

drates, a relation seems probable between the stimulation in synthesis of starch and the higher level of the phosphorylated sugars in the growing kernels. There is every reason to believe that in corn kernels, as in other plant structures, the enzyme primarily concerned with starch synthesis is phosphorylase, acting on glucose-1-phosphate. As there is some evidence of the common occurrence in plants of the enzymes necessary for the interconversion of the different hexose phosphates, a greater activity of phosphorylase should naturally be considered favorable for starch synthesis.

The close analogy between the effects on carbohydrate metabolism of the hormones naturally released after fertilization and those of the growth regulators artificially applied should be emphasized. This analogy suggests a corresponding similarity in regard to metabolism. The fact that the response following pollination is always somewhat delayed, compared to the response following hormone treatment, seems to indicate that fertilization, which in the corn follows pollination in about 24 hr, rather than pollination, is the process responsible for the maximum production of metabolically active hormones (10).

References

1. MURNEEK, A. E. *Plant Physiol.*, **1**, 3 (1926).
2. MARRE, E., and MURNEEK, A. E. *Ibid.* (in press).
3. MARRE, E. *Boll. soc. ital. biol. sper.*, **25**, 231 (1949).
4. BRITTON, E. J. *Am. J. Botany*, **37**, 345 (1950).
5. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. *Manometric Techniques and Tissue Metabolism*. Minneapolis, Minn.: Burgess (1949).
6. SLEIN, M. W., CORI, G. T., and CORI, C. F. *J. Biol. Chem.*, **186**, 763 (1950).
7. TRUBNER, F., and MURNEEK, A. E. *Science*, **116**, 39 (1952).
8. LARDY, A. H., and WELLMANN, H. *J. Biol. Chem.*, **195**, 215 (1952).
9. HSIANG, T. T. *Plant Physiol.*, **26**, 708 (1951).
10. MURNEEK, A. E. In Folke Skoog, Ed., *Plant Growth Substances*. Madison: Univ. Wis. Press (1951).

Manuscript received October 24, 1952.

Rate of Turnover of Epinephrine in the Adrenal Medulla

Sidney Udenfriend, Jack R. Cooper,¹
Carroll T. Clark, and John E. Baer²

Laboratory of Chemical Pharmacology,
National Heart Institute, National Institutes of Health,
USPHS, U. S. Department of Health,
Education, and Welfare, Bethesda, Maryland

Experiments designed to determine the precursors and intermediates involved in the biosynthesis of epinephrine have yielded the observation that the rate of formation and the normal rate of secretion of adrenal epinephrine are extremely slow. C¹⁴-labeled phenylalanine or tyrosine administered orally or intraperitoneally to rats or rabbits was incorporated into ad-

¹ Research Fellow of the United States Public Health Service, National Institutes of Health.

² Present address: Sharp and Dohme, Inc., Research Division, West Point, Pa.

TABLE 1
INCORPORATION OF C¹⁴ FROM PHENYLALANINE AND
TYROSINE INTO PLASMA PROTEIN TYROSINE
AND ADRENAL EPINEPHRINE OF RATS

Expt.	C ¹⁴ -amino acid administered*	Days	Time after last dose	Specific activity (cpm/μmole)		
				Free plasma tyrosine	Plasma protein tyrosine	Adrenal epinephrine
1	Phenylalanine	1	7 hr	80	34	< 5
2	Tyrosine	1	7 "	110	46	< 5
3	Tyrosine	6	24 "	700	—	280
4	Phenylalanine	6	24 "	930	340	180
5	Tyrosine	6	24 "	600	450	310
6	Phenylalanine	12	20 "	—	—	980
7	Phenylalanine	12	12 days	150	610	420

* 3-C¹⁴-D-L-phenylalanine (2.6×10^6 cpm/μmole) or 2-C¹⁴-D-L-tyrosine (2.0×10^5 cpm/μmole) were administered in doses of 1 mg/day for the number of days indicated.

renal epinephrine much more slowly than into the tyrosine of plasma protein (Table 1). The resulting radioactive adrenal epinephrine disappeared slowly after the administration of the labeled amino acid was discontinued; the half-life in rats was about 9 days. Estimates of half-life were based upon measurements of the specific activity of adrenal epinephrine³ in individual rats which were sacrificed under Evipal anesthesia at various time intervals after discontinuing administration of C¹⁴-phenylalanine (Fig. 1). In three additional experiments adrenal glands were compared in the same rats, the glands being removed one at a time several days apart (Table 2).

