tected" (B) titrimetric assay methods (*6*) also respond in a like manner, distinct from vitamin $B_{12}$. The term "unprotected" indicates that the medium is not protected with reductants (Table 1).

TABLE 1

| Sample | Units/μg A* | Units/μg B† |
|---|---|---|
| Thiocyanate analog | 2,300 | 6,200 |
| Vitamin $B_{12a}$ | 1,800 | 6,800 |
| Vitamin $B_{12}$ | 11,000 | 11,000 |

* *L. lactis.*
† *L. leichmannii* "unprotected" titrimetric assay.

Bioassays in rats reveal the same order of activity as vitamin $B_{12}$. Acute toxicity tests on mice failed to reveal any detectable toxicity at the equivalent level of 3.2 mg of the analog for a 70-kg man. Preliminary clinical reports have indicated that the thiocyanate analog of vitamin $B_{12}$ is fully active for pernicious anemia.

The ultraviolet absorption spectrum of the thiocyanate analog is practically identical with that of vitamin $B_{12a}$ from 6,000 to 2,200A (Table 2).

TABLE 2

| | E% at 3,520A | E% at 5,250A |
|---|---|---|
| Thiocyanate analog | 174 | 61 |
| Vitamin $B_{12a}$ | 174 | 59 |

The thiocyanate analog shows an absorption band in the infrared at 4.70 μ characteristic of thiocyanate compounds. Similarly, vitamin $B_{12}$ shows an absorption band at 4.60 μ characteristic of the cyano grouping. Vitamin $B_{12a}$, on the other hand, shows no absorption bands in the 4–5 μ region (*7*).

TABLE 3

| Distribution coefficient | Benzyl alcohol / water |
|---|---|
| Thiocyanate analog | 1.66 |
| Vitamin $B_{12}$ | 0.84 |
| Vitamin $B_{12a}$ | 0.13 |

Craig countercurrent studies of the thiocyanate analog show that our material is homogeneous and of high purity. The distribution curve is theoretical with the maxima in the fourth tube of an 8-tube study.

The thiocyanate analog is hygroscopic like vitamin $B_{12}$ and vitamin $B_{12a}$. The analog is not compatible with ascorbic acid at the level of 20 μg/ml of the analog to 20 mg/ml of ascorbic acid. Decolorization occurs within 24 hr. This is analogous to the observed behavior of vitamin $B_{12a}$, which also reacts with ascorbic acid, and in contradistinction to that of vitamin $B_{12}$ (*8*).

The properties of the thiocyanate analog of vitamin $B_{12}$ are shared in part by both vitamin $B_{12}$ and vitamin $B_{12a}$ (Table 3). Thus in the ultraviolet and in its reaction with ascorbic acid it resembles vitamin $B_{12a}$, whereas in the 4- to 5-μ region of the infrared and in its distribution behavior in the benzyl alcohol–water system it resembles vitamin $B_{12}$. Therefore, in the characterization of a related unknown, care must be exercised to examine as many properties as possible before any reasonably certain conclusions can be drawn regarding its relationship to known vitamin $B_{12}$ analogs.

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## Pulmonary Edema and Hemorrhage Induced by Hypothalamic Lesions in Rats[1]


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Pulmonary edema, often fatal, is a puzzling complication in a wide variety of clinical conditions. These include not only cardiovascular and pulmonary diseases but also allergy, thyroid crisis, beriberi, cirrhosis and degeneration of the liver, carcinoma, blood dyscrasias, septicemia, drowning, shock, heat stroke, head injuries, and brain tumors. In experimental animals, pulmonary hemorrhage and edema are reported to follow such seemingly unrelated and nonspecific procedures as epinephrine injection (*1*), thiourea poisoning (*2, 3*), feeding ammonium salts (*4*), insulin shock (*5*), cerebral concussion (*6*), increased intracranial pressure (*7*), intracarotid saline infusions (*8*), cisternal injection of veratrin (*9*), bilateral vagotomy (*10*), ligation of the aorta or compression of the left ventricle (*11*), positive pressure respiration (*12*), hyperthermia plus positive pressure respiration (*13*), and war-gas poisoning (*14*). Although the diversity of the clinical and experimental states leading to pulmonary edema suggests multiple causative mechanisms, a neural mechanism is thought by some to be the common denominator of some types of lung edema. The evidence is mostly indirect, since it is largely based upon the protective effect of autonomic blocking agents, narcotics, or surgical attacks on the autonomic nervous system. There is little uncontested evidence of pulmonary edema produced by peripheral or central neural lesions. The following experiments demonstrate that such a "neurogenic"


