arbeidsinspectie en Politie is gebleken, dat een monteur, in dienst van een firma in brandblusapparatuur, bij werkzaamheden in de dekhuiscabine waarin op dit schip de koolzuurgasbrandblusinstallatie staat opgesteld, een verkeerde handeling heeft verricht.

Aan boord van dit schip bevindt zich een brandblusinstallatie welke bestemd is om bij een eventuele grote brand in de machinekamer deze brand snel te kunnen blussen. Deze brandblusinstallatie bestaat uit ruim honderd in een speciale ruimte opgestelde stalen flessen, welke gevuld zijn met tot vloeistof verdicht koolzuur CO_2. Twee van deze flessen vormen de zogenaamde startbatterij. Nabij een toegangsdeur tot de machinekamer is er een bedieningskastje, waarin het mechanisme is aangebracht om de startbatterij in werking te stellen. Bij het openen van dit bedieningskastje worden de waarschuwingssirenes in de machinekamer [illegible] laten.

Het inwerking stellen dient te geschieden door aan een in het bedieningskastje aanwezig en naar de startbatterij doorlopend staalkabeltje te trekken, waardoor de snelafsluiters van de startflessen opgetrokken worden. Het uitstromende koolzuur bewerkstelligt daarna het openen van de afsluiters

van alle andere flessen, zodat in zeer korte tijd het koolzuur via een centrale pijpleiding naar de uitstroom-openingen in de machinekamer stroomt.

Regelmatig moet gecontroleerd worden, of alle flessen geheel gevuld zijn met koolzuur. Tijdens de reparatiewerkzaamheden, waarvoor het schip bij de werf in dok lag, werd deze controle verricht door twee arbeiders van een op brandblusinstallaties gespecialiseerd bedrijf. Zij waren in de CO_2 kamer bezig met het buiten werking stellen van de installatie door bij de startbatterij het staalkabeltje, waarmee de afsluiters van de startflessen worden opengetrokken, te ontkoppelen.

Bij het ontkoppelen van één der twee startflessen oefende de monteur te veel druk uit op het kabeltje, dat de hoedjes van beide flessen verbindt. Daardoor werd de afsluiter van de tweede fles geopend en stroomde het gas uit deze fles onder een druk van zestig atmosfeer in de leiding. Hierdoor trad de gehele installatie in werking en was het proces niet meer te stuiten. De machinekamer werd onmiddellijk met een gedeelte van de inhoud uit de CO_2 flessen gevuld. Daar CO_2-gas zwaarder is dan lucht, daalde het neer tot op de bodem van de machinekamer. Het gas was met een lichte reukstof, als van zeep, vermengd daar het gas van nature reukloos is.

De monteur heeft zich onmiddellijk de rampzalige gevolgen van zijn handeling gerealiseerd en rende naar de machinekamer om de daar aanwezige arbeiders te waarschuwen. Het personeel van de werf wierp onmiddellijk een persluchtslang in de machinekamerruimte en hieraan is het waarschijnlijk te danken, dat deze ramp niet aan nog veel meer mensen het leven heeft gekost.

Na afloop der ramp werd een onderzoek ingesteld naar de inhoud van de in de CO_2-ruimte opgestelde CO_2-flessen. Er is $\pm$ 1473,5 m³ gasvormig CO_2 vrijgekomen.

De flessen 1 t/m 23 waren gevuld elk met $\pm$ 33 kg CO_2; de flessen 24 t/m 112 waren ledig; startfles I was gevuld met $\pm$ 33 kg CO_2; startfles II was gevuld met $\pm$ 23 kg CO_2.

Als voordat het ongeval plaats vond, de flessen 24 tot en met 112 en startfles II elk 33 kg CO_2 zouden hebben bevat, dan zou totaal $89 \times 33 + 10 = 2947$ kg vloeibaar CO_2 de machinekamer zijn afgevoerd, hetgeen overeenkomt met $\pm$ 1473,5 m³ gasvormig CO_2. Ten opzichte van de gehele machinekamerruimte (5950 m³) bedraagt dit $\frac{1473,5}{59,5} = 24,76\,\%$. Aangezien CO_2 zwaarder is dan lucht zakt het dus bij voorkeur naar lager gelegen ruimten; onder in de machinekamer moet de concentratie dus nog hoger zijn geweest.

Voor Nederlandse schepen geldt een voorschrift van de Scheepsvaartinspectie, dat er een afsluiter moet bestaan tussen CO_2-kamer en de machinekamer, zodat bij reparaties deze afsluiter kan worden dicht gedraaid, zodat er geen directe verbinding meer bestaat tussen CO_2-kamer en machinekamer. Voor buitenlandse schepen geldt dit voorschrift echter niet. Het lijkt gewenst in de gevallen, waarin in de CO_2-leiding tussen deze twee ruimten geen afsluiter aanwezig is, te verlangen, dat er dan niet gelijktijdig gewerkt wordt in CO_2-kamer en machinekamer.

Er waren 30 personen bij betrokken. Hiervan waren er direct reeds 4 overleden, 3 anderen zijn tijdens het vervoer naar of in het ziekenhuis kort na binnenkomst overleden. Van de bemanning van het schip, meest Italianen, is één persoon overleden; 6 bemanningsleden zijn in ziekenhuizen opgenomen. De getroffenen, die uit de machinekamer naar boven werden gebracht door redders met zuurstofmaskers, waren bijna allen bewusteloos, ze zagen erg bleek en cyanotisch. De meesten zijn na toedienen van O_2 reeds op de werf bij bewustzijn gekomen. Bij opneming was een aantal patiënten onrustig en moest worden gesedeerd. Een aantal had veranderingen op het E.C.G. kort na binnenkomst, die later snel weer verdwenen.

Doordat vele personen onder in de machinekamer werkzaam waren, hebben zij door de lange afstand, die zij moesten afleggen om eruit te komen, toen zij zich bewust werden van de gevaarlijke situatie, de uitgang niet meer gehaald, doch zijn bewusteloos geworden. Daarbij zijn er velen gevallen of zijn zij bezeerd door anderen. Velen hadden blauwe ogen, schaafwonden en lichte inwendige kneuzingen. Fracturen zijn, voorzover ik weet, niet voorgekomen.

Kort na aankomst in het ziekenhuis zijn 11 personen naar huis gestuurd. In totaal werden er 21 opgenomen. Op 10.4.'61 waren nog 6 patiënten, op 12.4.'61 nog 4 patiënten in het ziekenhuis aanwezig. Op 28.4.'61 was iedereen ontslagen.

Verschijnselen bij ziekenhuisopneming

Bij opneming in het ziekenhuis werden de navolgende verschijnselen gevonden:

Afwijkingen	*aantal patiënten*
bewusteloosheid	5
retrograde amnesie	1
motorische onrust	3
suffheid	3
lage tensie	8
lage lichaamstemperatuur	3
hoofdpijnen	3
braken	3
conjunctivitis	2
perifere vaatcollaps	2
snelle pols	1
langzame pols	1
sterk gestoord E.C.G.	3
boezemfibrilleren	1
cyanotische tong	2
bloedafwijkingen: leukocytose	7
urine-afwijkingen: albuminurie	7
aceton (spoor)	2
reductie (sterk positief)	1
reductie (spoor)	1
sedimentafwijkingen	3
paraesthesieën	2
bloederig sputum op 1ste dag	2

Eén patiënt had aanvankelijk glucosurie van 2 %, in de loop van enkele dagen is dit percentage gedaald, doch het verschijnsel was bij ontslag nog niet geheel verdwenen.

Twee jonge mannen van ± 25 jaar hadden ernstige E.C.G.-stoornissen, namelijk negatieve T-toppen in vele afleidingen, wijzend op beschadiging van het myocard. Zij werden behandeld met bedrust van 3 weken. Na afloop van deze rustkuur werden zij ontslagen; het E.C.G. bleef afwijkingen vertonen zodat enige blijvende beschadigingen van het myocard toch wel aangenomen moet worden. Zij zullen onder specialistische controle moeten blijven.

Een man van 64 jaar had ook ernstige E.C.G.-afwijkingen, die wijzen op beschadiging van het myocard door O_2-tekort. Hij was een chronische longpatiënt. Hij werd behandeld met bedrust en anti-stollingstherapie. Hij kreeg het advies niet meer te gaan werken, mede in verband met zijn leeftijd.

Het is jammer, dat niet bij alle getroffenen een E.C.G. is gemaakt, vermoedelijk zouden er meer afwijkingen zijn geconstateerd en minder personen direct naar huis zijn gestuurd.

Sectierapport

Op één zo gaaf mogelijk lijk werd sectie verricht. Het betrof een man van 38 jaar oud.

Bevindingen:

A. Talrijke stipvormige bloedingen in het bindvlies van de oogleden en in het wit van de ogen; stipvormige bloedingen op hart en longen; idem in slijmvlies van nier bekkens; longen zwaar, bloedrijk en zuchtig; lever, nieren bloedrijk; zachte hersenvliezen bloedrijk; lijkvlekken paarsrood en -blauw; bloed in het hart donkerrood.

B. Een bloeding in het slijmvlies van de onderlip en onder het slijmvlies van de tong.

C. Geen verdere ziekelijke veranderingen van betekenis.

De sub *A* samengevatte veranderingen duiden op verstikking, welke de dood heeft teweeggebracht. Er was geen oorzaak voor deze verstikkingsdood aanwijsbaar.

Bij dodelijk verlopen koolzuurvergiftiging worden tekenen van verstikking aangetroffen, doch geen bepaalde specifieke veranderingen. De bij de sectie gebleken afwijkingen zijn dus niet in strijd met koolzuurvergiftiging, doch op zich zelf daarvoor geen bewijs. Scheikundig onderzoek bij koolzuurvergiftiging levert geen bruikbare resultaten op.

De letsels van de onderlip en de tong *(B)* kunnen door spierkrampen (tengevolge van verstikking) zijn ontstaan.

De eerste hulpverlening op het terrein van de werf was uitstekend georganiseerd. T.g.v. vakantie van de bedrijfsarts stond de eerste hulpverlening onder leiding van de waarnemend bedrijfsarts. Politie, Brandweer, G.G.D., Arbeidsinspectie en een aantal doktoren waren zeer spoedig na de ramp aanwezig. Ziekenauto's werden door politie geëscorteerd en alle belangrijke verkeersknooppunten werden vrijgehouden.

Discussie

Van Raalte: Blijvende T-topveranderingen in het electrocardiogram kunnen toch ook veroorzaakt zijn door een litteken na een bloedinkje in het myocard? Men kan dan niet meer spreken van een blijvende beschadiging van het myocard. Dit doet de prognose heel anders er uit zien.

Vogelenzang: Inderdaad.

Van Raalte: Bij een ernstige CO_2-vergiftiging, speciaal vaak op een werf, is de beste en snelste wijze van doelmatige therapie het toedienen van zuurstof onder overdruk en liefst in een drukkamer.

Vogelenzang: Een duikerklok was niet aanwezig.

Kaan: Is er iets bekend over het koolzuurgehalte in slecht geventileerde kantoorruimten? Wij hebben vrij veel mensen, die de hele dag aanwezig zijn en bij wie veel hoofdpijnklachten aan het eind van de dag optreden.

Vogelenzang: De CO_2-ophoping in slecht geventileerde ruimten kan weleens oplopen tot enkele procenten, maar dit verklaart de hoofdpijnklachten niet.

Op de vraag in hoeverre de CO_2-vergiftiging [illegible] troebeld door asphyxie vooral bij degenen die onder [illegible] het schip hebben gezeten waar de koolzuur een zodanige concentratie had aangenomen, dat de zuurstof werd verdrongen, antwoordt Vogelenzang, dat dit niet goed te scheiden is. Uit dierproeven blijkt, dat CO_2 een toxische werking heeft, al werkt het ook door verdringing van O_2.

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TOXIC PROPERTIES OF CARBON DIOXIDE* **

By RALPH M. WATERS, M. D.
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When I was a youngster I remember coming upon my father's hired man in the barn one day in the act of drinking a large glass of clear, amber colored fluid. He explained that he had been advised to drink a glassful of his own urine twice a day as a remedy for some slight ailment, the nature of which I have forgotten. I am often reminded of this observation when I see carbon dioxide used as a therapeutic agent. The two chief pathways for the elimination of waste products from our bodies are the kidneys and the lungs. Nitrogenous elements and water pass out largely through the kidneys and carbon dioxide largely through the lungs. Any interference with kidney function dams back nitrogenous waste substances, resulting, when complete, in death within a few days. The necessity for the elimination of carbon dioxide through the lungs is still more urgent and immediate.

