CARBON DIOXIDE AS A FACILITATING AGENT IN THE INITIATION AND GROWTH OF BUBBLES IN ANIMALS DECOMPRESSED TO SIMULATED ALTITUDES*

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(Received for publication, September 13, 1944)

Aviators, like divers, may be subject to decompression sickness following a marked drop in external barometric pressure of sufficient duration. In divers, the symptoms, known as the "bends" and the "chokes," were associated by Bert (1878) and by Boycott, Damant, and Haldane (1908), on the basis of animal experiments, with the liberation of dissolved nitrogen in the form of bubbles in the blood and tissues. Important applications of this principle have been made by Behnke and associates (summary, Behnke, 1942) in practical operations. Until recently, however, little evidence was available regarding bubble formation in animals decompressed from sea level to simulated altitudes (*i.e.*, to pressures of less than one atmosphere). Isolated early observations were made by Robert Boyle (1670), Hoppe (1857), and by Hill and Greenwood (1910), while the outstanding modern work antedating the war is that of Armstrong (1939). Using goats, Armstrong found bubbles in blood and tissues on decompression to simulated altitudes of 40,000 feet; smaller animals remained bubble-free under similar conditions.

Recently we have completed an extensive series of experiments on bubble formation in animals at reduced pressures, the results of which are reported in a separate communication (Whitaker, Blinks, Berg, Twitty, and Harris (1945)). The most important aspect of this work was the finding that muscular exercise in decompressed animals greatly favors bubble formation. Bubbles may appear in small animals (bullfrogs, rats) as well as in larger forms (rabbits, goats) following induced or spontaneous activity at simulated altitudes; in quiescent animals bubbles are absent. E. Newton Harvey and coworkers (unpublished) subsequently confirmed these results, using bullfrogs and cats as experimental animals.

The detailed mechanism of action of exercise on bubble formation has been the subject of further study. Blinks (unpublished), Dean (1944), and Harvey

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University. The authors are greatly indebted to Dr. John Fulton and other members of the Sub-Committee on Decompression Sickness of the Committee on Aviation Medicine for encouragement, advice, and support.

et al. (unpublished) have analyzed the rôle of purely physical factors (turbulence and other effects) which are involved in the action of exercise. They have shown that mechanical agitation causes bubble formation under conditions of supersaturation. We have studied the influence of certain metabolites resulting from muscular activity, particularly carbon dioxide. The possibility was early recognized that high concentrations of CO_2, present locally in the muscles during muscular exercise, might affect bubble development in these sites. This idea has been confirmed by numerous experiments, the results of which are reported in the following sections.

Materials and Methods.—For purposes of decompression, a small steel chamber, 7 × 7 × 28 inches, open at the top, was used. The top was covered with plate glass to permit direct observation of animals under reduced pressures. By means of a small pump and reserve vacuum tank, the pressure could be lowered to a simulated altitude[1] of 50,000 feet in approximately 45 seconds. Pressures within the chamber were measured with a mercury manometer, calibrated in terms of equivalent altitudes. In experiments involving exercise, frogs were placed on a wire grid and stimulated intermittently with 5 to 25 volts, 60 cycle A.C. For rats, this purpose was accomplished by applying electrodes, wet with saline, to the hind limbs and stimulating similarly.

Bubble Formation in Dead Animals

We were encouraged to investigate the rôle of CO_2 in bubble formation by the suggestive observation that in certain cases dead animals, if decompressed to 45,000 to 50,000 feet, exhibited widespread and profuse bubble formation in the vascular system. This finding was particularly interesting because of the absence of muscular activity or turbulence of the blood in dead animals. The first experiments of this type were performed with rats, killed by breaking the neck (without external rupture). The animals were then allowed to stand at atmospheric pressure and room temperature for 5 to 10 minutes longer to assure complete cessation of breathing and heart action. Rapid decompression to 50,000 feet followed, the rats remaining at altitude for 10 minutes. At the end of this time the animals were returned to atmospheric pressure and submitted to careful autopsy under a dissecting binocular. In typical cases the heart, arteries, and veins all contained large numbers of bubbles. With some variation between individual rats, the distribution of bubbles extended to all vascular branches, including all chambers of the heart, pulmonary arteries and veins, aorta and branches, as well as the precaval, postcaval, and hepatic portal systems. Often the lymphatic system, particularly the lumbar lymph nodes and cisterna chyli, also contained bubbles.