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TABLE 1

| Group | No. animals | Body wt (g) | Wet lung wt (g) | $\frac{WLW}{BW} \times 100$ | Dry lung wt (g) | $\frac{DLW}{BW} \times 100$ | Lung fluid (%) |
|---|---|---|---|---|---|---|---|
| Acute edema | 18 | 312.0 ± 15.5 | 3.311 ± 0.229 | 1.01 ± 0.06 | 0.447 ± 0.053* | 0.176 ± 0.005* | 82.04 ± 1.42* |
| Subacute edema | 15 | 332.2 ± 20.0 | 2.210 ± 0.137 | 0.68 ± 0.04 | 0.459 ± 0.055† | 0.140 ± 0.015† | 79.32 ± 0.60† |
| Operation control | 72 | 345.5 ± 7.7 | 1.500 ± 0.038 | 0.44 ± 0.007 | 0.334 ± 0.011‡ | 0.097 ± 0.002‡ | 78.10 ± 0.18‡ |
| Hyperthermia operative | 13 | 318.4 ± 14.6 | 1.694 ± 0.138 | 0.53 ± 0.03 | 0.348 ± 0.220* | 0.110 ± 0.004* | 78.28 ± 0.67* |
| Diathermy | 10 | 305.1 ± 6.5 | 1.466 ± 0.108 | 0.49 ± 0.04§ | 0.325 ± 0.017 | 0.107 ± 0.006§ | 77.53 ± 0.63 |

WLW = wet lung weight; BW = body weight; DLW = dry lung weight.
* Data on 5 animals. ‡ Data on 50 animals.
† Data on 7 animals. § Data on 8 animals.

edema can be produced in rats by restricted lesions of the brain.

In rats anesthetized with Evipal, electrolytic lesions were placed in the rostral hypothalamus with the Horsley-Clarke stereotaxic instrument. The animals were either observed until they died from the operation or were sacrificed 24 hr postoperatively. The lungs were examined macroscopically, weighed, and either sectioned for histological examination or desiccated to determine the dry lung weight.

Eighteen rats died in less than 24 hr after placement of rostral hypothalamic lesions (acute edema group), and pulmonary pathology was obvious in 15 others sacrificed 24 hr postoperatively (subacute edema group). In addition, lung and body weights were recorded for 72 other rats sacrificed after scattered hypothalamic lesions that did not produce pulmonary pathology. The data for this group (operation control group) serve as controls for the other groups. Hyperthermia, a well-known consequence of rostral hypothalamic lesions, developed in 13 operated animals. The data from this group (operative hyperthermia group) served to control the possibility that hyperthermia causes or contributes to the edema. As a further check, 10 rats were artificially rendered hyperthermic by diathermy. The mean data and their standard errors for all five groups appear in Table 1.

The animals of the acute edema group died 30 min–24 hr after operation. Fourteen died in less than 8 hr; 3 died at an undetermined time during the night; only one survived 24 hr. After recovering from the anesthesia, these animals displayed normal or hyperactive cage behavior and normal respiration for varying periods of time. Some quickening of respiration was usually observed just prior to death, and this rapidly developed into a severe respiratory crisis that shortly terminated in death. In the final stages, there was usually a copious flow of pink-tinged, frothy fluid from the mouth and nostrils. The final crisis usually developed explosively. Animals outwardly normal for several hours after operation suddenly developed tachypnea and dyspnea and died in a few minutes.

At autopsy the lungs were dark-red and appeared swollen; the cut surface exuded fluid. Usually all lobes were involved, but occasionally the discoloration was distributed irregularly. A pleural effusion of 1.5–2.4 ml was found in 7 animals; analysis in one instance revealed a total protein content of 4.2 g/100 ml. Fractional analysis gave values of 3.05 g/100 ml for albumin and 1.53 g/100 ml for globulin.

Table 1 shows that the lung weights of these animals were greatly increased, the mean lung weight/body weight ratio being 2.3 times that of the operation control group. The table also shows that the increased lung weight in the edematous animals was due to accumulation of both fluids and solids, since both the percentage of fluid and the dry lung weight/body weight ratio are significantly higher than in the operation control group. The increased fluid content suggests that increased lung weight is not entirely due to accumulation of whole blood (hemorrhage, congestion), for the fluid content of whole blood is not significantly different from that of the lungs. Only accumulation of a dilute transudate such as plasma, which contains a higher proportion of water than lung tissue does, could account for the increased percentage of lung fluid.

The histological appearance of the lungs of 12 animals in the acute edema group amply confirmed this conclusion. Without exception, severe hemorrhage and edema were found. The perivascular spaces were distended with erythrocytes and/or fluid. The alveoli were similarly distended, with large amounts of fibrous or granular transudate and often erythrocytes.