Complete rebreathing of a bag full of pure oxygen, permits ample oxygen supply while it interferes with the elimination of carbon dioxide. Under such circumstances, a normal individual makes an extreme effort to increase his breathing sufficiently to prevent increasing the carbon dioxide content of blood and tissues. Until the atmosphere inspired reaches a carbon dioxide concentration above that of the normal alveolar air (over five per cent), no increase in alveolar or blood carbon dioxide occurs. The minute-volume ventilation of the lungs is capable of increasing to ten or fifteen times the normal in an effort to maintain the balance, largely through increasing the depth of each breath though the rate increases as well. If rebreathing in the bag of oxygen is continued by a normal individual for some five or ten minutes, carbon dioxide in the bag will have accumulated to possibly seven or eight per cent at which concentration the overbreathing is unable to compensate and the blood and tissue content rises sufficiently to cause symptoms. The first feeling is one of inability to breathe deeply enough. As the carbon di-

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oxide accumulates to 10 per cent and beyond, loss of consciousness supervenes. Such loss of consciousness from excess carbon dioxide was recognized by Hickman early in the nineteenth century, and he used it before 1829 while he performed surgical operations upon dogs and recommended it for administration to human beings during surgery. During this period of hyperpnea, the blood pressure rises to extreme heights and the pulse rate increases only moderately. If the carbon dioxide concentration is continuously raised in the breathing bag and the oxygen replaced as it is used out by the subject, a concentration will be reached which no longer acts as a stimulant to blood pressure, pulse and respiration. Still later, depression of the circulatory and respiratory function occurs. Whether the convulsion which often supervenes during such an experiment is due to excess carbon dioxide directly, or whether it is a result of an interference with oxygen transport to the tissues, or to chemical changes of a more obscure sort, is not yet known.

SOME ARE HYPERSUSCEPTIBLE

An experience such as that described above is not ordinarily followed by serious consequences to life in a normal individual, although headache and malaise may last for many hours following. The importance of carbon dioxide toxicity lies in the fact that abnormal individuals, those who are ill from various causes, become in certain instances extremely hypersusceptible. Even the results of experiments with the administration of carbon dioxide enriched atmosphere to supposedly normal subjects show an extreme variation in the concentration of carbon dioxide required to produce a given change in blood pressure or respiratory activity. From what little we now know, we believe that previous conditions of disturbance in metabolic activity, temperature and certain drug effects predispose to hypersusceptibility.

The fact that carbon dioxide is one, if not the only, stimulus of respiratory activity has suggested its use in the therapy of depressed states. The ease with which pulmonary ventilation may be increased by permitting patients to inhale carbon dioxide-rich atmospheres has been spectacular. The procedure has been accepted without sufficient thought of possible dangers. If the administration of this gas were confined to its judicious use in sufficient concentration to bring depressed respiratory activity back to normal, no exception could be taken to the practice on any known physiologic ground. Certain misconceptions have arisen, however, as to the use of carbon dioxide for other purposes than that of restoring normal lung ventilation. Some of these misconceptions have arisen through the unfortunate practice of administering carbon dioxide mixed with oxygen. The supposed benefit of carbon dioxide therapy has often been due to the increase in oxygen which contacts the alveoli. In some circumstances, actual detrimental effects of the carbon dioxide have been masked in this manner.

The three most outstanding abuses of carbon dioxide as a therapeutic agent are based on three incorrect major premises.

1. The idea that shock is caused by a low carbon dioxide content of blood and tissues following overbreathing.

2. The belief that all inhalation anesthesia implies overbreathing, and hence results in low carbon dioxide.

3. Faith that administration of carbon dioxide and oxygen mixtures periodically, reduces the incidence and severity of postanesthetic and other pulmonary morbidity.

EFFECT ON ANIMALS

It has been shown that animals can be forced to overventilation until their blood contains less than fifteen volumes per cent of carbon dioxide, instead of the normal forty to fifty per cent, without extreme drops in blood pressure or other evidence of shock.[1] Animals suffering from very low blood pressure accompanying spinal anesthesia, when made to inhale excess carbon dioxide have shown a still lower blood pressure during such inhalation,[2] and we have many times observed a similar fall when carbon dioxide was

added to the inspired air of patients suffering from low blood pressure. On the other hand, oxygen alone administered by artificial respiration if necessary, has usually tended to restore the blood pressure toward normal.

MODERN PREANESTHESIA

The modern use of preanesthetic medication no longer permits of hyperventilation either before or during anesthesia. The tendency is rather toward a decreased minute-volume breathing and accumulation of carbon dioxide in the alveoli as was shown by work reported in 1931,[3] which has been verified in a paper by Hathaway and Orcutt, describing experiments in which they have analyzed tracheal, arterial and venous blood samples before and during inhalation anesthesia. To the advantage of our patients, we have reduced the rebreathing of anesthetic mixtures to a minimum and seldom or never add carbon dioxide to the anesthetic mask. The only exception is an occasional unseemly hurry to make rapid inductions and the occasional need for rapid removal of a toxic or anesthestic agent. A small stream of pure carbon dioxide is then poured over the patient's face to produce the desired hyperpnea.

The administration of carbon dioxide and oxygen mixtures intermittently, either at the end of operation or in the wards following recovery has, in our hands, resulted in a greater incidence of atelectasis or massive collapse and pneumonia than we now find since such treatment has been discontinued. We depend upon voluntary deep breathing of the patient under the nurse's direction for lung exercise when possible. Unconscious patients are still subjected to periodic carbon dioxide hyperpnea with atmospheric air as a vehicle, not oxygen. Thus the remote alveolar spaces which become inactive after hyperpnea, are not left filled with a rapidly absorbed gas such as oxygen.[4]

Whenever carbon dioxide is administered to a patient, whether by means of rebreathing or from a compressed supply, the necessity for careful individualization of dosage must not be overlooked. Certain physical signs are of aid in recognizing the occasional case of hypersusceptibility. Whenever hyperpnea fails to occur with inhalation of excess carbon dioxide, danger is near. If irregular or depressed or gasping breathing accompanies such therapy, discontinue it. Muscle twitchings of the small muscles of the face often precede a more generalized convulsion which can be prevented by promoting carbon dioxide elimination rather than administering it.

If the therapeutic use of carbon dioxide can be confined to well supervised and individualized administration sufficient to restore depressed breathing to normal, thus promoting an adequate oxygen supply to the alveolar spaces, no harm will result. However, if we continue to prescribe carbon dioxide and oxygen mixtures administered by untrained individuals as a cureall for every case of obstructed or depressed breathing, we will continue as we are now doing to cause the needless death of many persons each year.

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KINETICS OF CO_2-HCO_3^- IN NORMAL ADULT MALES

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The importance of quantitating the interchange between various components of the body's CO_2-HCO_3^- pools is well appreciated by investigators studying *in vivo* metabolism by measuring the rate of expiration of $^{14}CO_2$ after the administration of ^{14}C-labeled materials. It is clear that the rate of $^{14}CO_2$ expiration in the breath following administration of ^{14}C-labeled materials is not only a function of the *in vivo* metabolism of the given material but also of the kinetics of the CO_2-HCO_3^- pools which the labeled CO_2 must traverse before its expiration in the breath (*1*). The present study was undertaken to provide quantitative information about the rates of interchange between various components of the body's CO_2-HCO_3^- pools in normal male subjects at rest.

MATERIALS AND METHODS

The apparatus for continuous measurement of the rate of $^{14}CO_2$ and CO_2 excretion in the breath is similar to that described previously (*2*). The ^{14}C-labeled sodium bicarbonate was obtained from New England Nuclear Corp. with a specific activity of 4.6 mCi/m*M*. Before use the labeled bicarbonate was dissolved in sterile saline solution made slightly alkaline by adding a small quantity of sodium hydroxide. In all studies (with the exception of Patient CJ) 1.4–6.3 μCi ^{14}C-labeled bicarbonate was injected rapidly as a single intravenous bolus while the patient was breathing normally into the breath analysis apparatus. The $^{14}CO_2$ and total CO_2 excretion in the breath were monitored continuously in each subject for a minimum of 2 hr following intravenous administration of the label.

The $^{14}CO_2$ excretion rate during any given time interval was divided by the total CO_2 excretion rate during the same time interval. This ratio was then multiplied by the average CO_2 excretion rate measured during the study to give a $^{14}CO_2$ excretion rate for the given time period which was corrected for minute-to-minute variations in total CO_2 excretion. The $^{14}CO_2$ excretion data obtained during the initial 3 min were discarded, and only the corrected $^{14}CO_2$ excretion rates obtained after the initial 3-min interval were analyzed. Such data were fit to a function consisting of sums of exponential terms where one of the exponential terms had a rate constant fixed at the value of the turnover rate previously measured for the $^{14}CO_2$ breath analyzer used in the study (**k**). Data values were weighted by a factor equal to the reciprocal of the data value. A variable metric minimization program in a CDC 6600 computer was used to fit the function to the data (*3*).

Solutions for the rate constants of the model shown in Fig. 1 were obtained by identifying the terms of the Laplace transforms of the differential equations describing this model with the terms of the Laplace transforms of the function used to fit the data. Taking the amount of radioisotope injected as equal to unity, the initial conditions were taken as: $N_{10} = 1 - 3\alpha_{1t}$; $N_{20} = 3\alpha_{12}$; $N_{30} = \frac{\Sigma Ai}{k}$ where N_{10}, N_{20} and N_{30} are the fractions of administered radioactivity in compartments 1, 2 and 3, respectively, (Fig. 1) 3 min after i.v. administration of labeled bicarbonate, $\alpha_{1t} = \alpha_{12} + \alpha_{13} + \alpha_{1e}$, Ai = constant coefficient of the ith exponential term, k = turnover rate of the $^{14}CO_2$ breath analysis device. The total CO_2-HCO_3^- content of Compartment 1 was taken as the average breath CO_2 excretion rate divided by α_{13}. If one assumes that none of the CO_2 produced in the body is introduced into Compartment 2, then the CO_2-HCO_3^- content of Compartment 2 is equal to the product of the CO_2-HCO_3^- content of Compartment 1 times α_{12}/α_{21}. If all of the CO_2 produced in the body is introduced into Compartment 2 then the CO_2-HCO_3^- content of Compartment 2 is equal to the value calculated above plus the total-body CO_2 excretion rate divided by α_{21}. These two values give a lower and upper limit to the CO_2-HCO_3^- content of Compartment 2.

RESULTS

Figure 2 presents an example of the pattern of $^{14}CO_2$ excretion in the breath of patient (JG) given a single i.v. bolus of ^{14}C-labeled bicarbonate. The

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TABLE 1. PARAMETERS OF EXPONENTIAL FUNCTION USED TO FIT $^{14}CO_2$ EXCRETION DATA FOLLOWING $H^{14}CO_3$ ADMINISTRATION

Patient	Age	Wgt. (kg)	A_1	A_2	A_3	r_1	r_3	Average CO_2 excretion rate (mM/kg*/min)
HS	36	86.4	0.01028	0.03687	0.00476	0.01426	0.201	0.1455
GC	40	78.4	0.00861	0.01382	0.01061	0.01138	0.1279	0.1356
JB	27	70.5	0.00634	0.01256	0.01361	0.0091	0.1119	0.1387
PW	29	79.5	0.00716	0.00989	0.01544	0.01022	0.1192	0.1286
BS	36	54.5	0.00853	0.01814	0.00149	0.01058	0.1496	0.1512
ML	23	75.0	0.00658	0.0226	0.00926	0.00972	0.1362	0.1312
MZ	21	70.5	0.00605	0.01266	0.01310	0.0103	0.1121	0.1252
RB	24	81.8	0.00785	0.01944	0.00333	0.01058	0.164	0.1538
WC	26	80.9	0.00806	0.01594	0.01374	0.0116	0.105	0.1491
ML	31	76.2	0.01096	0.02431	0.00403	0.01636	0.1792	0.2021
ES	43	74.1	0.00594	0.01729	0.02492	0.00881	0.1322	0.1448
JJ	21	63.2	0.00603	0.01933	0.01766	0.00915	0.1106	0.1687
CJ	81	56.2	0.00529	0.01003	0.00778	0.01013	0.0973	0.1183

* kg = kilogram body weight.

