

ADDITIONAL MECHANISMS FOR THE ORIGIN OF BUBBLES IN ANIMALS DECOMPRESSED TO SIMULATED ALTITUDES*

BY W. E. BERG, MORGAN HARRIS, D. M. WHITAKER, AND V. C. TWITTY

(From the Department of Biology, Stanford University)

(Received for publication, September 13, 1944)

In a series of studies, described separately (Whitaker *et al.* (1945), Harris *et al.* (1945)), it has been shown that muscular activity causes bubbles to form in decompressed animals, and that high blood concentrations of dissolved gases (*e.g.* CO₂ or air) facilitate this effect, decreasing the degree of muscular activity required. Aside from the facilitating effect of the CO₂ produced, the muscular activity is believed to exert its effect largely through the physical consequences of mechanical disturbance. Blinks (unpublished), Dean (1944), and E. Newton Harvey (unpublished) have shown in models that mechanical agitation, by creating "negative pressures," causes bubbles to form in fluids (including blood) that are supersaturated with gases. However, the presence of "nuclei" (*e.g.* minute bubbles or gas films) which serve as centers for bubble growth is apparently necessary.

Assuming that nuclei are involved in bubble formation in animals, it is important to investigate means by which they may form within the body, or be introduced from without. In the experiments to be described, a possible source of entry of nuclei has been explored and also new methods of producing nuclei and bubbles within the body in the absence of muscular activity have been found.

Ingestion of Frothy Fat Emulsions

Blinks (unpublished) and Dean (1944), have found in models that surfaces of hydrophobic substances such as lanolin, paraffin, etc., in water tend to retain minute air films with great tenacity. If the surrounding water is supersaturated with gases, these air films act as nuclei for the growth of visible bubbles. In addition to retaining air films, hydrophobic surfaces may possibly also be involved in actual *de novo* formation of nuclei (Harvey, unpublished).

These considerations suggested that heavy fat ingestion, especially of colloidal frothy fat with extensive air films, might introduce bubble nuclei into the lymph or blood stream by direct transference through the intestinal mucosa.

*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.

Furthermore, increased fat in the vascular system might favor *de novo* formation of nuclei merely because of the properties of hydrophobic surfaces.

Rats were forcibly fed a large amount (approximately 15 to 20 cc.) of frothy emulsion containing approximately 50 per cent water, 40 per cent beef suet, 10 per cent lanolin, and 0.1 to 1 per cent bile salts. Bile salts were included to facilitate absorption of the fat, and the emulsion was prepared in an electric mixer. At intervals varying from 4½ to 25 hours after the feeding, the rats were decompressed rapidly in pure O₂ to 50,000 feet,¹ where they were maintained for 2 minutes before recompression and autopsy. For stimulation during decompression, electrodes were attached to the hind limbs.

Eight of these rats engaged in a degree of muscular activity classed as "moderate" which is approximately threshold for bubble formation in normal control rats at this altitude (see also Table II in Whitaker, Blinks, Berg, Twitty, and Harris (1945)). Accordingly, any appreciable facilitating effect on bubble

TABLE I
Effect of Fat Ingestion on Bubble Formation in Rats

Time interval between feeding and decompression	Simulated altitude	Muscular activity	Bubbles present	Bubbles absent
	<i>ft.</i>			
4½-25 hrs.....	50,000	Moderate	0	8
19-20½ hrs.....	50,000	Violent	2	1
Controls.....	50,000	Moderate	0	3
(See also Table II in Whitaker <i>et al.</i>).....	50,000	Violent	5	0

formation resulting from the ingested fat should have been revealed by the formation of bubbles in these animals. Actually, however, no bubbles were found. The muscular activity of three of the experimental rats was violent, and two of these indeed formed bubbles, but this is also characteristic of violently exercised control rats. Therefore, the results (Table I) indicate that ingestion of fat does not favor bubble formation.

Examination at autopsy revealed large quantities of ingested fat in the lymphatic vessels of the experimental rats, especially in the cisterna chyli and mesenteric branches, while these vessels were relatively clear in the controls.

The effect of heavy fat ingestion was further tested in bullfrogs, which form bubbles more readily than rats. Ten animals were each forcibly fed approximately 15 to 20 cc. of an emulsion prepared by blending equal parts of melted nucoa and water, with the further addition of a small amount of NaCl. Eight other frogs were fed a beef suet emulsion, and six were fed an emulsion of beef suet, lanolin, and bile salts. Periods of 2 to 72 hours after feeding were per-

¹ All experimental altitudes referred to in this report are simulated in a decompression chamber.

mitted to elapse, for digestion and absorption, before the frogs were decompressed.

