

This work suggests that micoina and Terramycin interfere with distinct metabolic mechanisms of *Br. abortus* and *Br. suis*.

References

1. VANSELOW, H. J. Personal communication.
2. VON KENNEL, J., KIMMIG, J., and LEMBKE, A. *Klin. Wochsch.*, **16**, 321 (1943).
3. LEMBKE, A., and FRAHM, H. *Zentr. Bakt.*, **152**, 221 (1947).
4. LEMBKE, A., and KÖRNLEIN, M. *Ibid.*, **152**, 231 (1947).
5. LEMBKE, A., KÖRNLEIN, M., and FRAHM, H. *Ibid.*, **155**, 16 (1950).
6. BARON, F. *et al. Bull. soc. chim. France*, **18**, 526 (1951).
7. DAMADE, *et al. Le Concours Médical*, 915 (1951).
8. ROSSI, P. *Rec. méd. vét. Alfort*, **127**, 26 (1951).
9. ROSSI, P., and BRUYÈRE, A. *Bull. acad. vét. France*, **23**, 443 (1950).
10. ROSSI, P. Unpublished data.
11. CRISCUOLO, E., *et al. El Dia Medico*, **23**, 1 (1951).

Manuscript received June 12, 1953.

Observations on the Chemical Constitution of Inflammatory Exudate in Normal and Hypophysectomized Rats¹

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Chemical data on the inflammatory exudates, though numerous, are conflicting because it is difficult to induce exudate formation in animals, in an exactly reproducible manner, and avoid general systemic stress. Usually inflammatory exudate is obtained after inflammation has been caused by the injection of irritants into large body cavities. Animals that survive such treatment are ill and stressed. This technique can be applied only with difficulty to hypophysectomized animals whose resistance to stress is particularly low. The extent to which stress influences, directly or through humoral substances, the course of inflammation has been clearly demonstrated in numerous publications (1). It is evident therefore that we should attempt to learn more about the fundamental development of inflammation when it is free from interfering factors.

A method was recently devised (1-3) that overcomes most of the difficulties. This method, the "granuloma pouch" technique is applicable to small laboratory animals. Inflammation can be initiated in a reproducible and quantitative manner and is well tolerated even by hypophysectomized rats; therefore, it is relatively stress free, yet it is responsive to humoral influences (3). The exudate is abundant, homogeneous, and well delimited by the inflammatory granulomatous tissue.

Two groups of male Sprague-Dawley rats were used. Group I consisted of 6 normal rats and group II of 5 rats that had been hypophysectomized 120 days prior to the initiation of inflammation. Inflam-

¹ This work was supported in part by the Medical Research and Development Board, Office of The Surgeon General, Department of the Army, Contract No. Da-49-007-Md 125.

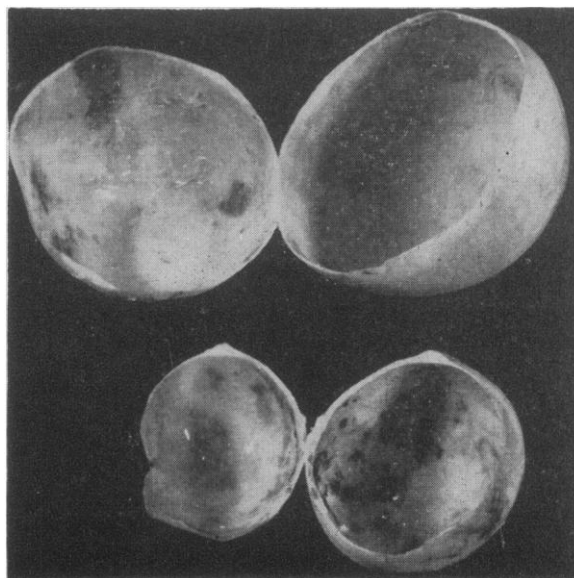


Fig. 1. (Upper) Granuloma pouch in a control rat. (Lower) Granuloma pouch in a hypophysectomized rat.

mation was obtained in both groups by injecting 25 ml of air under the dorsal skin, followed by 1 ml of a 1% solution of croton oil (irritant) in Mazola oil (vehicle). Thus an *in vivo* ampulla was formed. Fourteen days later, it had a granulomatous wall (Fig. 1) and was filled with a hemorrhagic exudate. At this stage, the rats were killed, the exudate was collected and analyzed. The completeness of hypophysectomy was carefully verified.