In an attempt to stimulate the synthesis of epinephrine, the adrenal glands of rabbits were depleted of epinephrine by the subcutaneous administration of insulin.⁴ Epinephrine was determined in the adrenals of individual rabbits sacrificed at various time intervals following insulin administration. The chemical method used was a modification of the fluorimetric procedure of Lund (1), which can measure epinephrine in the presence of nor-epinephrine and other catecholamines. It was found that about half of the epinephrine which had disappeared from the adrenal glands after insulin administration was restored in 72 hr (Table 3). No measurable quantities of nor-

³ A portion of an adrenal extract was assayed for epinephrine by the procedure of Lund (1). To the rest of the extract a measured quantity of nonisotopic l-epinephrine (about 10 mg) was added as a carrier. The carrier was recrystallized repeatedly until isotopic homogeneity was achieved. In a number of cases the recrystallized carrier was converted to a derivative, iodoadrenochrome (2), with no change in isotopic composition. The quantity of epinephrine in the adrenal extract (m), the quantity of added carrier (M), and the specific activity of the isolated carrier (C_c) permit calculation of the specific activity of the adrenal epinephrine (C_a) according to the equation $C_a = C_c M / m$, derived from isotope dilution principles.

⁴ After insulin administration the blood sugar levels fell to negligible values in 2-3 hr and returned to normal by 8 hr.

epinephrine were found in the glands at any time. In rats also the synthesis of adrenal epinephrine was found to be slow after its depletion by the administration of insulin.

These findings in rats and rabbits are in general agreement with the slow rate of synthesis found by the isotopic experiments. However, they are contrary to the observations of West (3) who, using physiological assay procedures, found that adrenal epinephrine in rabbits fell rapidly after insulin administration.

TABLE 2
COMPARISON OF SPECIFIC ACTIVITIES OF ADRENAL
EPINEPHRINE IN INDIVIDUAL GLANDS
REMOVED SEVERAL DAYS APART

Expt.	Adrenal	Time elapsed after discon- tinuing C^{14} -phenyl- alanine* (days)	Specific activity of adrenal epine- phrine (cpm/ μ mole)	Esti- mated half life (days)
16	Left	3	600	9
	Right	14	260	
17	Left	3	830	12
	Right	10	560	
18	Left	3	940	8
	Right	10	500	

* Rats were given 1 mg of C^{14} -D-L-phenylalanine/day for 12 days.

tion and returned to normal values within 8–10 hr. It is possible that the discrepancy between our findings and those of West lies in some other substance in adrenals which assays as epinephrine by physiological assay. This substance cannot be nor-epinephrine, because the rabbit adrenal gland contains only traces of this compound.

The basal rate of secretion of epinephrine from the adrenal gland may be calculated from the data on its rate of turnover. Assuming a half-life of 9 days in

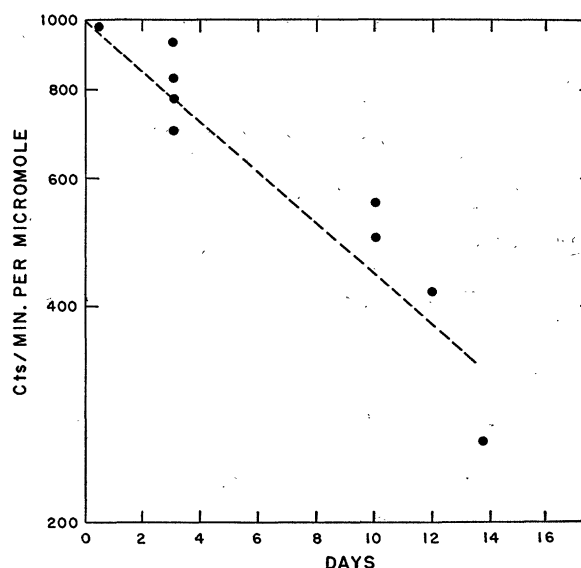


FIG. 1. Radioactive decay of adrenal epinephrine. Measurements were made on adrenals isolated from individual rats at various time intervals after discontinuation of administration of 3- C^{14} -D-L-phenylalanine. The curve was fitted to the data by eye, and it gives an approximation of the rate of radioactive decay.

the rat, a value of 0.002–0.004 μ g/kg of body weight/min is obtained. This value is a maximum one, since it is assumed that epinephrine in the adrenal gland is not chemically altered. The values determined from our isotopic studies are much lower than the basal values of 0.04–0.10 μ g/kg/min reported for dogs (4), but are in fair agreement with the values of 0.007–0.008 calculated from data reported by Vogt (5) for the denervated adrenal gland of the cat. It is conceivable that not all the epinephrine formed in the adrenal gland is pooled with the stored epinephrine before being secreted. A comparison of the specific activity of secreted epinephrine with epinephrine found in storage in the adrenal gland would decide this point. Studies in this laboratory have shown that the epi-

TABLE 3
EFFECT OF INSULIN ON THE CONCENTRATION OF EPINEPHRINE IN RABBIT ADRENAL GLANDS*

	Time in hr after insulin administration†					
	0	2–4	8–11	24	48	72
Micrograms of epinephrine/g of adrenal gland	749	390	113	445	263	640
	1220	210	531	220	370	687
	1010	479	523		800	534
	888	572	82		430	901
	989	342	163		440	
	921	225				
	1107	283				
	833					
	847					
	1400					
Mean $\pm$ standard error of the mean	1026 $\pm$ 61	357 $\pm$ 50	282 $\pm$ 100	333 $\pm$ 109	464 $\pm$ 87	690 $\pm$ 75

* Each value is based on an analysis of the combined adrenal glands of an individual animal. The animals were all males weighing about 2 kg.