TABLE 2. FRACTIONAL INTERCOMPARTMENTAL RATE CONSTANTS AND CO_2 CONTENT OF COMPARTMENTS SHOWN IN FIG. 1

Patient	α_{12}*	α_{21}*	α_{13}*	α_{10}*	N_1 (mM/kg†)	N_2‡ (mM/kg)
HS	0.0521	0.0513	0.1081	0.0039	2.43	5.13– 7.96
GC	0.0232	0.0547	0.0580	0.0034	5.84	6.19– 8.67
JB	0.0195	0.0426	0.0545	0.0044	7.11	9.11–12.35
PW	0.0175	0.0548	0.0524	0.0053	7.35	7.03– 9.38
BS	0.028	0.0531	0.077	0.0021	5.40	7.83–10.68
ML	0.0306	0.0370	0.0731	0.0052	4.29	8.53–12.02
MZ	0.0193	0.0423	0.0528	0.0079	6.49	8.10–11.06
RB	0.0289	0.0523	0.0891	0.0043	5.32	9.07–12.01
WC	0.0247	0.0421	0.0453	0.0042	6.04	8.04–10.04
ML	0.0433	0.0582	0.0870	0.0071	4.68	7.00–10.47
ES	0.0264	0.0367	0.0726	0.0053	5.49	10.85–14.81
JJ	0.0281	0.0311	0.0561	0.0045	6.00	10.83–16.25
CJ	0.0165	0.0383	0.0434	0.0092	4.21	4.73– 7.86
Mean	0.0275	0.0457	0.0670	0.0051	5.48	7.88–11.09
s.d.	0.0098	0.0084	0.0187	0.0018	1.27	1.81– 2.39
s.e.	0.0028	0.0024	0.0054	0.0005	0.37	0.52– .69

* Units of all α's are min^{-1}.
† Kilogram body weight.
‡ The lower limit of N_2 is calculated assuming no CO_2 is introduced into Compartment 2, and the upper limit assumes all CO_2 produced is introduced into Compartment 2. In these patients studied at rest, most of the CO_2 produced in the body would be expected to arise in abdominal and thoracic viscera and little would be produced in muscle and skin. Thus the lower value for N_2 listed is probably closer to the actual value of CO_2-HCO_3^- in the body in this compartment in these subjects.

data points are shown as open circles. During the initial 3 min following intravenous administration of labeled bicarbonate, the breath $^{14}CO_2$ excretion rate rises to a maximum and then begins to diminish. The biological events occurring during this 3-min time interval are thought to be related primarily to mixing of the label in the initial pool of equilibration. After the initial 3-min period, the rate of $^{14}CO_2$ excretion in the breath diminishes in a way that can be adequately described by a function consisting of three exponential terms. The exponential coefficient of one of these terms is equal to the measured turnover rate of the $^{14}CO_2$ detection apparatus (*1*). The values for the fitting parameters A_1, A_2, A_3, r_1, r_3 and average total CO_2 excretion rate obtained in 13 normal male subjects are given in Table 1.

The compartmental model used to analyze the $^{14}CO_2$ excretion data is shown in Fig. 1. Since three exponential terms were required to fit the $^{14}CO_2$ excretion data after initial equilibration had occurred, it was clear that there must be at least three compartments in any compartmental model used to an-

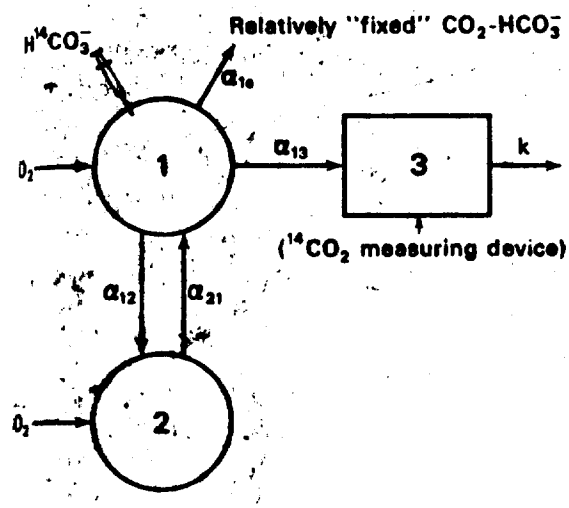

FIG. 1. Compartmental model used to analyze $^{14}CO_2$ excretion in breath before intravenous administration of ^{14}C-labeled bicarbonate. Compartment 1 represents those CO_2-HCO_3^- pools that are equilibrated with blood CO_2-HCO_3^- within first 3 min after intravenous administration of labeled bicarbonate. Compartment 2 represents those CO_2-HCO_3^- pools which show significantly delayed equilibration with blood CO_2-HCO_3^-. Compartment 3 represents activity in measuring device. Relatively "fixed" CO_2-HCO_3^- represents that labeled bicarbonate leaving Compartment 1 which is not significantly recycled back into Compartment 1 during time of study.

lyze such data. One of these compartments is the measuring device with a turnover constant (k). One of the two remaining compartments must be that compartment in which the initial equilibration of the label occurred after its intravenous administration. As such it must include the CO_2-HCO_3^- contained within the blood, and thus the $^{14}CO_2$ excreted in the breath must arise directly from this compartment. The second compartment must be in equilibrium with the first. It is possible for $^{14}CO_2$ to be relatively fixed from both the first and the second CO_2-HCO_3^- compartments, but in the present analysis the fixation of $^{14}CO_2$ from the second compartment was considered to be negligible.

Table 2 presents the values for the intercompartmental rate constants for the model in Fig. 1 obtained by analysis of the excretion rates of $^{14}CO_2$ and total CO_2 in the breath of the 13 normal male subjects listed in Table 1. The CO_2-HCO_3^- content of Compartment 1 and the upper and lower limits of the CO_2-HCO_3^- content of Compartment 2 are also listed in Table 2.

DISCUSSION

It is current opinion that CO_2 rapidly passes across cell membranes. If mixing equilibrium between the intravascular CO_2-HCO_3^- pool and the CO_2-HCO_3^- pool of any given tissue is complete on one passage of blood through the capillary bed of the organ in question, then the major parameter which influences the rate of CO_2-HCO_3^- equilibration between CO_2-HCO_3^- contained within the blood and the CO_2-HCO_3^- in various tissue compartments throughout the body is the ratio of regional blood flow rate to regional CO_2-HCO_3^- content. Using similar reasoning, Farhi and Rahn estimated that the mean equilibration time of bicarbonate in certain viscera with a large blood perfusion rate is on the order of 1–2 min, while that due to the relatively small vascular perfusion rate of muscle and the large muscle mass of the body the mean turnover time of the CO_2-HCO_3^- pool in resting muscle is on the order of 30 min (*4*). In view of such estimated turnover times for the CO_2-HCO_3^- pool in various organs, one would anticipate that CO_2-HCO_3^- in organs with high vascular perfusion (heart, liver, kidneys, intestinal tract) would be a component of Compartment 1 in the present model. On the other hand, CO_2-HCO_3^- contained in organs with a low ratio of blood flow rate to CO_2-HCO_3^- content, such as muscle, skin and fat, would be a component of Compartment 2 in the present model. This argument is consistent with the comparable calculated mean turn-

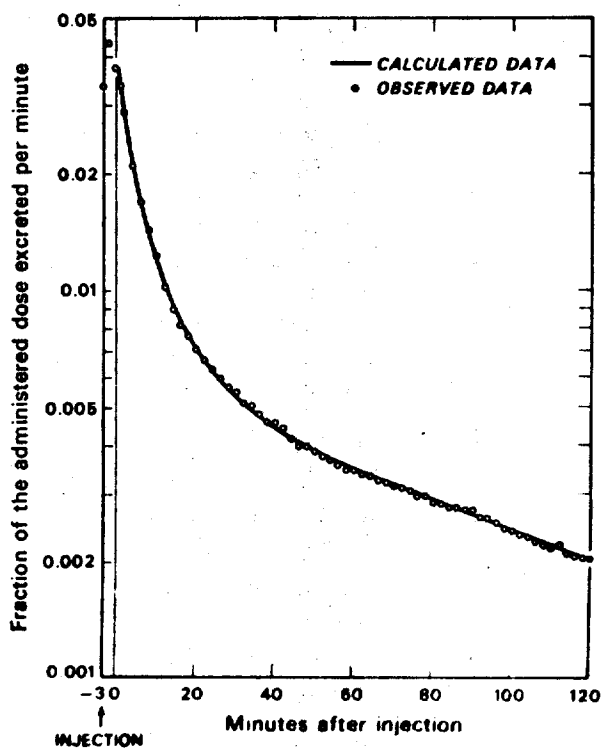

FIG. 2. $^{14}CO_2$ excretion rate in breath subsequent to intravenous administration of ^{14}C-labeled bicarbonate. Ordinate is expressed as fraction of administered dose excreted per minute. Abscissa is expressed as time in minutes minus 3 after intravenous administration of labeled bicarbonate. Measured $^{14}CO_2$ excretion rate is presented as open circles and solid line represents digital computer least squares best fit of three exponential function to data.

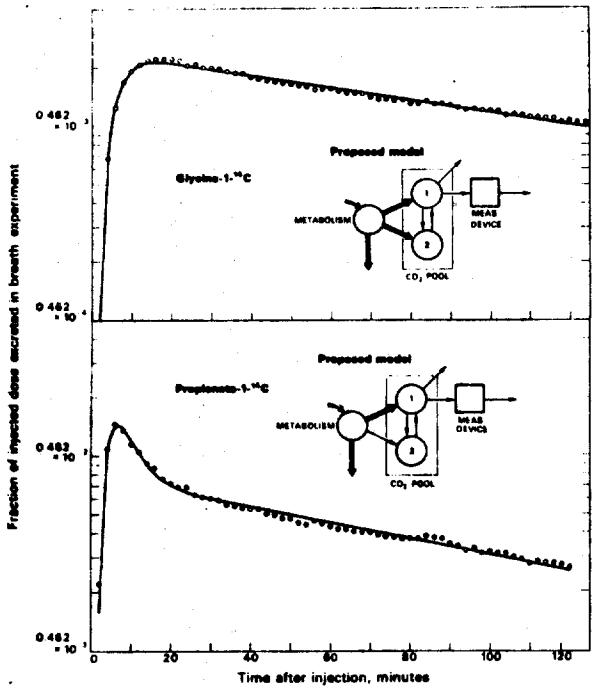

FIG. 3. $^{14}CO_2$ excretion rate in breath expressed as fraction of amount of radioactivity administered subsequent to intravenous administration of 1-^{14}C-labeled glycine (above) and 1-^{14}C-labeled propionate (below). Downslope of $^{14}CO_2$ excretion curves corresponding to turnover of $^{14}CO_2$ in body CO_2-HCO_3^- pools can be represented by single exponential function following administration of 1-^{14}C-glycine but requires two exponential terms following administration of 1-^{14}C-propionate.

over time of Compartment 2 (the average value for the 13 normal subjects in the present study is 22 min) and the mean turnover time of the CO_2-HCO_3^- pool in muscle estimated by Farhi and Rahn (30 min).

Although the brain is an organ with a low ratio of CO_2-HCO_3^- content to vascular perfusion rate, the presence of a blood brain barrier limiting the diffusion of bicarbonate into the brain may result in a delayed equilibration rate, similar to that calculated for muscle.

Blood CO_2-HCO_3^- is unquestionably in exchange with the bone bicarbonate. In a standard 70-kg man the bicarbonate content of bone is on the order of 5 moles (*4*). If the bone bicarbonate pool acts kinetically as a homogenous compartment, then labeled bicarbonate leaving the blood to enter this large bone bicarbonate pool would not be expected to significantly re-enter the blood during the time of the present study. Thus we may tentatively identify some of the CO_2-HCO_3^- leaving the blood destined for relatively "fixed" sites with bicarbonate entering bone. Further evidence that significant quantities of blood CO_2-HCO_3^- may be fixed in bone can be inferred from the studies of Myers and Hunter, in which they found significant bone fixation of ^{11}C given as bicarbonate in bones involved by tumor (*5*). Another possible site of trapping of body CO_2-HCO_3^- may be metabolic processes which result in incorporation of CO_2 into larger molecules (*6*). Other sites of bicarbonate loss in the body (feces, urine and sweat) probably account for only a small component of the bicarbonate entering relatively "fixed" sites from the blood when the subjects are at rest.

When labeled bicarbonate enters Compartments 1 and 2 of the present model in such a way that the specific activity of $^{14}CO_2$ in the two compartments is roughly comparable at all times, then both of these body CO_2-HCO_3^- pools act as though they were a single compartment. In such a case, the apparent mean turnover time for the equilibrated Compartments 1 and 2 for the 13 normal subjects studied in the present communication would be 89–110 min (fractional turnover rate of 0.0113–0.0091/min). Such a situation is approximated when a ^{14}C-labeled organic material is metabolized widely throughout the body (e.g., by muscle as well as by viscera). An example of this type of material is glycine labeled with ^{14}C in the #1 position (see upper curve, Fig. 3). When such ^{14}C-labeled materials are oxidized to $^{14}CO_2$, the breath $^{14}CO_2$ excretion curve shows only one exponential term corresponding to the turnover rate of the equilibrated CO_2-HCO_3^- Compartments 1 and 2 of the model. When a ^{14}C-labeled metabolite is metabolized primarily in one of the viscera whose CO_2-HCO_3^- content is a component of Compartment 1, then the body CO_2-HCO_3^- pools act kinetically as though they consisted of two discrete compartments. An example of this is shown in the lower portion of Fig. 3 for 1-^{14}C-propionate. The #1 carbon atom of intravenously administered propionate appears to be metabolized largely in the liver, and the $^{14}CO_2$ resulting from this metabolism is liberated into the blood in a similar way to an intravenous administration of labeled bicarbonate. The breath $^{14}CO_2$ excretion curve shows a definite peak in this circumstance with two exponential components in the downslope related to internal equilibration of the $^{14}CO_2$, thus liberated within the body's CO_2-HCO_3^- pools.

