The most ancient pottery heretofore reported from Mexico comes from Tehuacan. I have examined some of this material and have found that the pitted interiors and well-smoothed exteriors of the sherds are conspicuously similar to the Pox Pottery from Guerrero, a resemblance that has been commented on by others (*4*). The Tehuacan Pox Pottery occurs during the Purron phase which commenced in 2300 B.C., according to carbon-14 determinations (*5*). This date exactly equals the upper limit provided by the single carbon-14 determination available for the Guerrero Pox Pottery. Although this may indicate that the Guerrero ceramic antedates its Tehuacan counterpart, any claim to priority based on a single carbon-14 test has to be viewed with considerable skepticism. However, as both the Pox Pottery of Tehuacan and that of Guerrero were probably produced for a number of centuries, they must have been contemporaneous for part of the period during which each was made. The several hundred kilometers of rugged, mountainous terrain that separate these points of manufacture provide evidence for the existence of an extremely ancient horizon of closely interrelated cultures.

At both Tehuacan (*3*) and Guerrero, Pox Pottery overlies sherd-free cultural debris, making it highly probable that it constituted a portion of the first pottery made in both areas. As the fragile nature of ceramic containers make them of little use to nomadic peoples, the presence of pottery at an archeological site can imply a settled mode of subsistence. Consequently, Pox Pottery could prove an important horizon marker for the terminal stage in the change from a nomadic to a sedentary way of life. Additional examples of this pottery may be expected to turn up as more sites bridging this period of transition are discovered and excavated.

Coastal Guerrero and the arid Tehuacan Valley provide man with vastly different environments. The presence of Pox Pottery in such diverse regions might elucidate problems concerning the type of environment in which settled life first became established. Coe and Flannery (*6*) have advanced the hypothesis that this change took place at sites occupying lagoon- or estuary-dominated environments such as that enjoyed by Puerto Marquez and Zanja. Any evidence supporting the priority of the Guerrero over the Tehuacan Pox Pottery would lend credence to this hypothesis.

CHARLES F. BRUSH
American Museum of Natural History, Central Park West, New York, 10024

References and Notes

1. Carbon-14 determinations were made from samples of shell by the Humble Oil and Refining Company, Houston, Texas. Their interest in this project is greatly appreciated.
2. M. D. Coe, "La Victoria, an early site on the Pacific Coast of Guatemala," *Peabody Museum, Harvard, Papers, No. 53* (1961); K. A. Dixon, "Ceramics from two preclassic periods at Chiapa de Corzo, Chiapas, Mexico," *Papers New World Archaeol. Found. No. 8* (Orinda, California, 1959).
3. R. S. MacNeish, Second Annual Report of the Tehuacan Archaeological-Botanical Project (Robert S. Peabody Foundation for Archaeology, Andover, Mass., 1962).
4. M. D. Coe, *Am. Antiquity* **29**, 525 (1964).
5. R. S. MacNeish, *Science* **143**, 531 (1964).
6. M. D. Coe and K. V. Flannery, *ibid.*, p. 650.
7. These excavations were conducted under a permit from the Instituto Nacional de Antropologia e Historia of Mexico. The expenses incurred were defrayed in part by a grant from the Institute of Andean Research as part of the program "Interrelationships of New World Cultures."