† 1.8 units of insulin/kg of body weight were administered subcutaneously. The control animals were injected with saline.

nephhrine in the venom gland of the tropical toad *Bufo marinus* turns over even more slowly than the epinephrine in the rat adrenal. From the biochemical standpoint the slow rate of synthesis of epinephrine in the adrenal gland and in the toad venom gland discourages the use of these tissues as sources of active enzymes involved in epinephrine synthesis. It is possible that the rate of turnover of adrenal nor-epinephrine may differ from that of epinephrine. This is now being investigated.

Details of the chemical and isotopic procedures will be presented in a subsequent paper.

References

1. LUND, A. *Acta Pharmacol. Scand.*, **6**, 137 (1950).
2. RICHTER, D., and BLASCKO, H. *J. Chem. Soc.*, 601 (1937).
3. WEST, G. B. *Brit. J. Pharmacol.*, **6**, 289 (1951).
4. WADA, M., and KANOWOKA, K. *Taihoku J. Exptl. Med.*, **27**, 1 (1935).
5. VOGT, M. *Brit. J. Pharmacol.*, **7**, 325 (1952).

Manuscript received October 23, 1952.

Comments and Communications

On Some Recent Experiments with Supercooled Water

In a paper published in 1944 Rau (1) reported the supercooling of water to -72°C by the successive freezing and melting of water droplets in an apparatus which depended upon a dry-ice acetone mixture for cooling. This extremely low temperature prompted Cwilong (2) to repeat this work, but he was unable to confirm Rau's results.

During a program on ice physics conducted at the Commonwealth Engineering Company under the auspices of the United States Air Force (3) this experiment was repeated.

Using a dry-ice and acetone coolant, a drop of known size was frozen and melted with care to avoid evaporation. The drop was frozen a few times at the same temperature, frozen at a lower temperature, became elongated, and finally did not freeze when the temperature was lowered to -60°C . However, when the identical experiment was repeated using liquid nitrogen as the coolant, a freezing temperature could never be obtained which was lower than -6.5°C . The drop remained hemispherical throughout ten or more repeated freezings and did not elongate.

These data, therefore, indicate that the water in Rau's experiment was contaminated with acetone, as suggested by Cwilong; and that the determination of a -72°C nucleation temperature for pure water is in error. This conclusion is further substantiated by the work of Cohen and Van der Horst (4) who were able to obtain ice crystals of similar configuration to those obtained by Rau, when they froze dilute acetone-water solutions.

Recent experimental findings by Smith-Johannsen (5) have established that considerable supercooling of bulk water is possible if one takes care to cool the water drop in an apparatus where no air-solid interface below 0°C is in contact with the bulk water. In the Smith-Johannsen apparatus, where a water droplet is cooled on the central region of a plastic surface, which in turn rests upon the top of a cooling bar, nucleation has been found to begin at about -20°C . Using this apparatus we were able to obtain data simi-

lar to that reported by Smith-Johannsen and by modifying the apparatus to accommodate considerably larger volumes of water, we found that freezing temperatures of -35°C could frequently be obtained. These extremely low values, we feel, are due to heat-transfer effects within the bulk water and will be the subject of further investigation.

CARL BERGER

CHARLES M. SAFFER, JR.

Commonwealth Engineering Company of Ohio
Dayton, Ohio

References

1. RAU, W. *Schrift. deut. Akad. Luftfahrt*, **8**, 65 (1944).
2. CWILONG, B. M. *J. Glaciol.*, **2** (1947).
3. United States Air Force Contract, AF-33(038)-18687.
4. COHEN, E., and VAN DER HORST, J. *Z. physik. Chem.*, **B40**, 27 (1938).
5. SMITH-JOHANNSEN, R. *Science*, **108**, 652 (1948).

A Substitute for Drawing Ink in the Preparation of Diagrams for Photographic Reproduction

In seeking a substitute for India or drawing ink in the preparation of graphs, charts, line drawings, and similar items for photographic reproduction, it was found that the pencil "Mars Lumograph" (J. S. Staedtler, Inc.) was most satisfactory. Material prepared with this pencil photographed exceedingly well because of the flat, black line it produced. This was especially true if a high contrast film was used, such as Reprolith or Kodolith. These pencils come in varying degrees of hardness, but the one giving the best over-all results was the EXB grade. For filling in solid areas, as in histograms, however, the EX-EXB grade is recommended. Major advantages over ink are as follows: (1) neatness, in that erasures may be made without frayed or fuzzy lines; (2) increase in speed of preparation, because the need for inking the penciled drawing is eliminated; (3) ease in handling, because the amount of drawing equipment is reduced, and consequently less knowledge of drafting is required; and (4) the degree of blackness produced matches typewriting done with a fresh ribbon, and