SUMMARY

Intravenously administered ^{14}C-bicarbonate is felt to rapidly mix with the CO_2-HCO_3^- contained in the blood and tissues with a high ratio of blood flow rate to tissue CO_2-HCO_3^- content (abdominal and thoracic viscera, etc.), together having a total CO_2-HCO_3^- content of 5.5 m*M*/kg body weight. In 13 normal subjects, an average fraction of 0.067/min

f this first pool left to enter a second pool with a otal CO_2-HCO_3^- content of 7.9–11.0 m*M*/kg body eight. This second pool is felt to consist of CO_2-HCO_3^- contained in tissues with a small ratio of lood flow rate to tissue CO_2-HCO_3^- content, such s muscle. In these subjects an average fraction of 1.046/min left this second CO_2-HCO_3^- pool to feed ack to the first (corresponding to a mean turnover ime of 22 min). An average fraction of 0.028/min f the first pool left to be expired in the breath, while n average fraction of 0.005/min of the first pool eft to become "fixed" somewhere in the body. This "fixed" fraction is felt to be in part related to deposition in bone and possibly metabolic processes resulting in incorporation of CO_2 into larger molecules. Additionally, there is a small portion of the "fixed" fraction which is lost from the body by excretion in urine, feces and sweat. The relative rates of internal quilibration of ^{14}C-labeled HCO_3^- within the body's CO_2-HCO_3^- pools appears to depend largely on elative vascular perfusion rates of different tissues, nd the kinetic values herein obtained in 13 normal male subjects would be expected to change in nonesting or pathological states.

Analysis of the kinetics of the body's CO_2-HCO_3^- pools should aid in understanding patterns of $^{14}CO_2$ excretion in the breath following administration of various ^{14}C-labeled substrates.

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Diagnostic Carbon Dioxide: Its Use in Review

It has been recognized for at least 40 years that injections of air or oxygen into the body can be of appreciable diagnostic or therapeutic value. Especially important examples of this are the diagnosis of adrenal tumors by perirenal or presacral injection of gas, intrauterine insufflation for diagnostic and therapeutic purposes, and the induction of pneumoperitoneum and pneumothorax. Progress along these lines has been seriously impeded by the occurrence from time to time of fatal, or nearly fatal, accidents due to the inadvertent entrance of gas into the circulatory sys-

tem. The value of injections of carbon dioxide as a safe substitute for air or oxygen has been recognized for many years (Rubin, Carelli, Cahill, Moore, Braselton). Its safety is dependent on its high degree of solubility in plasma (approximately twenty times that of air or oxygen), but its rapid absorption and dissipation have negated its use when long persistence of gas in any area is desired (e.g., pneumothorax). The safety of carbon dioxide was demonstrated in 1947, by Durant, Long and Oppenheimer, by means of large intravenous injections of this gas into dogs. Since then, further experiments in animals and injections into human beings have shown that pure carbon dioxide is safe whether injected into systemic veins or into the left ventricle or systemic arteries. As a result, at Temple University and in some other institutions, carbon dioxide has been substituted for air or oxygen for presacral injections, and it has been found to be just as satisfactory and completely safe. To my knowledge, there have been no fatalities reported from such use of carbon dioxide.

Aortic and pulmonary valves, atrioventricular rings, mitral and tricuspid valves, and even atrial septal defects, artificially created in dogs, have been viewed with remarkable clarity, depending upon the location of injection. At no time has any significant physiologic or chemical alteration been noted in the animals. Little or no work has been done in human beings in order to evaluate the role that carbon dioxide might play in the detection or demonstration of acquired or congenital intracardiac defects.

The major use in human beings, in addition to presacral injections, has proved to be in the study of pericardial disease, namely, pericardial effusions and pericardial constriction. The technique is simple and safe and may be done with a minimum of x-ray exposure. Thus, multiple studies may be done with safety. It may also be performed where the more expensive and complex angiocardiographic equipment is not available. Fifty to 100 cubic centimeters of *pure* carbon dioxide is injected into a large vein in the left arm, with the patient in the left lateral decubitus position. An ordinary posteroanterior film with a horizontal beam is taken immediately following injection, and is repeated as soon as the cassette can be changed. In the majority of instances a thin right atrial wall measuring 2 mm. or less is visible between the gas bubble in the right atrium and pulmonary parenchyma. In the presence of pericardial effusion this right heart border is thickened. Pericardial constriction or fibrosis is demonstrated by a straight margin or actual concavity of this region. The gas bubble in the right heart disappears rapidly through solution in pulmonary blood and excretion by expiration.

The total diagnostic value of carbon dioxide has yet to be explored. The implications are protean. But at least the veil has been removed from another "Dare Not" of the past.

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THE ROLE OF CARBON DIOXIDE IN THE NERVOUS SYSTEM

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CHANGES in P_{CO_2} in an organism are capable of producing marked alterations in the functioning of the central nervous system. The gross actions of CO_2 upon central nervous system activity, however, are not simple because they include both depression and excitation, ranging from anesthesia to seizures. Carbon dioxide is not the only gas capable of affecting the activity of the central nervous system, but CO_2 is of special interest and importance because it is one of the principal end products of metabolism.

The mechanism of action of CO_2 on the nervous system is not understood; however, the gas participates in several reaction systems. The most recognized function of CO_2 is in the principal buffering system of the body; changes in P_{CO_2} result in changes in acid-base balance. Some of the significance of physiological variations in pH is probably derived from the dependence of enzyme activity on hydrogen ion concentration, and hence changes in pH can influence the rates of various metabolic reactions.

Carbon dioxide may also serve as a metabolic substrate in such known reactions as "CO_2-fixation" and carbamate formation. The rate of incorporation of CO_2 is at least partially dependent upon P_{CO_2}; therefore, any situation altering P_{CO_2} could conceivably affect the kinetics of such reactions. The significance of these reactions is not understood, except in the case of carbaminohemoglobin formation.

Carbon dioxide is not chemically or physiologically inert; consequently, the maintenance of a constant level of CO_2 is an important physiological function. The steady-state level of CO_2 in tissue depends upon two factors: (1) the rate of its metabolic production, and (2) the rate of its elimination. Any condition which influences either one of these factors can change the level of CO_2 and hence can produce profound functional alterations.

Numerous substances are capable of influencing either the rate of production or of elimination of CO_2. This review will present evidence that such substances exert a profound effect on the nervous system due to the fact that they alter the concentration of CO_2 in the brain. The brain is particularly sensitive to changes in CO_2 concentration and slight variations produce marked neurophysiological, neurochemical, respiratory, and vascular responses. Only neurophysiological and neurochemical responses will be discussed in this paper.

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THE EFFECTS OF EXOGENOUS CARBON DIOXIDE ON BRAIN FUNCTION

NEUROPHYSIOLOGICAL EFFECTS: *Effects of Various Concentrations of Carbon Dioxide on Brain Excitability.* The effects of various concentrations of CO_2 on brain excitability as measured by the threshold for electrically- and chemically-induced seizures have been tested. The effects on electroshock seizure threshold (EST) are shown in figure 1.[1] The EST of both mice and rats was elevated by increasing the concentrations of inhaled CO_2 until a peak was reached at 12.5 per cent CO_2 for mice and 15 per cent CO_2 for rats. Thereafter the EST declined until at 30 per cent CO_2 it reached the pretreatment level. In mice, a concentration of 40 per cent CO_2 lowered the EST. In rats approximately 30 per cent CO_2 induced seizures and hence the EST could not be determined. The seizures produced by moderate concentrations of CO_2 first appeared at a concentration of 25 to 30 per cent, reached a peak incidence at 35 per cent, and thereafter rapidly declined concomitant with the marked central depression which occurred at higher CO_2 concentrations. The animals exposed to concentrations of 40 per cent CO_2 appeared to be anesthetized.

The experiments on the effects of various concentrations of CO_2 on EST and overt activity indicate that with regard to excitability of the nervous system CO_2 exerts a triphasic effect which can be explained as follows: The

first phase, that of decreased cortical excitability, appears to be due to direct cortical depression and occurs with low concentrations of the gas; cortical excitability progressively decreases with increasing concentrations of CO_2. The *second phase*, that of increased cortical excitability, appears to be due to the activation of subcortical centers by CO_2. Gellhorn and co-workers[2,3] have demonstrated that cortical excitability can be enhanced by stimuli which activate the hypothalamic-cortical system, a part of the reticular activating system[4] involved in arousal mechanisms and activation of the EEG. It thus appears likely that CO_2 stimulation of the hypothalamic-cortical system enhances cortical excitability to the extent that localized clonic seizures occur. Although the concentrations of CO_2 required for activation of subcortical centers are higher than those necessary for cortical depression,[5] the stimulation seems to be sufficiently intense to overcome mild cortical depression and to produce seizures. The hyperexcitability in this phase is apparently enhanced by the release of epinephrine and of adrenal cortical hormones of the cortisol type as a result of CO_2-induced stimulation of the hypothalamus. Previous work has shown that both of these classes of hormones enhance excitability of the cortex.[6,7] The *third* or anesthetic phase in response to CO_2 inhalation is attributed to the marked cortical and subcortical depression caused by high concentrations of the gas. The inhibition of subcortical centers by high CO_2 concentrations is probably due to inhibition of the central reticular activating system in a manner similar to the blockade produced by barbiturates and certain other anesthetic agents.[8,9]

Other evidence for an effect of CO_2 on excitability is provided by the fact that low concentrations of this gas abolish the tonic extensor phase of the maximal electroshock seizure (MES) pattern in experimental animals (mice, rats, and rabbits)[1,10-14] and in man.[15] This can be addressed as evidence for a decrease in excitability (first phase of CO_2 action). In addition, low concentrations of CO_2 protect against chemically-induced seizures produced by such agents as pentylenetetrazol,[20,24,26] strychnine,[16,27] insulin,[18,19] picrotoxin,[16] camphor,[16] coriamyrtin,[16] and absinthe.[16]

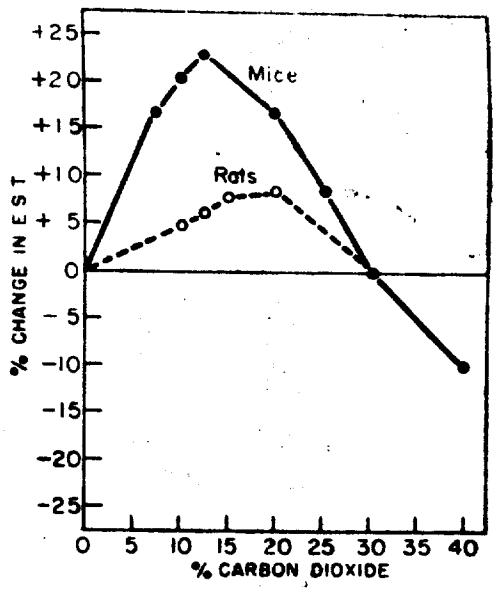

FIG. 1. Effect of inhalation of various concentrations of CO_2 on electroshock seizure threshold (EST) of mice and rats.[1] *Ordinate* is percentage change in EST referred to control values. *Abscissa* is inhaled CO_2 concentration in per cent. See text for discussion.

Effects on the EEG. Inhalation of CO_2 in low concentrations usually results in an increased frequency of cortical potentials and can interrupt petit mal seizure discharges as well as the clinical seizure.[20,21] On the other hand, decreased P_{CO_2} (induced by hyperventilation) results in slowing of frequency with the appearance of high voltage, slow-wave discharges particularly in the frontal leads. Exposure to high (anesthetic) concentrations of CO_2 usually causes flattening of the EEG in man and in experimental animals.[1,17] For example, in rats, 50 per cent CO_2 sharply depressed the EEG activity, and produced flattening of the normal waves. In contrast, when 30 per cent CO_2 was inhaled the EEG record showed intermittent bursts of high-voltage discharges. These bursts were of short duration and coincided with the episodes of overt clonic seizure activity which characterizes the hyperexcitable phase induced by CO_2.[1] Thus the biphasic effect of CO_2 on the nervous system is also evident from the EEG measurements.