The possibility was originally considered that these bubbles found at autopsy

[1] It should be understood that all altitude levels referred to in this paper are *simulated* altitudes (*i.e.* equivalent barometric pressures).

were introduced during the decompression through rupture of capillaries, either by expanding intestinal gases or in the lungs. In order to determine the site of origin of these bubbles, therefore, a number of dead rats were dissected open with minimal damage to blood vessels and decompressed. The animals were prepared so as to permit direct or microscopic observation at altitude of all the main vessels of the circulation. Observation in these cases revealed that bubbles were not apparent for a short interval after reaching the simulated altitude (50,000 feet); then suddenly a stream of bubbles appeared, coming from a small branch into a larger vessel, followed by bubbles arising similarly in other loci. Local fluctuations in the rate of bubble formation led to an irregular ebb and flow of bubbles in the vessels. In a short time the larger vessels and heart contained a froth or became entirely filled with gas from the coalescence of massive accumulations of bubbles. The pressure from this expanding gas phase resulted in forcing the bubbles throughout the vascular system, in arteries as well as veins. No bubbles were seen to arise in larger vessels, but invariably appeared to come from the smallest branches, predominantly from muscles of the legs and back. In no case did the primary source of bubbles involve the vessels from either the lungs or the digestive tract.

In order to test this point further, additional experiments were performed with dissected dead rats, in which the postcava and dorsal aorta were clamped off just anterior to the diaphragm. Several of these animals on decompression had great numbers of bubbles posterior to the clamps before any bubbles had yet appeared in the heart or lung regions, thus eliminating in these cases the lungs as a source. Likewise, when clamps were placed on postcava and aorta posterior to vessels supplying the digestive tract, bubbling occurred abundantly in the isolated posterior region. These observations indicate that bubbles in dead rats are not derived by "leaking" into the vascular system, but come from small vessels, deep within the tissues, and most commonly from muscular regions.

The question also arose whether bubbling in the decompressed dead rats might be due to some slight muscular activity (*e.g.*, peristalsis) still persisting after breathing and heart action had ceased. To check this point, six rats were killed as before and allowed to stand for 4 hours at room temperature. At the end of this time the animals were in rigor mortis and the body was cool. On subsequent decompression and autopsy, three rats contained great numbers of bubbles throughout the circulatory system, indicating that the cause of bubbling in these animals could not be attributed to lingering muscular activity.

Other experiments showed that the phenomenon of bubble formation in decompressed dead animals was not confined to rats. Several rabbits killed and decompressed according to the same procedure showed a similar widespread occurrence of bubbles at autopsy. It is difficult to perform comparable experiments with bullfrogs, since the heart continues to function even after the cen-

tral nervous system is destroyed. However, a number of frogs which had died from "red-leg" were used for this purpose. These were decompressed, 24 to 48 hours after death, to 50,000 feet for 10 minutes. Of fifteen frogs treated in this manner, bubbles occurred in eight.

In general, while the majority of decompressed dead animals contained bubbles, a certain number of individuals remained bubble-free. A rather sharp difference existed between the two groups of animals: typically, bubbling occurred profusely, spreading throughout the body, or not at all. Other experiments were performed to study this variability further. Preliminary work revealed no simple correlation of size or amount of fat with occurrence of bubbles in rats when killed and decompressed, although a trend existed toward a somewhat higher frequency of bubble formation in large, well fed animals.

Attention was also directed to the method of killing used in the first experiments (breaking the neck) to see if any factor inherent in this procedure was responsible for the variability noted. In some cases rats were killed by simple manual suffocation, to minimize the effect of trauma and attendant possibility of admitting air through injured tissues. This treatment did not, however, alter the incidence of bubbles found at autopsy, done after decompression. Another group of animals was killed by acute anoxia at simulated altitudes. These animals were decompressed in air, while still alive, to 50,000 feet for 4 minutes. Death occurred within a minute or two from oxygen lack. After the 4 minute period, the rats were recompressed and immediately autopsied. As before, the greater number, but not all, of these rats contained bubbles. In addition, the last mentioned experiments suggest that under certain circumstances the characteristic capacity noted for bubble formation may be present almost immediately after death, without the necessity of any intervening latent period. The effects of pure nitrogen and illuminating gas as lethal agents were also investigated and gave results similar to those already described.