The results are shown in Table II and indicate that the incidence of bubble formation in the experimental frogs with slight muscular activity, which is approximately threshold for bubble formation in normal control frogs at this altitude (see also Table I in Whitaker, Blinks, Berg, Twitty, and Harris (1945)), is not measurably greater than in the controls.

It thus appears that bubble nuclei do not cross the intestinal wall with digested fat in rats and bullfrogs. Furthermore, high fat concentration *per se* in the lymph and blood stream did not facilitate bubble formation. It should be emphasized in passing that the fat in these experiments is concentrated in

TABLE II
Effect of Fat Ingestion on Bubble Formation in Bullfrogs

Time interval between feeding and decompression	Simulated altitude	Type of fat	Muscular activity	Bubbles present	Bubbles absent
	<i>ft.</i>				
2-24 hrs.....	50,000	Nucoa emulsion	Slight	1	6
2-24 hrs.....	50,000	Nucoa emulsion	Moderate	2	1
7-25 hrs.....	50,000	Suet emulsion	Slight	0	6
5-25 hrs.....	50,000	Suet emulsion	Moderate	2	0
28-72 hrs.....	50,000	Suet and lanolin emulsion and bile salts	Slight	2	4
Controls (see also Table I in Whitaker <i>et al.</i>)	50,000	—	Slight	2	6

the lymph and blood, and the results do not bear on the effects of tissue adiposity that might result from a protracted high fat diet.

Crystallization in the Body

Blinks and Pease (unpublished) have observed in models that crystallization of a number of substances in water, including caprylic acid, induces formation of bubble nuclei *de novo*. Under decompression with mechanical agitation the nuclei grow to form visible bubbles, or in some cases may grow spontaneously without agitation. These results suggested experiments on the rôle of crystallization within the body.

Nuclei-free liquid caprylic acid (melting point 16°C.) was injected into veins of frogs at 25°C. which were then at once cooled at 10°C. The cooling caused caprylic acid crystals to form in the blood stream, and immediately after decompression to 40,000 feet numerous bubbles appeared in the blood vessels

containing the crystals, but not elsewhere. The bubbles grew spontaneously, without mechanical agitation, presumably from centers or nuclei produced by crystallization of the caprylic acid. Controls that were not cooled, in which injected caprylic acid remained liquid, did not form bubbles.

This experiment suggested the possibility that bubble nuclei might arise in normal animals by crystallization of substances occurring naturally in the body. Since cooling might crystallize some unknown organic compound, bullfrogs were cooled in an ice bath until the body temperature dropped to 1–2°C., after which they were decompressed without exercise to 50,000 feet. The results were negative, in that no bubbles were found on subsequent dissection.

In the course of the experiment just described it was discovered, however, that bubbles appeared spontaneously, without muscular activity, when frogs cooled below 0°C. were subsequently decompressed. Frogs cooled to –5° to –8°C., warmed to 20°C. (body temperature), and then decompressed, contained many bubbles in the vascular system. Dissection of frogs immediately after removal from the cold bath disclosed ice crystals in the blood stream.

To test the apparent relation between freezing and formation of bubble nuclei, a bullfrog was anesthetized and wrapped in a towel with one leg extending free. The foot of this leg was placed in a bath of mineral oil at –5° to –10°C. for nearly an hour. The frog was then dissected to expose the femoral veins, and decompressed to 50,000 feet without muscular activity. The frozen foot was warmed by means of a strong light, to restore circulation, and after a few minutes bubbles appeared in the femoral vein draining blood from the treated foot. This experiment was repeated a number of times and without exception bubbles came from the frozen appendage but not from the other leg. Microscopic examination of frozen feet before decompression revealed that blood in the smaller vessels was frozen solid. In several cases bubbles were observed at sea level (*i.e.* without decompression) in these small vessels after the blood melted.

Small segments of blood-filled veins (ventral abdominal, renal portal) were tied off with thread and removed from the frog. No bubbles formed in any of these segments after decompression. However, when ice crystals were formed in the blood by bringing the segment in contact with dry ice, and the segment was then decompressed, it immediately filled with gas bubbles.

The effectiveness of freezing in forming bubble nuclei was further tested with evacuated test tubes containing frog's blood. Usually no bubbles appeared if the test tube was very clean, indicating that no bubble nuclei were present. However, if the lower portion of the tube was placed in contact with dry ice, a heavy stream of bubbles appeared as soon as ice crystals formed, and it came from the region of the crystals. If the ice crystals were allowed to melt, the heavy stream of bubbles ceased.