25 May 1965

Glutamic-Oxaloacetic Transaminases in Reticulocytes and Erythrocytes

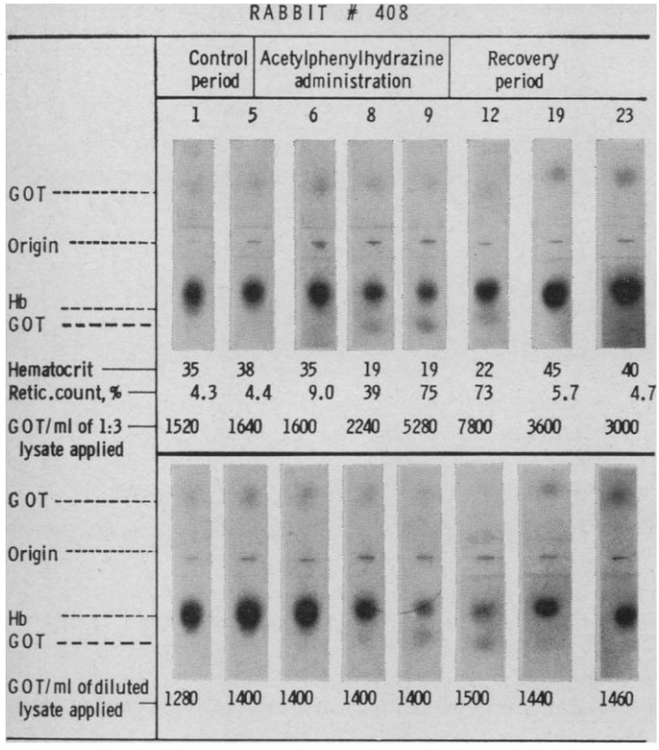
Abstract. *In rabbits, mature erythrocytes contain only the anionic isozyme of glutamic-oxaloacetic transaminase. Reticulocytosis induced by massive bleeding or by treatment with acetylphenylhydrazine is characterized by a five- to sixfold increase in red-cell transaminase and the appearance of a cationic transaminase isozyme that apparently resides in the mitochondrial fraction of reticulocytes.*

The anionic and cationic isozymes of glutamic-oxaloacetic transaminase (GOT) of human and animal heart and liver have been investigated in some detail (*1, 2*). Mature erythrocytes of man contain only anionic GOT; the kinetic and immunochemical characteristics of this component have been reported (*3*). The present communication is concerned with the electrophoretic characteristics of GOT in rabbit erythrocytes and reticulocytes.

The hematocrit, hemoglobin, reticulocyte content, activity of red-blood-cell transaminase, and the electrophoretic pattern of red-cell GOT were determined in each of two rabbits during a 5-day control period. Each rabbit was then injected subcutaneously with 1.0 ml of a 2.5-percent solution of acetylphenylhydrazine hydrochloride in 47.5 percent ethanol (*4*) on each of 5 successive days. Similar determinations were made throughout this period and during a subsequent recovery period of 2 weeks. The GOT activity of appropriate dilutions of hemolyzates were determined by the method of Karmen (*5*). The units of activity were expressed, however, per milliliter of hemolyzate per milliliter of reaction mixture.

The electrophoretic characteristics of GOT in hemolyzates of human red cells were examined in starch gels made up in 5.0 m*M* succinate-tris buffer, *p*H 7.2. The final *p*H of the gels was 6.8 to 7.0. Electrode vessels contained 0.1*M* phosphate buffer, *p*H 7.2. Electrophoresis was carried out in a cold room (4° to 6°C) for 18 hours at 4.0 volt/cm and 9 to 13 ma. The gels were sliced and stained specifically for GOT by the method of Schwartz *et al.* (*6*) (Fig. 1). As the reticulocyte counts rose, the GOT activity of the red blood cells increased about five- to sixfold, and a cationic zone of GOT appeared on gel electrophoresis. The appearance of the cationic band paralleled the fall in hematocrit and the rise in reticulocytes. There is also some indication from Fig. 1 that the intensity of the anionic band was inversely related to the intensity of the cationic band. Similar, though less marked, results were obtained when two rabbits were subjected to five successive bleedings, 25 to 30 ml each, over a period of 8 days. These results were not related to the possible presence of white cells since none were detectable per 1000 red cells through the 8th day of the experiment. The white-cell counts on days 9 and 12 were 0.1 percent and 0.5 percent, respectively, and decreased again to zero for the remainder of the observation period. Leukocytes have about the same transaminase activity as reticulocytes (*7*).