Since all of the above mentioned methods of killing involve violent or paroxysmal muscular activity as a terminal phase, experiments were designed to determine whether this was an indispensable condition for bubble formation after death. A number of rats were given lethal doses of nembutal (100 mg.) intraperitoneally, with a minimum of disturbance to the animals. Several of these were entirely quiescent during and after injection. Fifteen to 30 minutes after breathing and heart action had stopped, the animals were decompressed to 50,000 feet for 10 minutes. At autopsy a number of rats, including the quiescent animals, contained bubbles. It is evident, therefore, that extensive muscular activity just prior to death is not a necessary factor for bubble formation in dead rats. The modifying effect of prolonged violent exercise before death will be considered later.

Electrocution was found to be the most effective lethal agent, from the standpoint of a high incidence of bubble formation on subsequent decom-

pression. At first, electrodes wet with saline were attached diagonally to one front and one hind leg and current (110 volts, 60 cycle A.C.) applied for 1 minute. Later the animals were merely dropped into a saline bath into which the two electrodes had been immersed. As a control procedure for this method, six rats were left in the circuit for 30 minutes and then immediately autopsied (no decompression). No bubbles were found on careful search in these animals, indicating that electrocution *per se* is not a cause of bubble formation. Using this method, twenty rats were killed and subjected to decompression, of which only three failed to give bubbles. These results are summarized in Table I together with those obtained with other lethal agents. It is evident that a higher percentage of positive cases is obtained with electrocution, compared to other methods. This difference may conceivably be connected indirectly

TABLE I

Bubble Formation in Decompressed Dead Rats

Cause of death	Time between death and decompression	Duration of decompression	Simulated altitude	Rats with bubbles present	Rats with no bubbles
	min.	*min.*	*ft.*		
Broken neck	5–10	15	50,000	9	5
Manual suffocation	10	15	50,000	5	2
Anoxia (at altitude)	0	4	50,000	6	3
Nitrogen	5	15	45,000	3	2
Illuminating gas	10	4	50,000	1	1
Nembutal	15–30	10	50,000	14	6
Electrocution, 110 V.A.C.	10–30	10	50,000	17	3

with the demonstrable heating effect caused by passage of current through the tissues. Such heating might tend to delay the rate of cooling in the body after death. In support of this view is the fact that if rats are killed by nembutal and maintained in an incubator at 37°C. during the interval before decompression, the incidence of bubble formation tends to increase on later low pressure treatment. Furthermore, if rats are anesthetized and then killed by cold treatment without freezing, and are decompressed in ice water, bubble formation does not occur. If, however, rats killed by cold are warmed to 37°C. in an incubator for 45 minutes before decompression, bubbles form as usual with low pressure treatment. Hence it seems possible that the lower percentage of positive results in dead rats with lethal agents other than electrocution may be due to the cooling process after death, with consequent greater solubility of dissolved gases.

While temperature may constitute a cause for a certain number of negative results in the dead animals, it is not a complete explanation. Both in rats killed by electrocution and those killed by nembutal but maintained at 37°C.,

negative cases were still apparent. This finding, together with the essentially all-or-none character of bubble occurrence in the dead animals, suggests a threshold phenomenon, possibly in relation to the degree of supersaturation of dissolved gases in the body. This idea is supported by evidence from rats decompressed from high barometric pressures to sea level (Harris, Berg, Whitaker, and Twitty (1945)) where bubbles may form in quiescent living rats without any muscular exercise, if the degree of decompression (which influences supersaturation) is 60 pounds per square inch or greater.

It seems unlikely that variation in N_2 supersaturation between individual dead rats could account for the occasional negative results in question, since all animals were equilibrated similarly with air throughout the experiments. On the other hand the amount of dissolved free CO_2 in blood and tissues probably reaches high levels in dead animals, and might be subject to a considerable variation between individual rats. After death, the normal CO_2 tension is augmented not only by residual cellular respiration, the products of which are not removed, but also still further by anaerobic glycolysis. The latter process involves formation of lactic acid which reacts with bicarbonates in blood and tissue fluids to liberate additional free CO_2.

The extent of anaerobic glycolysis in dead animals is indicated by Voegtlin's studies (1933) with a glass electrode on pH changes in muscle, in which post mortem values as low as 6.2 were recorded. That anaerobic glycolysis may markedly increase the amount of free CO_2 is indicated further by experiments *in vitro*. Rat blood in a clean test tube will not bubble under decompression to 50,000 feet unless given strong mechanical agitation. If lactic acid is added in physiologically possible concentrations (0.4 per cent), bubbling likewise will not occur at simulated altitudes if the tube is undisturbed, but a slight tap will induce violent frothing.