Effects on the Spinal Cord. The effects of CO_2 inhalation on spinal cord activity and on polysynaptic and monosynaptic pathways have been studied by a number of investigators. Inhalation of 10 per cent CO_2 abolished the knee-jerk reflex of intact but not of spinal or decerebrate animals, and hence the inhibitory effect was thought to be of cerebral origin.[22] However, a direct effect of CO_2 on the cord itself has been demonstrated by other workers. Carbon dioxide in a concentration of 8.9 per cent induces a greater depression of monosynaptic than of polysynaptic spinal reflexes.[23] This observation was confirmed and extended by Esplin *et al.*[24] who found that low concentrations of CO_2 or acetazolamide, in striking contrast to other known depressant drugs, selectively depress monosynaptic (2N) pathways in the spinal cord of cats. With 10 per cent CO_2, 2N spike was reduced about 60 per cent, whereas polysynaptic response area was reduced only 5 per cent. In spite of their marked depressant effect, these agents are without influence on synaptic recovery, and they depress post-tetanic potentiation (PTP) only slightly. These data have been interpreted[25] to mean that CO_2 (and acetazolamide) affects some neuronal process which is more critically concerned in the spinal cord monosynaptic pathway than in polysynaptic circuits. The most outstanding difference between the component synapses of these two systems is that the safety factor with which transmission occurs is lower for monosynaptic than for polysynaptic pathways.[26] It was tentatively suggested, therefore, that CO_2 (and acetazolamide) decreases the safety factor of transmission, and that depression would be produced by this drug at all synapses in which the safety factor is low, as it is in the spinal monosynaptic pathways.

Carbon Dioxide-Withdrawal Seizures.[1] Rats exhibit clonic seizures usually within one minute after removal from anesthetic concentrations of CO_2 (35 per cent or higher). These seizures involve the face, jaw, and foreleg muscles and are accompanied by salivation, lacrimation, and often piloerection. The seizures appear to result from the fact that on withdrawal from anesthetic doses of CO_2 the rats pass through the hyperexcitable (stimulant) phase of CO_2 effect, and [illegible] seizures are prevented by anticonvulsant agents like phenobarbital, trimethadione, and acetazolamide, and are enhanced by diphenylhydantoin in low doses.[1]

METABOLIC EFFECTS OF EXOGENOUS CARBON DIOXIDE: *Effects on Electrolytes and Acid-Base Balance.* Inhalation of a concentration of CO_2 (12.5 per cent) which elevates EST (first phase) results in the following changes in brain electrolyte and acid-base composition.[27] There is a decrease in intracellular Na concentration (increase in ratio of extracellular to intracellular Na, Na_e/Na_c) and an increase in intracellular K concentration. Cellular HCO_3^- concentration increases; however, H_2CO_3 concentration increases to a greater extent and hence brain cell *p*H decreases. Such changes are consistent with the decrease in excitability which is observed in the animal exposed to this concentration of CO_2, and add additional support to the observations previously described (see Woodbury and Esplin[25] for summary) of a direct correlation between brain excitability and the Na_e/Na_c.

When rats were exposed to 50 per cent CO_2, there were changes in electrolyte and acid-base balance in the brain.[1, 28] There was a decrease in brain intracellular Na and K (increase in Na_e/Na_c and decrease in K_c/K_e), and a cellular acidosis (*p*H 6.41) resulted which was characterized by a large increase in both HCO_3^- and H_2CO_3. The total cation (assumed to be Na plus K) content in the brain cells was decreased, probably as a consequence of the reduction in organic anions resulting from the intracellular acidosis produced by CO_2. Nonbicarbonate, organic polyvalent anions (assumed to be total cation minus bicarbonate) decreased markedly in the brain of rats exposed to 50 per cent CO_2. The decrease in organic anions is probably due to a shift in the ionization of the cellular proteins toward the acid side of their isoelectric point as a result of the acidosis. Furthermore, additional observations from our laboratory have demonstrated that 50 per cent CO_2 decreases the brain concentrations of glutamic and aspartic acids (see below). Thus less total anion is present, necessitating a decrease in cation in order to maintain electrical neutrality. [illegible]

ats from an atmosphere of 50 per cent CO_2, he lowered intracellular brain Na concentra-ion increases rapidly, but K concentration emains decreased; seizures appear at this ime. Thus there is a markedly increased Na influx into brain cells associated with the apid loss of CO_2 and the occurrence of eizures. The rapid loss of CO_2 is associated with an increase in *p*H of the brain cells to the level of that in the cells of rats exposed to nly 30 per cent CO_2 (*p*H 6.76). In addition to the Na change which occurred on withdrawal from 50 per cent CO_2, there were changes in H_2CO_3 and HCO_3^- concentrations. The values for HCO_3^- and H_2CO_3 in plasma decreased below those observed in rats exposed to 30 per cent CO_2, and the brain intracellular H_2CO_3 and HCO_3^- concentrations fell into the same range of values as observed in the animals exposed to 30 per cent CO_2. The intracellular HCO_3^- concentration in the withdrawal animals was increased over that seen in the rats exposed to 50 per cent CO_2. Since rats exposed to 30 per cent CO_2 and those abruptly withdrawn from 50 per cent CO_2 exhibit seizures, and since the acid-base and presumably the electrolyte changes are similar in the two groups of rats, it is tempting to ascribe the seizures to either the electrolyte changes or the acid-base changes, or both. The seizures in the CO_2-withdrawal animals are thus associated with a rapid influx of Na and probably HCO_3^- into brain cells and consequently a decrease in brain Na ratio. This influx of Na into the brain appears to be of sufficient magnitude to precipitate the seizures and may be presumed to be the same process that renders the peripheral nerve axon unstable; the passage across the membrane of sufficient charge explosively increases permeability to Na and an action potential is generated. In the brain, where millions of neurons are involved, seizure activity might be the consequence.

The cause of the influx of Na into the brain during CO_2-withdrawal is unknown, but the following hypothesis can explain the results. The sudden and marked loss of CO_2 from the cell is unaccompanied by an equal loss of HCO_3^- because the cell membrane is less permeable to HCO_3^- than CO_2; as a result, [illegible] The increased cellular

TABLE I

EFFECT OF INHALATION OF VARIOUS CONCENTRATIONS OF CARBON DIOXIDE ON FREE AMINO ACID LEVELS IN CEREBRAL CORTEX OF RATS *

Treatment	Glutamic Acid (mg. %)	Aspartic Acid (mg. %)	Glutamine (mg. %)	GABA (mg. %)
Controls	103±4†	54±7	47±4	21.5
12.5% CO_2	110±6	70±9 (0.05)	39±4	36
50% CO_2	50±13 (0.02)	48±5	39±4	32
Withdrawal from 50% CO_2	58±1 (0.01)	42±4	38±6	19

* From unpublished observations of Woodbury, D. M., Vernadakis, A. and Timiras, P. S.

† Values are mean ± standard error. Numbers in parentheses are *P* values for significance of treated animals from the controls.

*p*H probably decreases the ionizable calcium of the cell; such a decrease is known to enhance the permeability of the cell to Na. Therefore Na pours into the cell as the *p*H increases in order to satisfy the increase in anions resulting from the excess HCO_3^- in the cell and the increase in the anion charges on the protein. Evidence for a role of calcium in precipitating the seizures is provided by the fact that administration of calcium chloride to rats protects against both withdrawal seizures and 30 per cent CO_2-induced seizures (unpublished observations). Also, Carey and Schaefer [29] have shown that the plasma Ca level markedly decreases immediately after withdrawal of rats from an atmosphere of 50 per cent CO_2.

Effects of Carbon Dioxide on Carbohydrate and Amino Acid Metabolism. The effects of inhalation of various concentrations of CO_2 on free amino acid concentrations in brain of rats are shown in table 1. Inhalation of 12.5 per cent CO_2 resulted in a slight increase in glutamic acid, a marked increase in aspartic acid, and a slight decrease in glutamine concentrations. In addition, the concentration of γ-aminobutyric acid (GABA) was increased considerably but the data are insufficient to establish the level of significance of this change. The increase in glutamic acid plus aspartic acid is consistent with an increase in

total cation found in the brain of rats treated with 12.5 per cent CO_2. However, this accounts for only part of the increase in cation; the remainder is due to the marked increase in HCO_3^- concentration.[27] Thus quantitatively the increase in cation content of the cell is accounted for mainly by (or is a consequence of) an increase in the labile anions (glutamic acid, aspartic acid, bicarbonate).

In contrast to the animals receiving 12.5 per cent CO_2, the rats exposed to 50 per cent CO_2 showed a marked decrease in glutamic acid, a small decrease in aspartic acid, a slight decrease in glutamine, and an increase in GABA concentrations. The decrease in glutamic acid plus aspartic acid concentrations counterbalances the marked increase in HCO_3^- concentration [28] induced by this concentration of gas. However, the decrease in total cation was accompanied by a marked cellular acidosis (*p*H 7.04 to 6.41) which would result in a decrease in polyvalent anion, consequently maintaining electrical neutrality.

On abrupt withdrawal from 50 per cent CO_2 there was a slight increase in glutamic acid, a slight decrease in aspartic acid and glutamine, and a striking decrease in GABA concentrations. Since the withdrawal animals were killed and the values measured just prior to the onset of seizures, the values for GABA would be expected to decrease even more as the seizures develop.

The values for GABA in the different experimental situations correlate fairly well with the level of excitability of the animals in the same situation. Both 12.5 per cent and 50 per cent CO_2 decrease excitability and increase GABA concentration, but the increase in GABA is greater in the 12.5 per cent CO_2 group which is the more excitable of the two groups. On withdrawal from 50 per cent CO_2, excitability increases markedly and the animals exhibit seizures, at which time the GABA concentration decreases markedly. Therefore, in all three of these experiments there is a rough inverse correlation between the level of excitability and the GABA concentration in the brain. These data are consistent with additional observations by us [30] and others (see Tower [31] for summary) which suggest the same correlation.

It is evident from these data that CO_2 has a profound influence on the amino acid metabolism of the brain, and that such effects are related to the electrolyte and acid-base metabolism of the brain and ultimately to the level of excitability of the nervous system.

Since selective electrolyte accumulation in cells has been shown to be an active process which requires energy, and since CO_2 affects electrolyte metabolism, it is likely that CO_2 exerts an influence on reactions yielding or transferring energy. It is of interest in this regard that Bain and co-workers [32, 33] have shown that inhalation of CO_2 (10 to 30 per cent) in normal cats resulted in lowered brain lactate and pyruvate levels with little or no effect on glucose concentration and on levels of high-energy phosphate. However, in cats undergoing electroshock seizures during the inhalation of CO_2, the expected rise in brain lactate, pyruvate, and inorganic phosphate concentrations was limited; the expected fall in high-energy phosphate level was prevented, and a rise in brain glucose concentration occurred. The brain/plasma glucose ratio, nevertheless, remained the same. These results might be interpreted to mean that CO_2 inhibits the utilization of glucose by the brain, with a consequent decrease in brain pyruvate and lactate concentrations. Inhibition of a major energy-yielding process would delay recovery from an energy-consuming process, such as occurs during a maximal electroshock seizure. It is of interest that CO_2 does indeed delay recovery from MES in mice.[10] The fact that the glutamic acid decreases markedly in rats exposed to 50 per cent CO_2 is also consistent with the hypothesis that CO_2 inhibits glucose utilization because glutamic acid can be used as a substrate for oxidative metabolism in the brain. It is likely that, under conditions which inhibit glucose oxidation, glutamic acid substitutes for glucose and thereby maintains cellular respiration and brain function.