It is evident, therefore, that in the animal as *in vitro* crystallization of water forms bubble nuclei and small bubbles. Dissolved gases are forced out of

solution, as in the manufacture of ice. The small bubbles persist in animals for some time after thawing.

Fracturing of Bones

Harvey (unpublished) has found with cats that crushing the leg muscles results in the formation of bubble nuclei. This is confirmed with bullfrogs, but breaking the leg bones is found to be still more effective.

Leg muscles of anesthetized bullfrogs were severely crushed by repeated pounding with a hammer at sea level. The skin was not broken, but hemorrhagic areas were observed in the muscles. The frogs were decompressed to 45,000 feet for 10 minutes, recompressed to sea level, and autopsied. As shown in Table III, bubbles were found in a few of the frogs. Since 45,000 feet is approximately threshold for this effect it is a favorable altitude for comparing the relative effectiveness of bone breaking. When the tibia or femur is broken by hand with a minimum of tissue damage, without breaking the skin, and essentially without injury to the muscles, decompression to 45,000 feet results in the formation of many bubbles in almost all of the frogs. The results are

TABLE III
Effect of Crushing Muscles and Fracturing Bones on Bubble Formation in Bullfrogs

Treatment prior to decompression	Simulated altitude	Bubbles present	Bubbles absent
	<i>ft.</i>		
Leg muscles crushed.....	45,000	2	8
Leg bones (femur or tibia) fractured.....	45,000	9	1

shown in Table III, and indicate that bone breaking is considerably more effective than crushing the muscles. If a tibia is broken and the overlying skin is removed, the broken ends of the bone are visible through the intact fascial layers. When such a preparation is decompressed the bubbles can clearly be seen to originate in the region of the bone fracture.

Bubble nuclei are evidently produced by the breaking of the bone. They have been shown to persist at sea level for $\frac{1}{2}$ to 1 hour, depending in part on the size of the bone, and may be carried to other parts of the body. The mechanism of formation of these nuclei has not been established, but they are probably produced by the intense mechanical disturbances developed when the bone snaps.

SUMMARY AND CONCLUSIONS

1. A heavy ingestion of frothy emulsified fat by rats and bullfrogs does not increase susceptibility to bubble formation when the animals are decompressed 2 to 72 hours later. This indicates that gaseous films (bubble nuclei) initially present do not pass across the intestinal wall with the digested fat, and also

that high fat content *per se* in the lymph and blood does not increase susceptibility to bubble formation.

2. Liquid caprylic acid injected into veins of bullfrogs crystallizes when the frogs are cooled. The crystallization causes bubbles to form without muscular activity on subsequent decompression. Cooling normal bullfrogs to 1–2°C. fails, however, to crystallize any substances occurring naturally in the animals that might act in a similar manner.

3. When bullfrogs are cooled (*e.g.* to –5° to –10°C.) until ice forms in the blood vessels, and are then warmed and decompressed, bubbles form in the absence of exercise. Crystallization of water in the body thus forms nuclei or even small bubbles that persist. If only one foot is frozen, bubbles originate in the frozen foot. In some cases visible bubbles were observed in thawed feet at sea level (*i.e.* without decompression). When frog's blood is partly frozen in test tubes or in tied off sections of veins, bubbles will appear on decompression in the absence of mechanical agitation. The practical relation of this phenomenon to flight at high altitude should not be overlooked.

4. Fracturing a leg bone (tibia or femur) in a frog induces bubble formation on subsequent decompression. Bubble nuclei, which persist for $\frac{1}{2}$ to 1 hour, are probably formed as a result of the intense mechanical disturbance when the bone snaps. Fracturing of bone is considerably more effective than crushing muscles for producing bubbles in frogs.

REFERENCES

- Dean, R. B., The formation of bubbles, *J. Appl. Physics*, 1944, **15**, 446.
Harris, M., Berg, W. E., Whitaker, D. M., Twitty, V. C., and Blinks, L. R., Carbon dioxide as a facilitating agent in the initiation and growth of bubbles in animals decompressed to simulated altitudes, *J. Gen. Physiol.*, 1945, **28**, 225.
Harris, M., Berg, W. E., Whitaker, D. M., and Twitty, V. C., The relation of exercise to bubble formation in animals decompressed to sea level from high barometric pressures, *J. Gen. Physiol.*, 1945, **28**, 241.
Whitaker, D. M., Blinks, L. R., Berg, W. E., Twitty, V. C., and Harris, M., Muscular activity and bubble formation in animals decompressed to simulated altitudes, *J. Gen. Physiol.*, 1945, **28**, 213.