Animal experiments were also devised to test the importance of CO_2 liberated by lactate formation for bubble development under decompression. For this purpose rats were used in which anaerobic glycolysis had been largely inhibited. The experimental animals were given lethal doses of sodium iodoacetate (370 to 550 mg.) intraperitoneally; a control series received lethal doses of nembutal instead. At the time of death, both experimental and control animals were placed in an incubator at 37°C. to eliminate any possible effects of cooling before decompression. After intervals varying from 10 minutes to 2 hours, the rats were decompressed to 50,000 feet for 10 minutes. Table II summarizes the results obtained at autopsy. It is evident that the reduction or inhibition of anaerobic glycolysis by iodoacetate poisoning is correlated with a definitely lower incidence and extent of bubble formation in decompressed dead rats.

Additional information regarding the rôle of CO_2 liberated from bicarbonates by lactic acid is suggested by experiments dealing with the effect of strong exercise just prior to death on bubble formation in dead rats. These animals,

through electrical stimulation (5 to 25 volts, 60 cycle, A.C.) and associated spontaneous reactions, underwent 15 minutes of violent and maximal activity, resulting in a state of exhaustion. The rats were then immediately killed (electrocution) and after 30 minutes decompressed to 50,000 feet for 10 minutes. Control animals were electrocuted without previous exercise and decompressed similarly. Table III presents the results obtained on the two groups, and indicates a definite trend toward protection against bubble formation in the pre-

TABLE II

Effect of Sodium Iodoacetate on Bubble Formation in Decompressed Dead Rats

	Lethal agent	Time between death and decompression	Bubbles at autopsy		
			None	Very few	Many
Experimental series.....	NaIA, (370–550 mg.)	10 min. to 2 hrs.	5	6	0
Control series.....	Nembutal (100 mg.)	10 min. to 1 hr.	2	1	6

Experimental and control animals maintained at 37°C. before decompression to 50,000 feet for 10 minutes.

TABLE III

Effect of Violent Muscular Exercise, Just Prior to Death, on Bubble Formation in Decompressed Dead Rats

Previous exercise	Bubbles at autopsy		
	No bubbles	Bubbles in venous system and right side of heart only	Bubbles in arteries, veins, and heart
Violent.....	4	6	0
No exercise.....	1	0	11

Rats stimulated intermittently for 15 minutes (5 to 25 volts, 60 cycle A.C.) before death by electrocution. After 30 minutes, decompressed to 50,000 feet for 10 min.

exercised rats. This finding on the surface appears paradoxical in view of the predisposing effect of pre-exercise on bubble formation in living bullfrogs (Whitaker, Blinks, Berg, Twitty, and Harris (1945)). However, it is possible that the results obtained here with rats may be attributed to the extreme degree of exercise used. In this connection, the studies reviewed by Dill (1936) on blood lactate and bicarbonate during muscular exercise in man are of interest. He reports that under conditions of mild or moderate exercise, no appreciable rise in blood lactate is observed. After extremely violent exercise, however, large quantities of lactate are found in the blood and a drop of bicarbonates to low levels is observed. The latter process is apparently due to liberation of free

CO_2 by lactate formation, which is lost *via* the lungs, and to the severe hyperpnea and increased loss of CO_2 from this source *per se*. It seems probable that these factors may operate similarly in the rat, especially in view of the small size and consequent rapid respiratory turnover. Thus, it is possible that in the present experiments, the extremely violent exercise and attendant hyperventilation may have so reduced the bicarbonate reserve that anaerobic glycolysis after death did not liberate sufficient CO_2 to produce the usual effect on bubble development. As seen in Table III, this view is supported by the characteristic distribution of bubbles in the positive cases among the experimental animals: bubbles occurred only on the venous side of the circulation, where the CO_2 tension is highest.

The sluggish bullfrog, on the other hand, has by comparison with the rat a highly inefficient lung and ventilation rate, so that the free CO_2 produced in muscular exercise may not have been eliminated to any great extent before decompression, and could therefore facilitate bubble formation at altitude. In living mammals (cats) Harvey (unpublished) have shown that pre-exercise facilitates bubble formation on later decompression, a result which harmonizes well with our earlier findings on bullfrogs, but differs from our preliminary results in a few experiments on living rats, where a protective effect was suggested. In view of Dill's statement, mentioned above, that a drop in bicarbonate reserve occurs in severe but not in moderate exercise, such variability might conceivably result if somewhat different levels of muscular activity were involved in the two cases. Also the cat, being larger than the rat, may possibly eliminate CO_2 less rapidly than the rat (as in the frog).