The intracellular distribution of



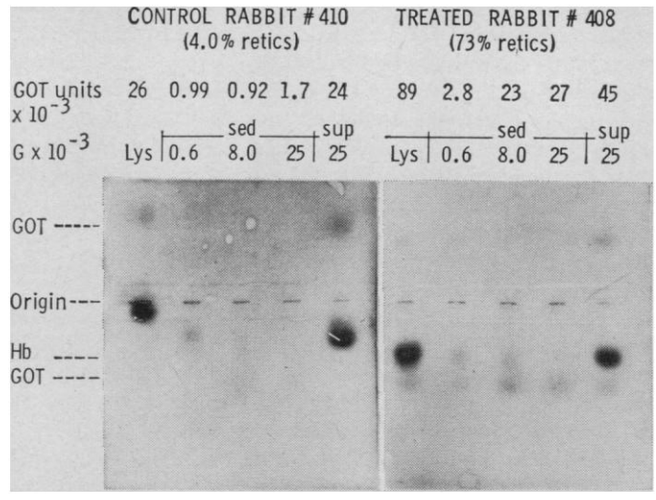


Fig. 1 (far left). Changes in electrophoretic distribution of glutamic-oxaloacetic transaminase of rabbit red blood cells as a function of reticulocyte count. Rabbits were injected subcutaneously with 1.0 ml of 2.5 percent acetylphenylhydrazine hydrochloride daily for 5 days starting on the 5th day. The washed cells were lysed by adding 2 volumes of water and then treating with high-frequency sound for 10 seconds. In the top series undiluted lysates were applied to the starch gels. In the bottom series the lysates were diluted to a constant enzyme activity and then applied to the starch gels. In all cases the anode is at top. Fig. 2 (right pair). Electrophoretic distribution of glutamic-oxaloacetic transaminase in the centrifugal fractions described in Table 1. The anode is at the top. The hemoglobin (spot) in the hemolyzate of the control blood of rabbit 410 moved more slowly than those in approximately 30 electrophoretic runs of hemolyzates of red blood cells in various stages of reticulocytosis (*10*), but this finding is not relevant to the present study.

Table 1. Centrifugal fractionation of GOT from red blood cells of reticulocytotic and normal rabbits. Blood (25 to 50 ml) was taken from rabbit No. 208 2 days after four daily injections of acetylphenylhydrazine and again 2 days later. Similar volumes of blood were taken from rabbits Nos. 408 and 409 2 days after a series of five injections. Three blood samples were taken from two control animals. Blood was collected in either ethylenediaminetetraacetate or heparin and centrifuged; the buffy coats were then removed. The cells were washed three times with three volumes of 0.9 percent NaCl, and then lysed with four volumes of 0.005*M* $MgCl_2$ in the cases of rabbits Nos. 208 and 209, or with 1.5 volumes of 0.005*M* $MgCl_2$ in the cases of rabbits Nos. 408, 409, and 410. The lysates were sonicated for 10 seconds, and isotonicity was then restored by the addition of 1.0 or 0.5 volume, respectively, of 1.5*M* sucrose to 0.15*M* KCl exactly 10 minutes after addition of the 0.005*M* $MgCl_2$. The sedimented fractions were suspended in cold distilled water, treated with high-frequency sound for 1.0 minute, and assayed for transaminase activity.

Rabbit	Reticulocytes in washed cells (%)	GOT/ml of packed cells	Percentage of original GOT recovered			
			0–600*g*	600–8000*g*	8000–25,000*g*	25,000*g* supernatant
		Control animals (non-injected)				
209	3.5	4440	2.8	2.8	2.3	107
209	3.9	4440	4.1	2.2	1.7	102
410	4.0	3780	3.7	3.5	6.4	91
		Average recovery:	3.5	2.9	3.5	100
		Injected animals				
208	37	13600	2.1	11.0	12.6	95
208	56	16200	4.2	26.0	11.0	52
408	73	26600	3.2	26.0	30.0	52
409	65	30200	6.6	24.9	9.0	50
		Average recovery:	4.2	22.0	17.0	60

