ministration of the drug was continued for 2 days. No difference in distribution between cells and plasma could be detected on the second as compared with the first day.

A similar comparison of the acetylated fractions of both drugs of the same patient showed an average ratio of 0.755 ± 0.30 of acetylated sulfathiazole in whole blood to that in serum while that of sulfapyridine was 0.973 ± 0.48. However, this difference is not statistically significant. Low concentrations of acetylated drug made the error of analysis appreciable and the failure to demonstrate a difference may be explained by this fact.

Sulfathiazole treated patients have shown considerably lower concentrations of drug in whole blood than patients treated with equal amounts of sulfapyridine. This difference between the drugs is explained in part by the smaller amount of sulfathiazole present in blood cells. The use of plasma or serum for relating drug concentration to therapeutic activity thus is to be preferred.

Our observations are in accord with those of Sadusk, Blake, and Seymour[4] who found in cerebrospinal fluid less sulfathiazole than sulfapyridine when equivalent amounts of drug were administered. It appears, therefore, that other types of cells besides red blood cells are likewise less permeable to sulfathiazole than to sulfapyridine.

Summary. Sulfathiazole is present in human red blood cells in lower concentrations than is sulfapyridine employed under identical conditions.

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Carbon Dioxide Tension and its Relation to the Quiescence of Spermatozoa *in vivo*.

L. B. SHETTLES.* (Introduced by N. J. Eastman.)

From the Department of Obstetrics, the Johns Hopkins University and Hospital.

It is generally agreed (Hartman,[1]) that spermatozoa are immotile within the male generative tract. Hartman found the tubules of

[4] Sadusk, J. F., Jr., Blake, F. G., and Seymour, A., *Yale J. Biol. Med.*, 1940, **12**, 681.

* Fellow, National Committee on Maternal Health.

[1] Hartman, C. G., Ovulation, fertilization and the transport and viability of eggs and spermatozoa, Chapter IX in Allen's *Sex and Internal Secretions*, 1939, p. 630.

the cauda epididymidis of the bat filled with spermatozoa during the winter, but he could observe no movement by means of transmitted light. It is his opinion that when spermatozoa are massed, *e.g.,* in the cauda epididymidis, carbon dioxide production maintains the environment on the acid side, thus immobilizing them.

Roemmele,[2] Belnoschkin,[3] and Granzow,[4] however believe that spermatozoan motility commences in the testis and progressively increases in the excretory tubules. Redenz[5] reports that the secretion of the rete tubules is rich in electrolytes which are favorable for motility, but that the epididymis contains a colloidal suspension which is poor in electrolytes and unfavorable for motility.

In 1940, the present writer[6] found that, *in vitro,* carbon dioxide produces complete immotility of human spermatozoa within a few minutes, the exact time varying with the donor, the freshness of the specimen, the frequency of ejaculation, and certain other factors as yet unknown. The effect of this gas was due neither to its acid character nor to the anoxia produced by it. Motility of the cells was restored when the carbon dioxide was replaced by nitrogen, air, or oxygen immediately after all movement had ceased. However, fewer and fewer cells were capable of restoration of motility after each additional exposure to carbon dioxide.

The present study deals with the question: are high carbon dioxide tissue tensions the actual cause of the quiescence which spermatozoa exhibit *in vivo?* We determined: (1) The carbon dioxide content of whole fresh testis, epididymis, vas deferens, liver, and skeletal muscle of the rat. (2) The concentration of carbon dioxide required to immobilize spermatozoa *in vitro.* (3) The toxicity of carbon dioxide in varying concentrations. (4) The length of time which spermatozoa can be immobilized by carbon dioxide and yet be resuscitated later.

The method of Danielson and Hastings[7] was used for the determination of tissue carbon dioxide. The tissue studied was placed in a side tube connected to a Van Slyke apparatus within 30 seconds after the dissection was begun. The carbon dioxide was liberated by the addition of acid and the application of heat at reduced pressure. The carbon dioxide produced was absorbed by alkali in the reaction chamber. The technic used with blood by Van Slyke and

2 Roemmele, O., *Zool. Jahrb. Abt. allg. Zool. u. Physiol.,* 1927, **44**, 85.

3 Belnoschkin, B., *Z. f. Biol.,* 1932, **92**, 542.

4 Granzow, J., *Arch. f. Gynäk.,* 1932, **198**, 149.

5 Redenz, *Bioch. Ztschr.,* **257**, 234.

6 Shettles, L. B., *Am. J. Physiol.,* 1940, **128**, 408.

7 Danielson, I. S., and Hastings, A. B., *J. Biol. Chem.,* 1939, **130**, 349.

TABLE I.
CO_2 Content of Various Tissues of the Rat in mM per Kilo.

Animal No.	Liver	Muscle	Testis	Epididymis	Vas deferens
1	16.0	11.2	6.7	5.7	7.5
2	15.8	10.8	3.5	6.3	4.7
3	18.7	9.2	6.4	4.9	4.5
4	18.0	12.9	5.0	5.8	6.4
5	13.3	10.5	3.9	6.0	8.0
6	17.0	11.4	6.6	4.8	9.3
7	15.9	13.9	4.1	5.9	8.7
8	17.5	10.7	2.9	8.3	6.6
9	16.8	14.0	7.3	7.3	5.6
10	14.3	13.9	5.8	8.0	9.8
Mean	16.5	11.9	5.2	6.3	7.1
Standard error	±2.7	±2.7	±2.2	±1.3	±3.1

Neill[8] was then employed to determine the carbon dioxide contained in the alkali. A ferric fluoride solution (1 volume of 4% NaF + 1 volume of 10% $Fe_2(SO_4)_3$) was used to prevent further production of carbon dioxide by enzymatic action. The results obtained are given in Table 1.