Considering the complex group of factors involved, it is not unreasonable to expect a certain random variation in the final level of CO_2 attained in dead rats, although a moderate increase could result in a condition of supersaturation with respect to CO_2 when the animals were decompressed. For example, if the normal venous CO_2 tension is roughly approximated at 5 per cent (38 mm.), then, to select an arbitrary figure, a mere threefold increase in the dead animals could result in a CO_2 level of 114 mm. At 50,000 feet (87 mm. barometric pressure), from Henry's law that solubility is a function of partial pressures, this CO_2 figure represents a state of supersaturation in the dead rats. This condition follows from the fact that the dead animal is not a rigid object and the pressure within bubbles inside the body will be substantially identical with external barometric pressure. Thus at 50,000 feet (87 mm. barometric pressure) a bubble in the blood stream would have an internal pressure of approximately 87 mm. and would grow constantly from CO_2 alone, if the CO_2 tension in its surroundings exceeded this level, as in the example given above. Nitrogen would likewise augment bubble growth, for similar reasons.

Furthermore, CO_2 under supersaturation, when compared to N_2 under similar conditions, exerts an effect all out of proportion to the degree of super-

saturation involved. In experiments with models, Blinks (unpublished) has shown that bubbles develop more readily in water saturated with CO_2 than with air. We have likewise studied this effect *in vitro* by saturating water in clean test tubes with one atmosphere of CO_2 and N_2 respectively, and decompressing the tubes to the equivalent of 50,000 feet. Bubbles form with much less mechanical agitation in the CO_2 water and grow in size at a higher relative rate. These effects may be related to the relatively high solubility of CO_2, with consequent large numbers of dissolved molecules. Thus even a relatively small degree of CO_2 supersaturation in the dead animals might be significant in bubble formation.

With regard to the mode of action of CO_2 on bubble formation in dead animals, one possible explanation is as follows: The experiments *in vitro* mentioned above, with CO_2-saturated water, and with lactic acid added to rat blood, suggest that if the concentration of supersaturated gas molecules is greatly increased, there is a corresponding marked reduction in the degree of mechanical agitation needed to induce bubble formation. It is therefore possible that while a high level of CO_2 in the dead animal does not in itself directly initiate bubble formation, it may predispose in this direction to the point where the very slight mechanical disturbances unavoidably present in decompression of the dead animals (mere expansion of the body under lowered pressure) may be sufficient to produce bubbles. In occasional animals with a lesser accumulation of CO_2 these slight strains might prove insufficient to initiate bubble formation, thus accounting for the threshold nature of the phenomenon.

The reasoning given above may also provide an explanation for certain results obtained by applying a tourniquet to the legs of decompressed animals. It has been found with young goats (Blinks and Reed, to be published) that considerably less muscular activity is required to produce bubbles in a leg if a tourniquet is used. The CO_2 content is undoubtedly increased by the tourniquet, and this may explain the reduced threshold of activity for bubble formation.

Effect of CO_2 Administration on Bubble Formation

It should be emphasized that the rôle of CO_2 as a means of facilitating bubble formation is in no way restricted to dead animals or extreme pathological conditions. The prime purpose of the analytical work presented thus far lies in revealing the importance of CO_2 for bubble formation in the living, intact animal. As already pointed out, the data thus obtained support the idea that the favoring effect of pre-exercise on bubble formation in decompressed bullfrogs can be explained by the accumulation of CO_2 in the body. We have in addition put this theory to a more direct test by treating living frogs with high concentrations of CO_2, in an effort to simulate the effects of pre-exercise on bubble formation. Bullfrogs were placed in an atmosphere of 60 to 70 per

cent CO_2 (balance air) for 2 to 3 hours. Following this treatment, which the frogs tolerate fairly well, they were decompressed to various pressure levels for 30 minutes. At altitude some remained quiescent; others underwent varying degrees of spontaneous activity. Still others, previously urethanized, were completely inactive. As controls, frogs not treated with CO_2 were decompressed similarly. The results are shown in Table IV. At 60,000 feet, and without any appreciable muscular activity (quiescent or urethanized frogs), bubbles formed in a majority of the CO_2-treated animals, while only three out of eighteen frogs in the control group contained bubbles. Bubble formation in many of the CO_2-treated frogs was very extensive and almost explosive in

TABLE IV

Bubble Formation in Decompressed Frogs Previously Treated with CO_2 (Compared with untreated Controls Decompressed Similarly)

