reverse it. Preliminary experiments utilizing the manometric technique of Zamecnik *et al.* (*8*) for the determination of lecithinase have yielded identical results.

These results suggest that Ca^{++} activates but is not essential for the activity of lecithinase whereas Zn^{++}, Co^{++}, and Mn^{++} activate and are essential for the activity of the enzyme. They can be explained on the basis that Zn^{++}, Co^{++}, and Mn^{++} are preferentially chelated over Ca^{++} (*9*) and thus remain chelated, even in an excess of Ca^{++}.

The action of EDTA in protecting mice from the toxin is undoubtedly due to its ability to chelate metal ions, inasmuch as it no longer protects when an "essential" ion is present in excess. The protection afforded by EDTA and the reversal of this protection by an "essential" ion are rather dramatic. The mice that received the toxin plus EDTA manifested no ill effects and no decrease in their normal activity. In those mice in which the protective action of EDTA was reversed by either Zn^{++}, Co^{++}, or Mn^{++}, the pattern of death was identical with that of the controls that received toxin alone. They all died in the same time interval, and had an extensive area of inflammation at the site of injection.

The action of the toxin is not confined to the injection site. Therefore the EDTA exerts more than a local effect. An experiment was performed in which the toxin and EDTA were injected into different sites on opposite sides of mice, and five of the ten mice survived. The greater amount of protection that resulted when the toxin and EDTA were injected simultaneously indicates that inhibition of the toxin at the site of injection is involved in this enhanced protection. A study of the dosage levels of EDTA and Ca-EDTA that are required to protect animals when they are given by intravenous and other routes has been started.

EDTA is not toxic for animals and human beings until an amount is given that induces a hypocalcemic state with resultant tetany (*10*), but Ca-EDTA can be given in large quantities without any apparent toxicity and has been used clinically in cases of lead poisoning (*11*). The possible use of Ca-EDTA in the therapy of gaseous gangrene is obvious.

MERWIN MOSKOWITZ
MERWYN W. DEVERELL
RAMON MCKINNEY
Division of Bacteriology, Department of Biological Sciences, Purdue University, Lafayette, Indiana

References and Notes

1. M. G. McFarlane and B. C. J. G. Knight, *Biochem. J. London* 35, 884 (1941).
2. M. G. McFarlane, in *Mechanisms of Microbial Pathogenicity* (Cambridge Univ., London, 1955).
3. E. A. Meyer and M. Moskowitz, *J. Bacteriol.* 69, 111 (1955).
4. Used as Di-Sodium Di-Hydrogen Versenate (analytic reagent). Kindly supplied by the Bersworth Chemical Company and their successor, the Dow Chemical Company.
5. Supported in part by a grant from the Purdue Research Foundation and grant G-3444 of the National Institutes of Health, U.S. Public Health Service.
6. Kindly supplied by Lederle Laboratories Division, American Cyanamid Company.
7. Borate-buffer was made up as follows: (i) 1.25 g of boric acid was dissolved in 100 ml of 0.16*M* NaCl, and (ii) 1.9 g of Borax was dissolved in 100 ml of 0.16*M* NaCl; 10 ml of ii mixed with 90 ml of i to give a *p*H of 7.6. The final *p*H of the mixtures used for injection was 6.9 to 7.1.
8. P. C. Zamecnik, L. E. Brewster, F. Lipmann, *J. Exptl. Med.* 85, 381 (1947).
9. *The Versenes* (Bersworth Chemical Co., Framingham, Mass., 1953).
10. A. Popovici *et al.*, *Proc. Soc. Exptl. Biol. Med.* 74, 415 (1950).
11. H. L. Hardy *et al.*, *J. Am. Med. Assoc.* 154, 1171 (1954).

19 December 1955

Simplotite, New Calcium Tetravanadite from the Colorado Plateau

Simplotite, $CaV_4O_9 \cdot 5H_2O$, is a new mineral found in several vanadium-uranium mines on the Colorado Plateau (*1*). The mines are located in the Salt Wash sandstone member of the Morrison formation of late Jurassic age. The new mineral is named in honor of J. R. Simplot (*2*), former owner of the Peanut Mine, Montrose County, Colo., where the material used for this description was collected.

Simplotite occurs as hemispherical aggregates of dark-green, platy crystals. It is found in comparatively unoxidized ore and is associated with montroseite, paramontroseite, vanadiferous silicates, uraninite, and coffinite. At the Peanut Mine it occurs as coatings on fractures in the ore-bearing sandstone with duttonite, $VO(OH)_2$ (*3*), melanovanadite, native selenium, and an undescribed vanadium oxide.

The color of simplotite varies from nearly black, in coarse aggregates, to yellow-green, in thin flakes. It is biaxial negative; 2V is about 25°; and dispersion is $r > v$, weak and crossed. $X = b$ (yellow); Y (green); $Z \wedge c = +58°$ (green); $\alpha = 1.705 \pm 0.002$, $\beta = 1.767 \pm 0.002$, $\gamma = 1.769 \pm 0.002$. The specific gravity, as measured by immersion in a mixture of bromoform and acetone, is 2.64 ± 0.02.