The average carbon dioxide content of the liver, muscle, testis, epididymis, and vas deferens was 16.5, 11.9, 5.2, 6.3, and 7.1 mM per kg, respectively. The standard error ranged from 1.3 to 3.1. The content of the gas in the liver was over 3 times as great as in the testis and over twice as great as in either epididymis or vas deferens. Also, the carbon dioxide tension of skeletal muscle was over twice as great as that of the testis and considerably greater than that of the epididymis or vas deferens. The relatively low carbon dioxide content of testis, epididymis, and vas deferens will become of more interest when it is compared with the *in vitro* concentration of the gas required for immobilization. It will also be noted that of the generative organs the carbon dioxide content of the vas deferens was greatest, the epididymis less, and the testis least.

The *in vitro* effect of carbon dioxide on spermatozoa was studied in the following manner: Fluid from the vas deferens and physiological saline solution were mixed in equal volume. A hanging drop of the mixture with constant volume was placed on a cover glass. This glass was sealed with vaseline on a small gas chamber. The chamber was provided with an inlet and outlet so that a known mixture of carbon dioxide and oxygen could be passed through. This mixture was obtained by means of flow meters. Various con-

8 Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

centrations of carbon dioxide were tried until a threshold for immobilization of the spermatozoa was obtained. The spermatozoa were kept quiescent for various lengths of time in different concentrations of the gas. Then pure oxygen was passed through the compartment of the chamber and any resuscitation noted. Control tests were made in the same way without carbon dioxide. These experiments were conducted at room temperature. The results are presented in Tables II and III.

Table II shows that the concentration of carbon dioxide necessary for the immobilization of spermatozoa in a carbon dioxide-oxygen

TABLE II.
In Vitro Concentration of CO_2 Required for Immobilization of Spermatozoa.
Immobilization, +; no immobilization, —

	Concentration of CO_2 in carbon dioxide-oxygen mixture in %			
Animal No.	15	18	20	25
1	—	—	+	+
2	—	+	—	+
3	—	—	+	+
4	—	—	+	+
5	—	+	—	+
6	—	—	+	+
7	—	+	+	+
8	—	—	—	+
9	—	—	+	+
10	—	+	+	+
% immobilized	0	40	70	100

TABLE III.
Relation Between Concentration of Carbon Dioxide, Time of Immobilization and Revival of Spermatozoa with Pure Oxygen.
Resuscitation, +; no resuscitation, —.

	Concentration of CO_2 in carbon dioxide-oxygen mixture in %											
	20				60				100			
	Time of immobilization in minutes											
Animal No.	3	5	10	15	3	5	10	15	3	5	10	15
1	+	+	+	+	+	+	+	—	+	—	—	—
2	+	+	+	—	+	+	+	—	+	+	—	—
3	+	+	+	—	+	+	+	—	+	+	—	—
4	+	+	+	+	+	+	+	—	+	—	—	—
5	+	+	+	—	+	+	+	—	+	+	—	—
6	+	—	—	—	—	—	—	—	—	—	—	—
7	+	+	+	+	+	+	—	—	—	—	—	—
8	+	+	+	+	+	—	—	—	+	+	—	—
9	+	+	+	+	+	+	+	—	+	—	—	—
10	+	+	—	—	+	—	—	—	+	—	—	—
% revived	100	90	80	50	90	70	60	0	80	40	0	0

mixture is approximately 20%. Campbell[9] reports that the subcutaneous carbon dioxide tension for the rat and guinea pig is approximately 7%. When the carbon dioxide content of the various portions of the generative tract are compared with this *in vitro* concentration of 20%, the former concentrations appear too low to produce quiescence.

Table III demonstrates that the per cent of spermatozoa revived depends upon the length of time that they were immobilized, and also the concentration of the carbon dioxide. Moreover, all concentrations of the gas producing immotility, even the minimum, proved toxic provided the spermatozoa were exposed long enough. If spermatozoa are non-motile *in vivo,* factors other than carbon dioxide must be responsible.

The fact that motility is usually absent after 24 hours *in vitro* exposure at room temperature, indicates that some unusual factors allowing for survival must be present in the intact generative tract. A sample of seminal fluid collected after a week has passed without emission, must contain spermatozoa which are already several days old. And yet there are no significant differences in the length of *in vitro* spermatozoan survival in specimens collected at daily or weekly intervals. Whether the condition within the generative tract which preserves spermatozoa is associated with their immotility is still unknown.

Summary. There is conflicting opinion concerning the motility of spermatozoa within the generative tract. It has been suggested that a high local tension of carbon dioxide prevents them from becoming motile in the epididymis and vas deferens. The carbon dioxide concentration of various tissues of the rat was studied and found even lower in the generative organs than in liver or skeletal muscle. Furthermore, the concentration of carbon dioxide found in the tissues of the generative tract was much lower than that which is required to immobilize spermatozoa *in vitro.*

[9] Campbell, J. A., *Physiol. Rev.,* 1931, **11**, 1.