Drugs Which Influence Endogenous Carbon Dioxide Metabolism: Their Effect on Brain Function

Drugs Which Increase Carbon Dioxide Production: Further evidence for a role of carbon dioxide in regulating the excitability of the nervous system is provided by the use of

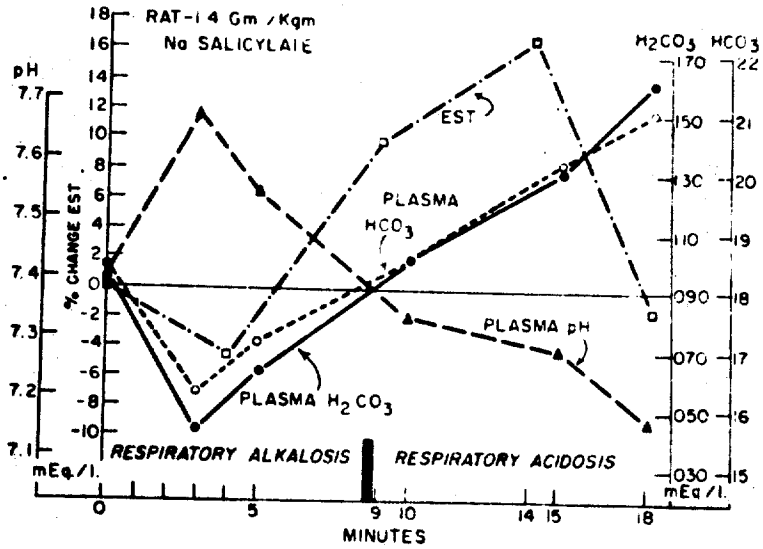

FIG. 2. Effect of acute intraperitoneal administration of Na salicylate on electroshock seizure threshold (EST) and acid-base balance of rats at various times after injection. *Ordinates* on left are blood *pH* values and percentage change in EST referred to pretreatment control values; on right, plasma H_2CO_3 and HCO_3^- concentrations in mEq./l. *Abscissa* is time in minutes. See text for discussion.

agents which influence the endogenous production of CO_2. For example, salicylates enhance both the oxygen uptake and the carbon dioxide production of the body as a whole and of various tissues (see review by Smith [34]). The increase in CO_2 output is generally accompanied by a stimulation of respiration which more than compensates for the increased production and lowers P_{CO_2}. As a result, a respiratory alkalosis develops. However, if the dose of salicylate is large, respiratory depression soon follows and P_{CO_2} increases since CO_2 production exceeds the capacity of the respiratory system to eliminate the excess CO_2. As a result, a respiratory acidosis develops. It was of interest, therefore, to study the acute effects of sodium salicylate on the central nervous system during the state of respiratory alkalosis when the P_{CO_2} was low and during the state of respiratory acidosis when the P_{CO_2} was high. If the CNS effects are related to the P_{CO_2}, then such effects should follow changes in endogenous P_{CO_2}. The experiments were carried out in rats given an LD_{85+} of Na salicylate (1.4g./kg.).[35] The EST was measured and electrolyte and acid-base values were determined at various times after intraperitoneal administration of the salicylate.

The results are shown in figure 2. An initial respiratory alkalosis developed within 2 minutes and lasted for 9 minutes; this was followed by a respiratory acidosis which was severe at 15 minutes when seizures followed by death occurred. Consequently, the seizures appeared during the stage of respiratory acidosis, not during respiratory alkalosis. The EST decreased in the alkalotic phase, increased in the acidotic phase and decreased suddenly just prior to the onset of seizures. The changes in EST paralleled changes in plasma (and brain cell) carbonic acid concentration. The results from the salicylate experiments indicate that a lower than normal P_{CO_2} increases brain excitability, whereas a higher than normal P_{CO_2} decreases brain excitability up to a certain point and thereafter increases it to the extent that seizures occur. Thus the curve for the EST changes produced by salicylate, after the initial excitatory phase due to the low P_{CO_2}, resembles exactly the first two stages of the effect of exogenous CO_2 on EST as shown in figure 1. These data, therefore, suggest that the EST changes associated with high doses of salicylate are secondary to the changes in carbonic acid concentration in plasma and brain cells. In

addition, with the doses of salicylate used, there is also some inhibition of brain carbonic anhydrase (unpublished observations), and this effect tends to enhance the accumulation of CO_2 in the brain.

Other drugs which increase CO_2 production also have effects on the nervous system. 2,4-Dinitrophenol (DNP) produces central nervous system depression in rats but does not produce spontaneous seizures even in lethal doses. DNP also lengthens the tonic flexor phase and shortens the tonic extensor phase of the MES, facts indicative of anticonvulsant activity. Apparently DNP possesses the first phase of the CO_2 triphasic response (unpublished observations); however, in combination with other drugs which alter CO_2 metabolism, DNP produces more profound CNS effects (see next section).

DRUGS AFFECTING CARBON DIOXIDE RELEASE FROM BRAIN (CARBONIC ANHYDRASE INHIBITORS): *Neurophysiological Effects.* Although carbonic anhydrase is found in the central nervous system,[36] its precise role in this tissue has not been elucidated. However, the fact that carbonic anhydrase inhibitors, such as acetazolamide, have marked effects on central nervous system function suggests that this enzyme is involved in the regulation of brain excitability.

Thus, acetazolamide has been shown to be a potent anticonvulsant agent, both in laboratory animals and in epileptic patients. In experimental animals, acetazolamide abolishes the tonic extensor component of the MES in mice and rats,[13, 37-40] and potentiates the effects of CO_2 on MES.[37] The anticonvulsant action of acetazolamide is not abolished by nephrectomy and hence this action is independent of the systemic acidosis which results from the inhibition of carbonic anhydrase activity in the kidney.

In addition, acetazolamide, in higher doses than are necessary to modify MES, elevates EST about 16 per cent. But still higher doses diminish the elevation in EST, thus indicating as is the case with moderately high concentrations of CO_2, an excitatory component of action.[13] However, acetazolamide even in extremely large doses does not produce seizures as do moderate concentrations of CO_2, but the drug does produce seizures when combined with small doses of CO_2 (see below). Fabriard and Gangloff[41] studied the effects of acetazolamide on the threshold voltage for stimulation of the cortex, diencephalon, and rhinencephalon in rabbits. They observed that there was a slight decrease in the excitability of the cortex and a marked decrease in the excitability of the diencephalon. Thus acetazolamide, like CO_2, exhibits effects on cortical and subcortical centers. The drug has also been reported to obtund audiogenic seizures.[42]

A number of reports attest to the efficacy of acetazolamide against various types of epilepsy. The drug was first introduced for the therapy of epilepsy by Bergstrom *et al.*[43] and subsequent reports[44-47] confirmed its effectiveness in all types of epilepsy (grand mal, petit mal, and psychomotor seizures).

In addition, acetazolamide, like CO_2, protects against seizures evoked by various pharmacological agents. It reduces the intensity of spinal discharges induced by convulsive doses of strychnine (Esplin, personal communication), and it antagonizes pentylenetetrazol induced seizures, although only to a limited extent (unpublished observations). Finally acetazolamide protects against seizures produced by inhalation of 30 per cent CO_2 or by withdrawal from 50 per cent CO_2.[1] It is evident from the comparative observations that acetazolamide and CO_2 possess a wide and similar spectrum of anticonvulsant action on the CNS.

Further evidence that the action of acetazolamide on the CNS is identical to that of CO_2 is derived from the influence of the drug on spinal cord activity. As discussed previously acetazolamide produces exactly the same effect on the spinal cord as does CO_2, and potentiation occurs when it is given in combination with CO_2.[24] Thus acetazolamide, like CO_2, selectively depresses monosynaptic (2N) pathways in the spinal cord without affecting synaptic recovery or post-tetanic potentiation.

There is much experimental[13, 27, 48] and clinical[47, 48] evidence that tolerance develops to the anticonvulsant effects of acetazolamide. If the anticonvulsant activity of acetazolamide is due to a localized increase in P_{CO_2} in neuronal cells, it follows logically that the tolerance which is known to develop to the anticonvul

sant effect of acetazolamide should be accompanied by tolerance to the anticonvulsant effect of CO_2. That this assumption is correct was demonstrated by Koch and Woodbury who found that tolerance developed to the repeated administration of acetazolamide and that cross tolerance concurrently developed to CO_2.[13] These data provide further strong evidence for a common mode of action of CO_2 and acetazolamide.

Since nitrate ion (NO_3^-) in rather high concentrations (24 mEq./l.) has been shown by Roughton and Booth[49] to inhibit carbonic anhydrase *in vitro*, it was of interest to test the anticonvulsant effect of this ion which is chemically unrelated to acetazolamide. If carbonic anhydrase inhibitors are acting by causing accumulation of CO_2 in brain cells, then it follows that such agents should be effective anticonvulsants and tolerance should develop to the anticonvulsant effect on repeated administration. Such is the case for NO_3^-; it protects against MES in mice and rats in the same ratio as does CO_2 (5.7) and tolerance develops to its anticonvulsant effect.[13]

Millichap[50, 51] and Millichap and co-workers[52] have presented convincing evidence that carbonic anhydrase is of functional significance in the development of the electroshock seizure pattern in developing rats and guinea pigs. They suggested that this enzyme is important in the development of the ability to exhibit maximal tonic convulsions, in which a generalized spread of the seizures discharge is probably involved, but that the enzyme probably is not involved in the capacity to exhibit clonic seizures, which involve localized neuronal discharges. This suggestion is supported by their observations that the hyperkinetic behavior induced by electroshock in rats 10 days old or less is refractory to acetazolamide, that the clonic seizures induced by electroshock in 10 to 20-day old rats were abolished only by very large doses of this drug, and that the tonic type of seizures seen in 21-day or older rats was abolished by low doses of acetazolamide.

Further evidence that carbonic anhydrase is involved in the spread of discharges was presented by Davenport[53] who observed in rabbits treated with a carbonic anhydrase inhibitor, thiophene-2-sulfonamide, that local stimulation of the cerebral cortex did not affect the general electrical activity of the stimulated area.

Carbonic anhydrase inhibitors other than acetazolamide have also been shown to have effects on the nervous system. Sulfanilamide abolishes the tonic extensor component of the MES in experimental animals[38] and possesses a weak antiepileptic effect in man.[54, 55] Meththazolamide and ethoxzolamide, congeners of acetazolamide, exhibit anticonvulsant activity in experimental animals[39, 40] and in man.[56] Various other substances which are carbonic anhydrase inhibitors *in vitro* or *in vivo* have also been demonstrated to possess anticonvulsant activity. These include salicylate, NO_3^-, I^-, and Br^-,[57] but it is yet to be established conclusively that they exert their CNS activity through inhibition of this enzyme.

Metabolic Effects of Carbonic Anhydrase Inhibitors. The observations of Millichap *et al.*[38] have established that there is a direct relation between anticonvulsant effect and inhibition of brain carbonic anhydrase in mice. They compared the activity of acetazolamide with that of sulfanilamide, a much less potent inhibitor, and found that acetazolamide was approximately twice as potent as sulfanilamide, both as an anticonvulsant and as an inhibitor of brain carbonic anhydrase. In addition, the time of maximum degree of enzyme inhibition produced by these two drugs corresponded with their time of peak anticonvulsant effect.

These data support the hypothesis that carbonic anhydrase is integrally involved in regulation of CNS excitability. If this is the case, then inhibition of the enzyme should cause metabolic changes which are related to CNS activity. The following data show that this assumption is correct and lead to a theory of the mechanism of action of acetazolamide.

The evidence presented above indicates that the CNS effects of acetazolamide are mediated through CO_2 accumulation. Hence treatment with acetazolamide should increase the concentration of total CO_2 in the brain. The data presented in table 2 show that this drug does indeed elevate the CO_2 content of brain. In high doses, however, the CO_2 level is lowered and this is consistent with the lower EST of rats given high doses of acetazolamide.[13] Therefore, it appears from these data, and from those of the effect of salicylate on EST

TABLE 2

EFFECT OF ACETAZOLAMIDE ON TOTAL CO_2 CONCENTRATION IN CEREBRAL CORTEX OF RATS

Treatment	Total Intracellular CO_2 in m*M*/kg. Cell H_2O	Reference
Adults		
control	14.7	27
acetazolamide (20 mg./kg.)	17.3	
12.5% CO_2	22.7	
	Total CO_2 in m*M*/kg. wet brain	
24 day old		
control	18.6	53
acetazolamide	21.7	
Adults		
control	11.5	Heninger and Woodbury, unpublished observations
acetazolamide (10 mg./kg.)	13.2	
acetazolamide (200 mg./kg.)	10.7	

presented previously, that the excitability of the brain as measured by EST is inversely proportional to the CO_2 level in the brain.

Inasmuch as exogenous CO_2 profoundly influences electrolyte metabolism of the brain and the resulting changes in electrolytes are correlated with brain excitability, it was of interest to determine the effects of acetazolamide on brain electrolytes. The results[27] will be only briefly summarized here. The effects of acetazolamide on brain electrolytes were studied in nephrectomized animals in order to eliminate all renal effects of the drug. There was a significant decrease in the concentration of intracellular brain Na and an increase in brain cell K; hence both the Na_e/Na_c and K_c/K_e ratios were increased. These effects are the same as those produced by inhalation of 12.5 per cent CO_2 (see above). Animals rendered tolerant to acetazolamide showed an increase in both cellular Na and K concentrations and were hyperexcitable. These data lend support to previous observations that there is a correlation between Na_e/Na_c and brain excitability (see references 6, 25, and 27 for summary).

In order further to analyze the effect of acetazolamide on cellular Na, the influence of the drug on the rate of uptake of isotopic Na was studied in nephrectomized rats.[27] Acetazolamide decreased the uptake of Na^{22} by brain tissue as compared with the controls. The finding of an increased Na_e/Na_c coupled with a decreased rate of Na turnover has been interpreted to mean that acetazolamide decreases the rate of Na influx into brain cell, *i.e.*, decreases the permeability of the cell membrane to Na (see Koch and Woodbury[27] for evidence and review of literature). The relation of this effect of acetazolamide on brain cell Na metabolism to its mechanism of action is discussed below, after the role of carbonic anhydrase in the nervous system has been considered.