transaminase in the red cells of rabbits in which reticulocytosis had been induced with acetylphenylhydrazine was then studied. Blood was collected by cardiac puncture. The hematocrit, hemoglobin, and reticulocyte count were determined. The cells were treated as described in Table 1. The hemolyzates were then fractionated by centrifugation. The fraction that sedimented below 600*g* was about the same in the treated and control animals, whereas the fractions that sedimented between 600*g* and 25,000*g* contained an average of 39 percent of the GOT in the hemolyzates from the rabbits that had high reticulocyte counts. Only 6.4 percent of the activity was present in these fractions from control animals. Less than 1 percent of the GOT sedimented between 25,000*g* and 105,000*g* from the hemolyzate of either normal or reticulocytotic animals. The enzyme that sedimented between 600*g* and 8000*g* and between 8000*g* and 25,000*g* was predominantly the cationic type (Fig. 2). From treated animals the original hemolyzates and the 25,000*g* supernatants contained both anionic and cationic GOT.

Hallinan *et al.* (*8*) submitted electron-micrographic evidence that rat reticulocytes contain mitochondria. These intracellular structures are absent from the fully mature erythrocyte. The cationic component of rat liver GOT is confined predominantly to the mitochondrial fraction, whereas the anionic component is almost exclusively in the supernatant fraction (*2*). Our results with the rabbit are in agreement with the earlier findings. The mature erythrocyte contains only the anionic GOT, whereas the reticulocytes contain the cationic type. Ellis *et al.* (*9*) have reported that during the transition from reticulocyte to erythrocyte much of the cellular protein, other than hemoglobin, disappears and that the reticulocytes show substantial increases in acid cathepsin acting at *p*H 3.2, in several peptidases, and in acid and alkaline phosphatases. Hallinan *et al.* (*8*) have pointed out that in sections prepared for electron microscopy the mitochondria of rat reticulocytes frequently show signs of alterations, with disoriented cristae and even ruptured outer membranes, whereas the mitochondria of monocytes in the same section are relatively unaltered. As noted above, there was some indication that the intensity of the anionic band of GOT was inversely related to the intensity of the cationic band as reticulocytosis was induced and allowed to recede.

J. S. NISSELBAUM
OSCAR BODANSKY
Sloan-Kettering Institute for Cancer Research and *Graduate School of Medical Sciences, Cornell University Medical College, New York*

References and Notes

1. G. A. Fleisher, C. S. Potter, K. G. Wakim, *Proc. Soc. Exptl. Biol. Med.* **103**, 229 (1960); Y. Morino, H. Itoh, H. Wada, *Biochem. Biophys. Res. Commun.* **13**, 348 (1963); J. S. Nisselbaum and O. Bodansky, *J. Biol. Chem.* **239**, 4232 (1964).
2. J. W. Boyd, *Biochem. J.* **81**, 434 (1961).
3. J. S. Nisselbaum, *Federation Proc.* **24**, 356 (1965).
4. H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley, P. H. Lowy, *J. Biol. Chem.* **196**, 669 (1952).
5. A. Karmen, *J. Clin. Invest.* **34**, 131 (1955).
6. M. K. Schwartz, J. S. Nisselbaum, O. Bodansky, *Am. J. Clin. Pathol.* **40**, 103 (1963).
7. F. Belfiore, *Boll. Soc. Ital. Biol. Sper.* **39**, 709, 749 (1963).
8. T. Hallinan, E. Eden, R. North, *Blood* **20**, 547 (1962).
9. D. Ellis, C. E. Sewell, L. G. Skinner, *Nature* **177**, 190 (1956).
10. J. S. Nisselbaum and O. Bodansky, unpublished data.
11. Supported in part by American Cancer Society grants P-163 and P-164, and contract SA-43-ph-3716 from the National Cancer Institute.