Pre-treatment	Altitude	Activity	Treated animals		Controls decompressed similarly, without CO_2 pre-treatment	
			Bubbles present	No bubbles	Bubbles present	No bubbles
per cent CO_2	*ft.*					
60–70	60,000	None (urethanized)	6	3	3	15
60–70	50,000	None (2 urethanized)	0	3	0	2
60–70	50,000	Slight (spontaneous)	6	0	0	7
60–70	15,000	Violent (stimulated)	2	3	0	5
25	50,000	Slight (spontaneous)	1	3	0	5
25	50,000	Moderate	2	0	7	0

Experimental animals placed in CO_2 mixtures for 1.5 to 3.5 hours before decompression. Duration of decompression 2 to 10 minutes.

character, beginning abruptly after a short latent period at altitude, and causing rapid and pronounced swelling of the body. Such phenomena did not occur in control animals even when bubbles were formed. At 50,000 feet, the differences between experimental and control groups were even more clear cut; here bubbles formed in CO_2-treated frogs with slight muscular activity, although the same degree of exercise did not result in bubble formation in any of the untreated controls. It is evident that direct administration of CO_2 to living frogs facilitates bubble formation on subsequent decompression, just as does previous exercise at sea level. However, high concentrations of CO_2 are required to produce this effect; a few experiments showed that 25 per cent CO_2 has only a slight effect on subsequent bubble formation (Table IV). This may in part reflect the difficulty of reproducing the local CO_2 picture in capillaries and small vessels by diffuse external application. The CO_2 concentration may be very high locally in the normal exercised animal.

CO_2 treatment of frogs, besides facilitating bubble formation at relatively

high altitudes, also causes a lowering of the threshold altitude at which bubbles will form. Bubbles ordinarily will not form (or at least cannot be observed) in normal bullfrogs violently exercised at a pressure equivalent to 15,000 feet. However, bubbles will appear at this same altitude in frogs which have been in a 70 to 80 per cent CO_2 atmosphere for 3 to 4 hours and then violently exercised at 15,000 feet (see Table V). In two frogs bubbles appeared as low as 10,000

TABLE V

Effect of CO_2 Treatment on Threshold Altitude for Bubble Formation in Bullfrogs (R. catesbiana)

Altitude	Pre-treatment	Activity	Autopsy	
			Bubbles present	Bubbles absent
ft.	*per cent CO_2*			
15,000	60–70	Violent	4	3
10,000	60–70	Violent	2	3
5,000–7,000	60–70	Violent	0	6
15,000	None (controls)	Violent	1	6

Experimental frogs in CO_2 3 to 4 hours before decompression.

TABLE VI

Effect of CO_2 Treatment on Threshold Altitude for Bubble Formation in Grass Frogs (R. pipiens)

Altitude	Pre-treatment	Activity	Autopsy	
			Bubbles present	Bubbles absent
ft.	*per cent CO_2*			
25,000–30,000	60–70	Violent	3	1
20,000	60–70	Violent	3	6
10,000–15,000	60–70	Violent	0	8
70,000	None (controls)	Violent	5	0
60,000	None (controls)	Violent	1	6

Experimental frogs placed in CO_2 mixtures for 3 to 4 hours before decompression.

feet. There is an optimum concentration of CO_2 for demonstrating this phenomenon. Too much has a narcotic effect so that the frogs will not exercise violently, whereas if too little CO_2 is administered the facilitating effect disappears. Lowering of the threshold altitude for bubble formation is even more striking in grass frogs (*Rana pipiens*), which are smaller than bullfrogs. Ordinarily bubbles will not form in grass frogs, even under violent exercise at a pressure equivalent to 60,000 feet. But if these frogs are placed for 3 to 4 hours in 70 to 80 per cent CO_2, bubbles will appear on violent exercise at a pressure equivalent to 20,000 feet (Table VI).

The ratio of minimum barometric pressures at which bubbles may be formed in normal frogs of these two species is as follows.

$$\frac{\text{Bullfrogs}}{\text{Grass frog}} = \frac{349}{54} = 6.5$$

Thus the threshold pressure for bubble formation in the bullfrog is over 6 times that which is required for grass frogs. In frogs pre-treated with CO_2 this ratio becomes

$$\frac{\text{Bullfrog}}{\text{Grass frog}} = \frac{429}{349} = 1.2$$

Thus the effect of CO_2 is to reduce the threshold barometric pressures required for bubble formation in these frogs nearly to a common level. In general, it is more difficult to obtain bubbles in small frogs than in large ones. CO_2 treatment tends to make this size difference disappear, a fact which may throw some light on why the original difference exists. Assuming that CO_2 is important for bubble initiation and that it has to reach a certain molecular concentration to be effective, then it may be that small frogs do not form bubbles readily because the CO_2 diffuses out of their smaller muscles much faster than out of the larger muscles of bullfrogs. Thus a threshold concentration of CO_2 would not be reached as easily in small muscles and small animals as in large ones.