An impressive body of evidence has been collected which indicates that the neurophysiological and neurochemical effects of acetazolamide are mediated through CO_2 accumulation. This evidence will now be summarized. (1) Acetazolamide and CO_2 have the same spectrum of activity on experimental seizures and on the spinal cord. In addition, the effects of acetazolamide on the MES and on spinal cord synaptic transmission are potentiated by CO_2. (2) The changes in brain excitability induced by various concentrations of CO_2 are enhanced by acetazolamide (fig. 2). (3) Seizures induced by agents which enhance CO_2 production by brain cells (salicylate, DNP) are enhanced by acetazolamide. (4) Acetazolamide increases the total CO_2 concentration in brain cells. (5) Acetazolamide produces the same effects on brain electrolyte concentrations as does CO_2. (6) The effects of acetazolamide and CO_2 on brain amino acid concentrations are similar. (7) Rats which develop tolerance to the anticonvulsant effect of acetazolamide on MES are also tolerant to the anticonvulsant effect of CO_2.

COMBINATIONS OF DRUGS WHICH INCREASE CARBON DIOXIDE PRODUCTION OR INHIBIT ITS RELEASE: THEIR EFFECTS ON BRAIN FUNCTION. Further support for the concept that salicylate, DNP, and acetazolamide alter brain function through their influence on CO_2 metabolism is provided by experiments in which various combinations of these drugs were tested for their effect on CNS activity. The results of these experiments are summarized in table 3

The effects of the drugs, alone or in combination, were tested on the MES pattern and on the development of clonic seizures similar to those produced by 30 per cent CO_2 as described above. When either salicylate, DNP, or acetazolamide was combined with CO_2 there was a marked potentiation of the anticonvulsant effect of CO_2 on MES. The drugs were combined with CO_2 in doses which by themselves had very little effect on the MES (table 3). In addition, the drugs when combined in subconvulsive concentrations with a subconvulsive concentration of CO_2 (15 to 20 per cent) produced severe clonic seizures (table 3). Thus both the anticonvulsant effects of low CO_2 concentrations and the seizure-producing effects of moderate doses of CO_2 are enhanced by salicylate and DNP, both of which increase CO_2 production, and by acetazolamide which inhibits CO_2 elimination from brain cells.

The combination of salicylate and DNP also enhanced the anticonvulsant effect of either one of the drugs given alone, and DNP also enhanced the seizures produced by salicylate. Acetazolamide and DNP are similar in the respect that either agent by itself is only capable of producing depression and not seizures. The combination of acetazolamide and DNP in low doses results in an enhancement of their respective anticonvulsant effects; in contrast, the combination in high doses results in the production of mild clonic seizures. The rats receiving this combination were identical in all their responses with those of animals receiving salicylate alone (salivation, diuresis, piloerection, seizures). Since the combination of DNP and acetazolamide produces effects identical with those of salicylate, and salicylate enhances CO_2 production and also inhibits brain carbonic anhydrase, it appears that the CNS effects of these drugs are mediated through CO_2 accumulation. The reason salicylate produces powerful effects on the CNS is that it possesses both the ability to increase CO_2 production and to inhibit CO_2 release.

It might be argued that the enhanced effect on the CNS produced by DNP, salicylate, or acetazolamide in combination with CO_2 is due to the acidosis produced by the gas and not the P_{CO_2}. The resultant acidosis would reduce the charge on the drugs which are weak acids; consequently, a higher percentage of the molecules would be in the noncharged form. It has been demonstrated (see Brodie and Hogben[58] for summary) that uncharged molecules penetrate the brain more readily than do charged ones. Therefore, the acidosis would increase the concentration of DNP, salicylate, or acetazolamide in the brain and thereby enhance the CNS effect of CO_2. Although this may occur to a certain extent, it cannot be the explanation of the findings shown in table 2. When a degree of acidosis comparable to that caused by CO_2 was produced by ammonium chloride (NH_4Cl), only equivocal effects were produced on MES, and no seizures appeared. When NH_4Cl was combined with salicylate in a dose which produced the same degree of acidosis as does the combination of CO_2 and salicylate, neither the anticonvulsant nor the convulsant effect of salicylate was enhanced. It seems evident, therefore, that it is the CO_2 and not the H^+ concentration which produces the CNS effects of salicylate, DNP, and acetazolamide. In addition, the enhancement of effects produced by the combination of DNP with salicylate and

TABLE 3

CENTRAL NEROUS SYSTEM EFFECTS OF DRUGS WHICH ENHANCE CARBON DIOXIDE PRODUCTION OR INHIBIT ITS RELEASE FROM BRAIN CELLS

Drug Treatment	Anti-convulsant Effect on MES (Low Doses)	Effect on CNS activity (High Doses)	
		Clonic Seizures	Depression
15-20% CO_2	+ + +	−	+
30-40% CO_2	−	+	−
Salicylate	+	+	−
2,4-Dinitrophenol (DNP)	+	−	+
Acetazolamide	+ + +	−	+ (very high doses produce some excitation)
CO_2 (15-20%) + Salicylate	+ + + +	+ + +	−
CO_2 + DNP	+ + + +	+ + +	−
CO_2 + Acetazolamide	+ + + + +	+ + +	−
Salicylate + DNP	+ +	+ + +	−
Salicylate + Acetazolamide	+ + + +	+ + +	−
DNP + Acetazolamide	+ + + +	+ (resembles salicylate)	−
NH_4Cl*	±	+ (only in extremely large doses)	−
NH_4Cl + Salicylate	+	+	−

* Dose necessary to produce an acidosis equivalent to that produced by CO_2.

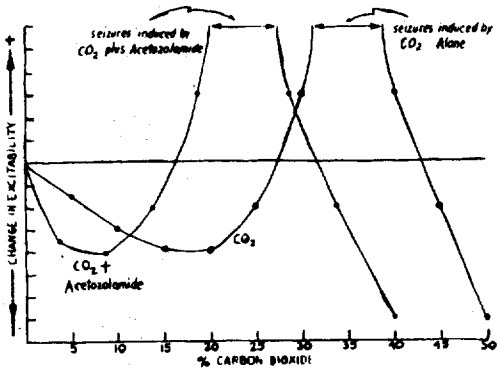

FIG. 3. Effect of CO_2 alone and in combination with acetazolamide, on brain excitability. *Ordinate* is change in excitability on an arbitrary scale. *Abscissa* is inhaled CO_2 concentration in per cent. See text for discussion.

by the combination of acetazolamide with salicylate or DNP cannot be due to acidosis because there was no appreciable change in plasma *p*H when these experiments were performed.

Finally, the observation that a combination of DNP and acetazolamide causes clonic seizures indistinguishable from those produced by salicylate or CO_2 cannot be explained by increased penetration of DNP or acetazolamide into the brain as a result of an acidosis produced by one of these agents. DNP by itself, in doses as high as an LD_{50}, causes only mild depression, as is indicated by its anticonvulsant effect on MES as well as by the overt signs and symptoms (unpublished observations). Also acetazolamide causes only depression, except in very large doses which produce some excitatory phenomena. In the doses used in the combination experiments, only depression would have been expected even if three times as much DNP or acetazolamide had entered the cell as a result of an acidosis caused by either of these agents. However, since seizures rather than depression occurred it appears that these two drugs increased the CO_2 level in the brain sufficiently to put the animals into the second or hyperexcitable phase of CO_2 effect.

Further evidence for a role of endogenous CO_2 in regulating CNS function is provided by experiments in which acetazolamide is combined with various concentrations of CO_2. These data are summarized in figure 3. The ordinate is brain excitability in arbitrary units and the abscissa is per cent CO_2. It can be seen from this figure that acetazolamide potentiated the anticonvulsant effect of a low concentration of CO_2 [37]; but, when given to rats which were exposed to 15 per cent CO_2 the drug caused seizures. However, when acetazolamide was administered to rats exposed to 30 per cent CO_2 the seizures were prevented, an indication that the animals were elevated to the third or anesthetic phase of CO_2 effect. Thus the addition of acetazolamide appears to be the equivalent of exposing animals to a CO_2 concentration about 10 per cent higher than the actual concentration. These data provide convincing evidence that acetazolamide and CO_2 have the same mode of action.

It appears from the available data that agents which enhance CO_2 production can affect CNS excitability if the rate at which the CO_2 is produced exceeds the rate of elimination of the gas from the brain cells, thus resulting in a net accumulation of CO_2. It has been shown that accumulation may occur by at least three mechanisms: (1) decreased rate of elimination of the CO_2 from the lungs secondary to respiratory depression; (2) decreased rate of release of CO_2 from the brain cells; and (3) rate of CO_2 production exceeding the normal excretory capacity of the respiratory system for CO_2. Examples of the first mechanism are effects produced by barbiturates, trimethadione, and morphine which depress respiration and enhance the toxicity of salicylate and DNP. Salicylate (unpublished observations) and DNP[58] have also been reported to enhance barbiturate-induced sleep time. This is probably due to CO_2 accumulation as a result of increased CO_2 production coupled with respiratory depression. Examples of the second mechanism are the effects produced by carbonic anhydrase inhibitors, such as acetazolamide, sulfanilamide, etc. Examples of the third mechanism are effects produced by combinations of two drugs such as salicylate or DNP, each of which enhances CO_2 production.

Role of Carbonic Anhydrase in the Central Nervous System

In the previous sections of this discussion some of the effects of CO_2 on the central nervous system were reviewed. It is clear that

any experimental condition which changes the CO_2 tension in the brain is also capable of affecting brain function. The nature of the mechanism by which a change in CO_2 tension can produce changes in function remains obscure. The effects of carbonic anhydrase inhibitors upon the central nervous system demonstrate that carbonic anhydrase is important in regulating CO_2 tension in the brain. An understanding of the role of carbonic anhydrase in brain may yield some information on the biochemical role of CO_2 in the central nervous system.

The following discussion of the role of carbonic anhydrase in the central nervous system involves a fundamental assumption that the enzyme catalyzes only one set of reactions which include the hydration of CO_2 and the dehydration of carbonic acid. In a physiological medium this equation is generally represented as follows:

$$CO_2 + H_2O \xrightleftharpoons{\text{Carbonic Anhydrase}} H_2CO_3.$$

It must be remembered that the assumption of specificity of action of carbonic anhydrase may not be a valid one; however, at present the hydration and dehydration of CO_2 are the only reactions known to be catalyzed by carbonic anhydrase, and therefore they are the only ones which can be considered.

In discussing a reversibly catalyzed set of reactions one must consider which direction the steady-state condition will favor; the direction depends solely upon the fate of the reaction products and the concentration of the substrate. For example, in the lung, CO_2 escapes the pulmonary vascular system, and hence this favors the generation of CO_2 and H_2O. However, the direction is reversed in blood at the tissue level because CO_2 cannot escape and the resulting high CO_2 tension in red blood cells drives the reaction in the direction of H_2CO_3 and HCO_3^-. Similarly, in organs which secrete H^+ or HCO_3^-, such as kidney or pancreas, carbonic acid formation can predominate.

The reactions, reversibly catalyzed by carbonic anhydrase, are somewhat unique among the enzyme-catalyzed systems in that they proceed at an appreciable rate in an uncatalyzed medium.[59] The fact that the uncatalyzed rate is significant is important because it implies that the inhibition of carbonic anhydrase in a sequence of reactions cannot completely block the sequence, but the resulting reduction in the rate may render the carbonic anhydrase catalyzed step a rate-limiting one. This is an important point to consider in evaluating the role of carbonic anhydrase in a physiological system.

It is relatively easy to appreciate the essentiality of carbonic anhydrase in activities associated with blood and other organs such as kidney and pancreas, but in brain a role for the enzyme has not been completely defined. In general, two functions for carbonic anhydrase are possible: one is the dehydration of carbonic acid to CO_2 and the other is the hydration of CO_2 to carbonic acid. The immediate question with respect to the brain is which of these reactions is utilized? In tissue as complex as brain, it is also possible that both functions are of equal importance, as in blood. In any event, the role of carbonic anhydrase in the brain revolves around the relation of the enzyme to endogenous CO_2 and H_2CO_3, the substrates of the enzyme.

CHARACTERISTICS OF CARBONIC ANHYDRASE ACTIVITY IN BRAIN: *Influence of Aging.* The influence of aging upon carbonic anhydrase activity in 2 to 38 day old rats was studied by Millichap *et al.*[50, 51, 52] who reported an increase in activity in developing animals. They observed an inverse relation between enzymatic activity in brain and excitability as measured by an electroshock method. An additional study of the relation of age to carbonic anhydrase activity has indicated that the activity in cerebral cortex continuously increases with increasing age from newborn rats to animals over two years old.[61] The relation between brain excitability and the progressive increase in enzymatic activity remains to be determined.