25 May 1965

Serotonin: Synthesis and Release from the Myenteric Plexus of the Mouse Intestine

Abstract. *After injection of its radioactive precursor, 5-hydroxytryptophan, radioactive serotonin was biosynthesized and bound in the myenteric plexus of the mouse intestine. Addition of nonradioactive serotonin to preparations in vitro caused a net release of radioactive serotonin from the plexus. This release appeared to result from activity in the intramural nervous system of the intestine. A neurotransmitter role between sensory and motor neurons in the peristaltic reflex pathway is suggested as a working hypothesis to explain the action of serotonin.*

A radioautographic method, utilizing the tritiated precursor of serotonin, H^3-labeled 5-hydroxytryptophan (5HTP-H^3), has recently been devised for the purpose of localizing tissue sites that synthesize and bind serotonin (*1, 2*). The method has shown the myenteric plexus of the mouse intestine to be such a site. Surprisingly, and in marked contrast with the stomach where the argentaffin cells of the mucosa also become labeled, the myenteric plexus is the only site shown to synthesize and bind serotonin in the small intestine after a single intravenous injection of 0.4 to 4.0 mc of 5HTP-H^3 (*2, 3*). We now report additional experiments intended to define the localization and fate of intestinal serotonin; certain results may be interpreted to suggest that serotonin is a neurotransmitter between sensory and motor nerve cells in the intramural nervous system of the intestine.

One hour after intraperitoneal injection of 60 mg/kg of β-phenylisopropylhydrazine (a monoamine oxidase inhibitor), mice were injected intravenously with 0.4 to 4.0 mc of 5HTP-H^3 or 5.0 μc of C^{14}-labeled 5HTP. Stomach and intestines were removed 4 hours later, and strips of gut were opened longitudinally and bathed in oxygenated Krebs solution at 37°C.

The resultant outflow of radioactive material can be described by a multicompartmental washout curve (*4*). Three components were resolved: the first was extremely rapid, with a half time ($t_{1/2}$) of about 40 seconds; the $t_{1/2}$ of the second averaged 9 minutes; the third component was very slow, having a $t_{1/2}$ averaging 560 minutes. The radioactive compounds present in the effluent were identified by paper chromatography. In addition, the radioactive compounds initially present, and those remaining after washout, were extracted from the intestinal strips with 70-percent ethanol and similarly assayed. Most of the material accounting for the radioactivity of the two faster components was either 5HTP-H^3 or tritiated serotonin-*O*-glucuronide. Radioactive serotonin was detected in the bath only in small amounts, briefly, during the first minute of washout, and not again until 60 minutes later.

Since very little radioactive serotonin leaves the intestinal strips, the proportion of tissue radioactivity due to tritiated serotonin increases with washout time (Fig. 1). It was concluded therefore that the rapid components represent washout of the serotonin precursor and metabolite which are unbound, whereas the slow component reflects the existence of a barrier to the free diffusion of serotonin from the tissues (*4*). The extremely rapid phase may depict washout from extracellular space and perhaps from blood platelets.

Since tritiated serotonin accounts for most of the radioactivity remaining in the gut after the slow component is reached, any label detected by radioautography in tissue fixed at this time probably indicates the presence of tri-

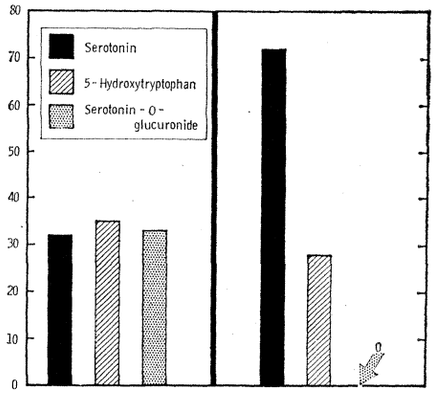


Fig. 1. Radioactive compounds extracted from intestinal strips with 70-percent ethanol 4 hours after injection of C^{14}-labeled 5-hydroxytryptophan and isolated and identified by paper chromatography; their amounts were determined by counting in a liquid-scintillation spectrometer. The compounds initially present, expressed as percentages of total extractable radioactivity, are shown at left; those present after 1 hour of washout, on the right.