

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

Julian B. Marsh and Karen L. Miller

Department of Physiological Chemistry,
Graduate School of Medicine,
University of Pennsylvania, Philadelphia

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.

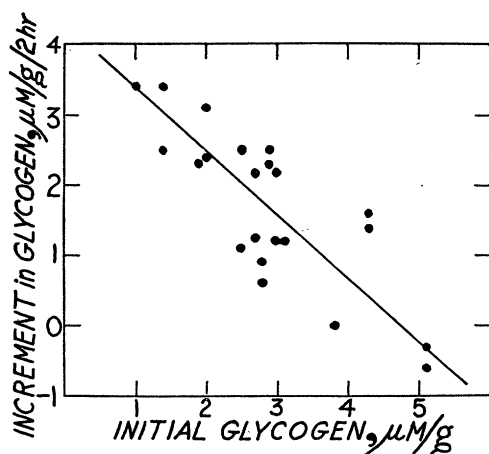


FIG. 1. Relationship of glycogen synthesis to initial glycogen content in rat kidney slices.

high glycogen content, but the physiological significance of this is not evident. The present experiments show that kidney slices are capable of synthesizing glycogen from glucose *in vitro*.

Approximately 10 slices, 0.5 mm thick, were obtained from each kidney of 150–200 g male albino rats. The slices were placed on filter paper moistened with buffer in a Petri dish set in a tray of ice. Three to five slices, chosen at random, were weighed on a torsion balance and placed in Warburg vessels containing 2.0 ml of medium and 0.2 ml of 5.4% glucose. The vessels were gassed for 3 min with a 95% O₂–5% CO₂ gas mixture and incubated for 2 hr at 37°. At the end of the incubation period, the entire contents of the vessel were transferred to a centrifuge tube and 1 ml of 80% NaOH added. Glycogen was determined by a modification (3) of the method of Good, Kramer, and Somogyi (4), using the method of Nelson (5) for the final determination of glucose equivalents.

In 23 experiments, the mean initial glycogen content was 2.4 ± 0.21 μM (as glucose) per g wet weight. The increment in glycogen after incubation for 2 hr was 1.6 ± 0.44 $\mu\text{M/g}$, demonstrating effective glycogen synthesis by kidney tissue. It was found that the synthesis of glycogen was inversely related to the initial glycogen content (Fig. 1). The coefficient of correlation for this relationship was 0.82 ± 0.071 . The significance of this correlation remains to be determined.

Optimal synthesis of glycogen was achieved with a medium of the following ionic composition, expressed in mM/l: K⁺, 110; Mg⁺⁺, 20 (or Ca⁺⁺, 10); HCO₃⁻, 40; CL⁻, 110; pH = 7.35. Sodium or phosphate ions were inhibitory. Except for the lack of a sharp pH optimum, these *in vitro* conditions are virtually identical with those found by Buchanan, Hastings, and Nesbitt (6) for rat liver slices, and contrast sharply with the optimal conditions for glycogen synthesis in muscle (7, 8). Further studies of the relationship of inorganic ions to carbohydrate metabolism in kidney tissue should be especially interesting in view of the recent work of Whittam and Davies (9) which indicates that metabolic energy is necessary for the maintenance of Na⁺ and K⁺ gradients in kidney slices.

References

1. EHRLICH, P. *Z. klin. Med.*, **6**, 33 (1883).
2. ROBBINS, S. L. *Am. J. Med. Sci.*, **219**, 376 (1950).
3. STADIE, W. C., HAUGAARD, N., and MARSH, J. B. *J. Biol. Chem.*, **188**, 167 (1951).
4. GOOD, C. A., KRAMER, H., and SOMOGYI, M. *Ibid.*, **100**, 485 (1933).
5. NELSON, N. *Ibid.*, **153**, 375 (1944).
6. BUCHANAN, J. M., HASTINGS, A. B., and NESBITT, F. B. *Ibid.*, **180**, 435 (1949).
7. STADIE, W. C., HAUGAARD, N., and PERLMUTTER, M. *Ibid.*, **171**, 419 (1947).
8. STADIE, W. C., and ZAPP, J. A., JR. *Ibid.*, **170**, 55 (1947).
9. WHITTAM, R., and DAVIES, R. E. *Biochem. J. (London)*, **54**, vii (1953).

Manuscript received June 30, 1953.

Comments and Communications

Hope Chest

MAX I propose that a "crackpot pot" be established by foundations and other agencies that grant funds for research?

This could help compensate for such organizations' too common regression toward traditional means in their criteria of research competence and promise. The intimate communication that exists among the various fund-granting groups, amounting at times essentially to interlocking of directorates, inevitably means that special points of view become accepted as orthodox by the makers of grants. In addition, if an individual comes from certain institutions, if he holds certain degrees, if he has certain types of backgrounds, or if

he uses certain methodology, he is among the blessed. When he asks he is likely to receive.

The history of science teems with episodes of outstanding discoveries of science accomplished by non-traditional methods and by *personae non gratae*. Crackpot pots could aid such individuals in this and future generations to get financial support for their creative heterodoxy.

Such pots of gold would consist possibly of no more than one-tenth of the total sum of money to be dispersed by an agency. Unlike the other funds, usually expended on the basis of the majority recommendations or votes of committees, officers, or trustees, these sums could be spent only on recommendation of a minority. And at least one member of the group would