Intracellular Distribution. A study of the intracellular distribution of carbonic anhydrase has added another consideration to the problem of the role of the enzyme. In cerebral cortex[62] and other parts of the brain (unpublished observations) the enzyme is present in two subcellular fractions, mitochondrial and supernatant. As the total enzymatic activity of the tissue increases with age so does the activ-

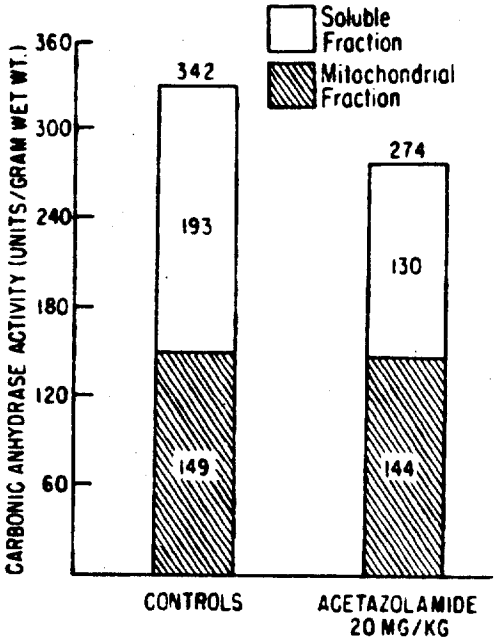

FIG. 4. Effect of acetazolamide on the intracellular distribution of carbonic anhydrase in rat cerebral cortex. Perfused cortex homogenized in 0.25 *M* sucrose solution (1:10) and the mitochondrial fraction separated from the supernatant by centrifuging 10,000 × *g* for 25 minutes a homogenate which had previously been centrifuged for removal of the nuclear fraction. Carbonic anhydrase activity was measured by a modification of the manometric method of Meldrum and Roughton. Acetazolamide dose was 20 mg./kg. intraperitoneally, and animals were sacrificed 4 hours after drug administration.

ity in the two subcellular fractions.[41] The presence of the enzyme in different subcellular components introduces the problems of the role of the enzyme in each locus and of the functional relation between the two enzyme loci.

Effect of Acetazolamide. A study of the effect of acetazolamide upon the intracellular distribution of carbonic anhydrase activity demonstrated that low doses of the drug *in vivo* preferentially inhibited the supernatant over the mitochondrial fraction of the enzyme. An example of the results of such an experiment is given in figure 4.

In-vitro experiments have indicated that acetazolamide is capable of inhibiting both cellular fractions containing carbonic anhydrase. As yet, there is no explanation for the greater vulnerability *in vivo* of the enzyme in the supernatant of the cytoplasm compared to that in the mitochondrial fraction. It is possible that the mitochondrial membrane *in vivo* is relatively impermeable to the drug; no information, however, is available on this point. Another possibility may lie in the biochemical nature of the two cellular enzymes. For example, it is not known if the enzymes in the two cell fractions are identical. The only biochemical characterization which has been made on these enzymes was a comparison of their activity-concentration curves. These curves have identical characteristics which provide some evidence that the enzymes are identical (unpublished observations). With respect to the problem of selectivity of drug action, one important biochemical characterization which is needed is the dissociation constants for the two enzyme-drug complexes. A difference in these constants could account for the preferential inhibition of one enzyme fraction over the other.

SOURCE AND NATURE OF CARBON DIOXIDE PRODUCED IN TISSUES: The principal source of CO_2 in tissues is probably the decarboxylation reactions associated with the tricarboxylic acid cycle. The enzymes involved in the tricarboxylic acid cycle are generally considered to exist intracellularly within mitochondria; therefore, the mitochondria are probably the major site of CO_2 production in cells. The presence of carbonic anhydrase in the mitochondrial fraction may indicate that there is an intimate relation between the enzyme and metabolically produced CO_2.

Another known source of CO_2 in cells arises from the decarboxylation of amino acids. In the central nervous system, the decarboxylation of glutamic acid to γ-aminobutyric acid is an example of such a reaction. Information on the intracellular localization of amino acid decarboxylases is meager; however, at least some of them are associated with the soluble fraction of cells. A qualitative study of glutamic decarboxylase in brain showed that it is also present in the soluble fraction (unpublished observations). It is again interesting to note that the intracellular distribution of carbonic anhydrase corresponds to a major site of CO_2 production.

Nature of Metabolically Produced Carbon Dioxide. The customary biochemical repre-

sentation of CO_2 produced during metabolism conveys the impression that CO_2 is split off directly from the decarboxylated product. It is true that, due to its volatility, CO_2 is an ultimate product of respiration, whether it is measured in the whole organism or in biochemical preparations; however, because of the kinetics of the hydration-dehydration reactions it is not known whether CO_2 or a hydrated product (carbonic acid or bicarbonate) is initially produced in metabolic reactions.

Attempts have been made to determine the original nature of the CO_2 liberated by decarboxylating enzyme systems by measuring the rate of CO_2 production in the presence and absence of carbonic anhydrase. On the basis of such experiments, Krebs and Roughton [63] concluded that reaction systems utilizing yeast carboxylase or urease produce CO_2 as the initial product. Conway and O'Malley [64] confirmed these results with respect to the urease system; however, with respect to the carboxylase system they maintained that the original nature of the CO_2 varied, being either the gas itself or H_2CO_3, depending upon experimental conditions.

Another approach to the study of the formation of CO_2 in enzymatic reactions was presented by Rothberg and Steinberg [65] who employed O^{18}-labelled water in reaction systems containing various bacterial amino acid decarboxylases. They observed that the CO_2 produced was essentially free of O^{18} and concluded that these pyridoxal-catalyzed decarboxylations were nonhydrolytic; consequently CO_2 was the initial product. The results support a mechanistic model for pyridoxal-dependent decarboxylase reactions proposed earlier by Metzler *et al.*[66]

The above-cited studies on the nature of CO_2 produced by decarboxylation reactions were necessarily restricted to isolated enzyme systems as opposed to tissue slices or homogenates because it was not technically possible to measure directly rates of CO_2 production in a respiring system. Ultimately, it is necessary to study the problem in respiring systems because they are closer to the situation *in vivo*. Some indirect studies of the effect of carbonic anhydrase inhibitors on the rate of CO_2 production in intact tissues have been reported. Shanes [67] suggested (on the basis of a hydrogen-potassium exchange hypothesis) that the increase in potential produced by sulfanilamide could be explained if H^+ and HCO_3^- were the first products of decarboxylation rather than CO_2.

Koch and Woodbury [27] observed that the total CO_2 content in the cerebral cortex of acetazolamide-treated animals was higher than normal values (table 2). They concluded that the inhibition of carbonic anhydrase reduced the rate of CO_2 escape from the tissue, an indication that CO_2 is derived from carbonic acid. It is of interest to note that the experimental conditions of these experiments were similar to those discussed earlier which demonstrated that the soluble fraction of brain carbonic anhydrase activity was selectively inhibited over the mitochondrial enzyme activity. The increase in total CO_2 content of cortex produced by acetazolamide, consequently, appears related to inhibition of carbonic anhydrase activity in the supernatant fraction of cells.

We have extended the study of the origin of CO_2 produced during respiration by employing a completely different system utilizing an infrared CO_2 analyzer. With a CO_2 analyzer it is now possible for the first time to record continuously the rate of CO_2 production

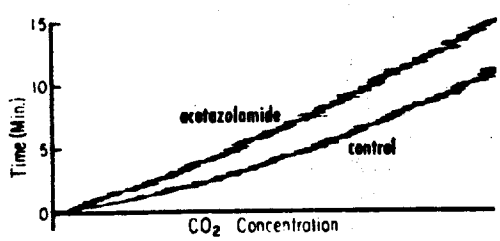

FIG. 5. Inhibitory influence of acetazolamide on the rate of CO_2 production in rat brain homogenate. Study conducted in a standard Warburg vessel at 38 C. Respiratory system contained 3.0 ml. of a 0.25 *M* sucrose homogenate (1:5) of brain, 0.6 ml. of 0.25 *M* glucose solution, and 0.4 ml. of either H_2O (control) or 10^{-4} *M* acetazolamide solution; final acetazolamide concentration equalled 10^{-5} *M*. This figure represents a photograph of the actual recording with the ordinate as time and the abscissa as CO_2 concentration. Note that rate of CO_2 production in the control system exceeds that of the system to which acetazolamide has been added. For example, the CO_2 concentration reached in the control system at 10 minutes is not attained in the acetazolamide inhibited system until approximately 15 minutes.

in tissue homogenates of brain during aerobic metabolism. Examples of such recordings are shown in figure 5. These recordings illustrate that the rate of CO_2 production decreased following the addition of acetazolamide. Furthermore, it was shown that the decreased rate of CO_2 production was not due to a decreased respiratory rate because oxygen consumption was unaffected by the concentrations of acetazolamide used. The fact that the *apparent* rate of CO_2 production decreased following the addition of acetazolamide supports the concept that respiratory CO_2 is derived from carbonic acid.

It is interesting to compare the relation of the depression of CO_2 production to the degree of carbonic anhydrase inhibition. The degree of depression in CO_2 production was approximately the same in the presence of either a 10^{-5} *M* or 10^{-7} *M* acetazolamide solution; however, a 10^{-5} *M* solution completely inhibited the carbonic anhydrase, whereas a 10^{-7} *M* solution inhibited only 10–15 per cent of the total tissue enzyme activity. Reactions containing a 10^{-7} *M* solution are comparable to the concentrations used in the *in vivo* experiments discussed earlier which demonstrated an increase in total CO_2 and selective inhibition of the supernatant carbonic anhydrase activity. The equal inhibition of CO_2 production obtained by widely different degrees of enzyme inhibition remains to be explained.

The studies on CO_2 production which have been made with the CO_2 analyzer were performed on whole tissue homogenates. However, since the major sites of CO_2 production in tissue are probably mitochondrial and supernatant fractions, it is important to study the nature of the CO_2 produced in these two isolated cellular fractions in order ultimately to understand the intracellular relationship of the carbonic anhydrase function in these two fractions. Such studies are now being carried out in our laboratory.

Mechanism of Anticonvulsant Action of Carbon Dioxide on the Nervous System

A hypothesis for the mechanism of anticonvulsant action of CO_2 on the CNS has been presented by Koch and Woodbury[27] and will only be summarized here. The effect of giving exogenous CO_2 or acetazolamide is to increase the steady-state concentration of H_2CO_3 in brain cells. In the case of acetazolamide, the inhibition of carbonic anhydrase reduces the rate at which metabolically derived CO_2, which is produced as H_2CO_3, is dehydrated. Because H_2CO_3 is much less diffusible than CO_2, the inhibition of carbonic anhydrase results in an increase in the steady-state concentration of H_2CO_3 in the brain. The increased H_2CO_3 concentration, produced by CO_2 and acetazolamide, reduces the permeability of the cell membrane to Na probably as a result of an increase in ionizable calcium which occurs from the local increase in acidity induced by H_2CO_3. Increased amounts of calcium ion are known to decrease cell permeability to Na. Inasmuch as Na-pump activity is not affected by H_2CO_3, the decreased Na influx results in a lower steady-state concentration of Na within the cells and a higher ratio of Na across the membrane. The decrease in permeability of the membrane to Na would be predicted to raise the threshold of firing of neurons and reduce, to some extent, the height of the propagated impulse. A very slight effect of this nature would be difficult to detect along the major portion of an axon, but could exert a marked effect on the postjunctional elements of a synapse.[68] Hence, carbon dioxide would be expected to block the abnormally high number of impulses impinging on the dendrites of central neurons and thereby to reduce considerably the probability of successful synaptic transmission. As a consequence, activity from an epileptogenic focus would tend to remain a localized phenomenon instead of progressively spreading to involve the whole cerebral cortex.

Summary

It has been shown that various concentrations of CO_2 exert inhibitory and excitatory effects on the central nervous system (CNS). In addition, several drugs which mimic the effects of CO_2 on the CNS possess this activity by virtue of their ability to alter the tissue level of CO_2. The mechanism of the CNS action of CO_2 appears dependent upon specific electrolyte and amino acid changes. These studies have also demonstrated that carbonic anhydrase is important in the regulation of the level of brain CO_2 and excitability and

that the role of carbonic anhydrase in brain is ultimately concerned with the conversion of metabolically produced H_2CO_3 to CO_2. Furthermore, this functional role of the enzyme appears associated with the soluble fraction of the cell.

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