The small residual difference in threshold altitudes for bubble formation in these frogs after treatment with CO_2 may be due to a difference in the degree of mechanical disturbance occurring during muscular activity in the two sizes of muscles, since the muscular activity of bullfrogs is more powerful than that of grass frogs. This idea is supported by negative results obtained in attempts to induce bubble formation in small flaps of exposed muscle in large frogs, where mechanical agitation would be less, due to the relatively weak contractions. However, the relatively high outward diffusion of CO_2 from these flaps may also be involved.

Gas Analysis of Bubbles

Another and even more direct approach to the importance of CO_2 in bubble formation lies in gas analysis of the bubbles themselves, formed under various conditions. For this purpose a new procedure has been developed by one of us (Berg, to be published) for the estimation of CO_2 and O_2 in small bubbles. The method adopted permits analysis of bubbles 0.4 to 1.5 mm^3. in volume. Briefly, it involves the use of a long piece of capillary tubing, of uniform bore, with a bell-shaped enlargement on one end and a piece of rubber tubing and screw clamp, to exert suction, on the other end. Bubbles placed in the bell with a pipette may be drawn in and out of the fine bore for measurement before and after reaction with KOH (to determine CO_2) and pyrogallol (to determine O_2).

The method for obtaining the gas sample from the blood stream is as follows: a bubble of sufficient size for analysis is located in a vein, which is then cut. As the bubbles come out, one is picked up in a pipette filled with saturated LiCl (this reagent is used to reduce diffusion of gas in or out of the bubble) and transferred to a pool of LiCl solution to free it of blood and then is introduced into the analyzer. The entire operation can be performed in 15 to 20 seconds.

Analyses of bubbles taken from the large veins of frogs exercised during decompression show a high N_2 content (average values: 95 per cent N_2, 3.5 per cent CO_2, 1 to 2 per cent O_2). In bubbles from frogs which had been exercised prior to decompression a higher CO_2 content was found (6.5 per cent CO_2).

TABLE VII

Analyses of the CO_2 Content of Bubbles from Decompressed Dead Rats and Dead Bullfrogs

Animal	Time dead before decompression	CO_2 content of bubbles
	hrs.	*per cent*
Rat	1	60
Rat	1	85, 70
Rat	1	60, 68
Rat	1	80, 73
Frog	48	54
Frog	48	36
Frog	30	26
Frog	30	15
Frog	24	26

All animals decompressed to 50,000 feet.

While these figures show a trend, they should not be taken as indicating the true values for bubbles at their site of origin. The limitations of the method give reason to believe that during the early development of these bubbles the CO_2 tension is much higher than that shown in the analysis. Even if the bubbles originate in a region locally very high in CO_2 (*e.g.* muscular regions during exercise), equilibration to a lower level undoubtedly occurs rapidly as they move outward into the large vessels, where they are first available for analysis. This was tested by injecting bubbles of 50 per cent CO_2 into the blood stream of a frog at sea level. These were removed at various intervals after their injection and it was found that the bubbles equilibrate with the blood in a few seconds. Since the interval of time between the first visible appearance of a bubble in a vein of a decompressed frog and its introduction into the analyzer is several minutes, much of the CO_2 originally present could diffuse out.

Considering these difficulties, the high values for CO_2 obtained in further

analyses of bubbles from dead rats are of particular interest. Rats were killed by electrocution (110 volts, A.C.), and kept at 37°C. for 10 to 30 minutes after death. After subsequent decompression, bubbles were removed at autopsy by the previously described method and analyzed. The CO_2 content of bubbles formed in dead rats is extremely high, ranging from 60 to 80 per cent (Table VII). In the muscles where the bubbles originate the CO_2 tension is undoubtedly still higher, since there is some loss of CO_2 in transferring the bubble to the analyzer, and also since the bubble, as it moved outward into the large veins, would tend to lose CO_2 to the blood and take up N_2.