Table 1 also lists the data on 15 animals classified in the subacute edema group. These rats survived operation and were sacrificed 24 hr later by carotid exsanguination. Most of these animals were depressed and dyspneic at the time of sacrifice and probably would have died if the postoperative observation period had been prolonged. In all instances, however, the depression or dyspnea was preceded by postoperative periods of normal or hyperactive behavior and normal respiration. Macroscopically, the lungs were hemorrhagically discolored, but the distribution of lesions was more often spotty than in the acute edema group. The ratios of both wet and dry lung weights to body weight were significantly elevated over those of the operation control group. The mean percentage of fluid in the lungs was slightly greater than that in the control group, but the difference was not statistically significant. This suggests that the increased weight of the lungs was due largely to an accumulation of whole blood (hemorrhage, congestion) rather than a cell-free transudate, which would have diluted the lung tissue.

Again, this was confirmed by histological examination of the lungs. The alveolar and perivascular transudate was predominantly cellular and therefore differed from that in the acute edema group. Frank hemorrhage was the rule, and transudates, if present, were generally sprinkled with erythrocytes. Since hemorrhage occurred in both groups, it seems not unlikely that it is the initial event. Transudation, when it occurs, may be a secondary event, depending on the degree of respiratory obstruction and capillary asphyxia induced by hemorrhage. This hypothesis requires further study.

The data on the hyperthermic rats were collected to test the hypothesis that fever, induced by the hypothalamic lesions, causes the pulmonary changes. Table 1 contains data on 13 rats with postoperative records of elevated body temperatures ranging from 40.6° to 42.7° C; 9 of these animals died, apparently as a result of the fever. In addition, 10 animals were heated by diathermy to body temperatures of 40°–44.2° C; 7 rats died. Although the lungs of these rats showed mild, uniform discoloration, they were clearly distinguishable from those of the edematous animals. Histologically, the lungs appeared normal save for some congestion; the hemorrhage and transudate characteristic of the edematous groups were conspicuously absent. The lung weights of the hyperthermic animals were only slightly elevated over those of the control animals (Table 1). On the other hand, severe edema and hemorrhage occurred in 3 rats in which the postoperative body temperatures never exceeded 38.4° C, 37.3° C, and 38.0° C, respectively. Thus, hyperthermia is not essential to the development of pulmonary edema and hemorrhage following hypothalamic lesions.

To identify the hypothalamic region, destruction of which caused the lung edema and hemorrhage, the brains of 30 of the 33 edematous animals were sectioned serially. In addition, serial sections were cut from 26 brains of the operation control group. These brains were selected because macroscopic examination suggested that the lesions bordered the critical region. In 26 of the edematous animals there was bilateral damage to the region just overlying the rostral half of the optic chiasm. The center of the lesions was usually a millimeter from the midline and dorsal to the rostral third of the chiasm. The lesions thus occupied what Gurdjian (*15*) has termed the "preoptic region." In 4 other edematous animals the lesions were more caudally placed and were asymmetrical. In each instance, however, one needle puncture had penetrated the midline and bilaterally damaged the periventricular system. This suggests that the executant cell bodies or fibers in their caudal course occupy the region around the ventricle. The localization of the lesions in the control animals demonstrated that neither bilateral damage bordering the preoptic region nor unilateral damage to the preoptic region produced pulmonary edema.

Although the phenomenon described is by definition neurogenic hemorrhagic edema, whether it is neurogenic in the sense that the action is exerted on pulmonary vessels as opposed to the heart remains to be determined. That restricted lesions of the hypothalamus can cause such edema holds the promise that some of the nonspecific edemagenic procedures such as cerebral concussion may be given a unitary explanation.

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## Physiological Availability of Dehydro-L-Ascorbic Acid and Palmitoyl-L-Ascorbic Acid[1]


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Previous studies of the biological activity of dehydro-L-ascorbic acid (DHAA) involved the use of solutions prepared by oxidation of natural or synthetic L-ascorbic acid (AA). The criteria for assessment of potency in guinea pig tests included cure of scurvy (*1–4*), weight gain (*3*), and increase in serum alkaline phosphatase (*5*). Johnson and Zilva (*6*) and Todhunter *et al.* (*7*) determined urinary excretions after oral dosage to humans. The relative activity of DHAA as compared to AA ranged from 80 to 100% in these tests. Recently, crystalline DHAA has been prepared by Pecherer (*8*) by a modification of the method of Kenyon and Munro (*9*) and made available to us; it was of interest to determine the activity of the pure compound in urinary excretion tests in humans similar to those described by Melnick *et al.* (*10*).

Seven male subjects participating in these tests consumed a self-selected diet, but followed a parallel diet on the 2 consecutive urine-collection days, particularly as regards foods high in AA. Since the diets were not standardized from week to week, considerable

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