The pH of the exudate was measured on a Beckman pH meter, model G, and the sodium and potassium on the Beckman flame spectrophotometer, model DU. The sugars were determined by the Folin and Malmros micromethod (4), the NPN (nonprotein nitrogen) by Folin and Wu (5), the inorganic phosphates by Fiske and Subbarow (6), chlorides by Van Slyke (7), and iron by Kennedy's method (8). The total proteins were determined by an adaptation of the micro-Kjeldahl procedure (9). The total fat was estimated by the following gravimetric method. Five milliliters of the exudate were adsorbed on Whatman filter paper No. 40 and dried in an oven at 75° C for 48 hr. The filter papers were then folded, weighed to constant weight, and extracted in a Soxhlet apparatus under reflux with 3:1, ethanol: ethyl ether mixture for 8 hr. The filter papers were then removed from the Soxhlet, again dried in an oven at 75° C for 48 hr, and reweighed to constant weight. The loss of weight of the filter papers during extraction was equal to the total ether-soluble fraction (fatty substances) of the exudate. Table 1 summarizes the results obtained.

The rats of the control group gained an overall 81 g in body weight during the 14 days the experiment lasted. The gain was due to normal somatic growth and to the weight of the granuloma pouch.

TABLE 1
CHEMICAL ANALYSIS OF THE EXUDATE

Group	No. of rats	Body weight of initial, g	Body weight final, g	Exudate, ml	Granu- loma wall, g	pH	Total reducing sugars, mg %	Total fat, mg %	Total proteins, g %	NPN, mg %	Total Cl meq/l	Total inorg. P, mg %	Total Na, meq/l	Total K, meq/l	Total Fe, mg %
I Control	6														
m	—	132.33	213.00 ± 6.30	28.00 ± 2.02	3.135 ± 0.344	7.53 ± 0.07	5.67 ± 3.09	85.00 ± 31.39	5.41 ± 0.28	41.44 ± 2.58	90.16 ± 2.43	8.94 ± 0.34	146.20 ± 1.35	6.34 ± 0.24	8.43 ± 1.66
± S.E.															
II Hypophy- sectomized	5														
m	—	130.00	145.00 ± 6.52	14.80 ± 1.37	2.026 ± 0.073	7.66 ± 0.05	61.80 ± 4.68	1030.00 ± 86.95	6.12 ± 0.31	64.16 ± 5.58	92.68 ± 0.56	6.14 ± 0.29	155.40 ± 2.27	9.78 ± 0.77	9.36 ± 1.22
± S.E.															
P	—	—	—	<0.01	<0.01	>0.1	<0.01	<0.01	<0.01	<0.01	>0.3	<0.01	<0.01	<0.01	>0.6

The hypophysectomized rats gained an overall 15 g in body weight in 14 days. This apparent gain in body weight was due solely to the development of inflammation.

A considerable amount of exudate was formed in rats of both groups. However, the control rats formed about twice as much exudate as the hypophysectomized animals. The chemical composition of the exudate varied little from one animal to the other within each group, but there were marked differences between the composition of the exudates from the control rats and from the hypophysectomized animals. Thus, the exudate from the control animals contained traces of total reducing sugars, little total fat, and less protein than the exudate from the hypophysectomized rats. The exudate of the latter, on the other hand, contained appreciable amounts of total reducing sugars, high amounts of total fat, and more inorganic phosphates than the exudate from the control rats. The differences are statistically significant.

It may be concluded: (1) The granuloma pouch technique permits the production of inflammation with large amounts of exudate in the rat. (2) This technique apparently does not cause much systemic stress since it is well tolerated by hypophysectomized animals. (3) The inflammatory exudate shows a high degree of constancy in its main chemical constituents within the control group and within the hypophysectomized group of rats. (4) Hypophysectomy decreases the volume and alters the chemical constitution of the inflammatory exudate.

References

1. SELYE, H., and HORAVA, A. *Second Annual Report on Stress*. Montreal: Acta Inc. (1952).
2. SELYE, H. In G. Pincus, Ed., *Recent Progress in Hormone Research*, 8, 117. New York: Academic Press (1953).
3. SELYE, H. *Proc. Soc. Exptl. Biol. Med.*, **82**, 328 (1953).
4. FOLIN, O., and MALMROS, H. *J. Biol. Chem.*, **83**, 115 (1929).
5. FOLIN, O., and WU, H. *Ibid.*, **89**, 81 (1919).
6. FISKE, C. H., and SUBBAROW, Y. *Ibid.*, **66**, 375 (1925).
7. VAN SLYKE, D. D. *Ibid.*, **38**, 523 (1923).
8. KENNEDY, R. P. *Ibid.*, **74**, 385 (1927).
9. HAWKE, P. B., et al. *Practical Physiological Chemistry*, 12th ed., p. 547. Philadelphia and Toronto: Blakiston (1949).

Manuscript received May 18, 1953.

Demonstration of Glycogen Synthesis by Rat Kidney Slices *in vitro*¹

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Glycogen accumulates in the cells of the kidney tubules in diabetes mellitus (1, 2). Whereas the concentration of glycogen in the kidney as a whole is low (approx. 0.04%), it is found mostly in the cells of the proximal convoluted tubules and the loop of Henle. These cells must therefore possess a relatively

¹ This work has been supported by the George W. Raiziss Research Fund in Biochemistry.