Analyses of bubbles from dead frogs gave essentially the same results as those obtained with rats, although in general the values were somewhat lower. The frogs had been dead from "red leg" for 12 to 48 hours before decompression (to 50,000 feet). The results of the analyses are listed in Table VII, and show that the CO_2 content of bubbles formed in dead frogs is high. Here again the values obtained are probably low, due to operation of the factors mentioned previously.

DISCUSSION

In the preceding sections we have approached the relation of CO_2 to bubble formation in a variety of ways. Decompression experiments have shown that bubbles form readily in animals under conditions involving high accumulation of CO_2, and that certain factors lowering the CO_2 tension in these animals also decrease the tendency for bubble formation. Administration of CO_2 directly to living frogs greatly increases the incidence of bubbles on decompression, and actual analyses of bubbles in living as well as dead animals show that CO_2 is present in appreciable amount. Taken together, these experiments indicate an important rôle for CO_2 in bubble development. From a synthesis of the results we offer the following picture with respect to CO_2 and the initiation and growth of bubbles in the normal, living decompressed animal. As long as such animals remain quiescent, CO_2 probably does not reach a state of supersaturation in the body, and has little if any predisposing influence toward bubble formation. With muscular exercise, however, the CO_2 tension may reach high levels locally in the muscles. At these points the accumulated CO_2 may lead to a marked local supersaturation, which in turn greatly increases the ease with which bubbles may be formed there. It does so by reducing the magnitude of mechanical disturbance necessary for creating bubbles *de novo* at that point. Thus the effects of mechanical agitation are greatly accentuated and facilitated. It should be understood, of course, that N_2[2] is

[2] Oxygen and water vapor would also enter into the bubbles, in proportion to their concentration and at a rate depending also on diffusibility, but the concentration of oxygen is much less than that of nitrogen. Direct analysis of bubbles taken from the veins of decompressed frogs and rats shows the presence of oxygen.

also concerned with the initiation of bubbles, but under these conditions may play only a minor rôle in their origin and early growth. It is possible that bubbles in early stages contain a high proportion of CO_2 and grow very rapidly in size because of the high concentration of CO_2 molecules in their immediate neighborhood. As bubbles move out into the larger vessels, they move away from local regions of high CO_2 and equilibrate rapidly with the relatively low tension of CO_2 in the larger vessels. This loss of CO_2 is compensated for by a steady increase in N_2, which is responsible for further growth and maintenance of the bubbles.[2] It is conceivable that the composition of the bubble may change from largely CO_2 at the point of origin to predominantly N_2 in the larger vessels and heart. We have, therefore, the concept of CO_2 as a facilitator in bubble formation. It greatly increases the ease with which bubbles may be initiated and may be responsible for their rapid growth in early stages of development. At later stages N_2 is more directly concerned with their further growth and maintenance.

SUMMARY

1. Rats killed in a variety of ways (broken neck, nembutal, anoxia, electrocution) may undergo extensive bubble formation when subsequently decompressed from atmospheric pressure to simulated altitudes of 50,000 feet. On autopsy at sea level, large numbers of bubbles are found throughout the vascular system in the majority of animals. These bubbles appear to originate in small vessels deep within muscular regions, later spreading widely in arterial and venous systems. Dead rabbits and frogs also bubble profusely on decompression.

2. Bubble formation in dead animals is attributed primarily to the accumulation of CO_2, derived from residual cellular respiration after death, and from anaerobic glycolysis with attendant decomposition of bicarbonates in blood and tissue fluids. If anaerobic glycolysis is inhibited by using sodium iodoacetate as a lethal agent, bubble formation is greatly reduced or lacking on subsequent decompression.

3. Experiments *in vitro* suggest that high concentrations of CO_2 favor bubble formation by reducing the degree of mechanical disturbance necessary.

4. Administration of CO_2 in high concentrations to living frogs lowers the minimum altitude (pressure equivalent) at which bubble formation occurs, with exercise, in untreated animals. Pre-treatment with CO_2 also reduces the degree of muscular activity necessary for bubbles to form in frogs at higher altitudes.

5. Analyses have been made of the gas content of bubbles taken directly from the large veins of decompressed frogs and rats. In living animals the figures obtained indicate rapid equilibration with gas tensions in the blood. Bubbles taken from decompressed dead rats may contain 60–80 per cent CO_2.

6. The bearing of these experiments on the mechanisms of bubble initiation and growth in normal living animals is discussed. Reasons are given for suggesting that CO_2, due largely to its high dissolved concentration in localized active regions, may be an outstanding factor in the initiation and early growth of bubbles which in later stages are expanded and maintained principally by nitrogen.

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