A microchemical analysis made by one of us (R. M.) on approximately 70 mg of simplotite showed the following composition: CaO, 11.6 percent; V_2O_4, 67.7 percent; V_2O_5, 0.5 percent; H_2O, 18.4 percent; and insoluble material, 0.5 percent; total, 98.7 percent. Qualitative spectrographic analysis by Katherine E. Valentine of the original material indicated the presence of Mg and Al in amounts of 0.1 to 0.5 percent and Na, K, and Fe in amounts 0.05 to 0.1 percent.

Simplotite is monoclinic and pseudotetragonal. It has a very easy micaceous cleavage on (010) and is very soft. The unit-cell constants were determined by M. E. Mrose of the U.S. Geological Survey as follows: $a_0 = 8.39 \pm 0.02$ A, $b_0 = 17.02 \pm 0.02$ A, $c_0 = 8.37 \pm 0.02$ A, $\beta = 90°25' \pm 5'$; $a:b:c = 0.4929:1:0.4918$. The space group is C $2/m$; the cell contents are 4 $(CaV_4O_9 \cdot 5H_2O)$; and the calculated specific gravity is 2.65.

Simplotite was first found in 1952 by Alice D. Weeks and other members of the U.S. Geological Survey field party at the Sundown claim, San Miguel County, Colo. The sample used for this description was collected by one of us (C. H. R.) in the course of a detailed study of the mineralogy, geochemistry, and geology of the Peanut Mine. The mineral has been identified in the following three other mines: the Shattuck-Denn lease on Club Mesa and the J. J. Mine in Paradox Valley, both in Montrose County, Colo., and the Vanadium Queen Mine at La Sal Creek, San Juan County, Utah.

MARY E. THOMPSON*
CARL H. ROACH
ROBERT MEYROWITZ
U.S. Geological Survey Grand Junction, Colorado

References and Notes

1. Publication authorized by the director, U.S. Geological Survey. The study of the mineralogy of the uranium deposits of the Colorado Plateau is part of a program being conducted by the U.S. Geological Survey on behalf of the Division of Raw Materials of the U.S. Atomic Energy Commission.
2. J. R. Simplot Mining Co., Boise, Idaho.
3. M. E. Thompson, C. H. Roach, R. Meyrowitz, *Science* 123, 990 (1956).
* Present address: 38 Clarendon St., Newtonville, Mass.

16 January 1956

Relation of Aromatic Amino Acids to Excretory Pattern of Schizophrenics

It has been shown that schizophrenics generally excrete a number of aromatic compounds that appear little, if at all, in normal urine (*1, 2*). The appearance of these abnormal metabolites may be related to the production in schizophrenia of some hallucinogen. Because of the well-known limitations on human biosynthesis of aromatic compounds, such abnormal metabolites are most probably derived from the dietary aromatic amino acids, phenylalanine, tyrosine, and tryptophan. Limiting the dietary intake of one or all of these aromatic amino acids should provide evidence concerning the origin of these aromatic compounds. With this in mind, we fed a

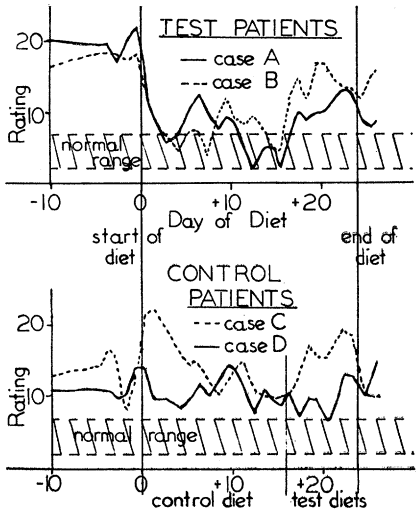

Fig. 1. Two-day running average of chromatographic ratings. Case C received a diet low in tryptophan from the 16th to the 24th day; case D received a diet low in phenylalanine and tyrosine during the same period.

diet low in the aromatic amino acids to a small group of schizophrenic volunteers (*3*). Biochemical changes were followed by the chromatographic method previously reported (*2*).

Four male chronic schizophrenics were kept under close supervision in an isolated ward for the period of the diet, as well as for a month preceding. In order to reduce protein reserves that might furnish aromatic precursors, we gave the patients a low-protein diet (40 g/day) for 1 week before the experimental period. Two patients, A and B, were then placed for 24 days on a test diet that lacked all three aromatic amino acids. This test diet was similar to those used in studies on the essential character of tryptophan and for the treatment of phenylketonuria (*4*). Patients C and D, who served as controls, were fed a control diet for 16 days; this control diet appeared exactly the same as the test diet, but it contained all the essential amino acids. For the remaining 8 days, case C was given the test diet with added phenylalanine and tyrosine (lacking only tryptophan), while case D was given the test diet with added tryptophan (lacking phenylalanine and tyrosine). Estimated daily intakes of the aromatic amino acids and of histidine are indicated in Table 1.

A concentrated (10:1, in a vacuum) sample of the daily morning urine of each patient was analyzed chromatographically for diazo-coupling compounds according to the method previously described (*2*); these chromatograms were compared with chromatograms of normal urine prepared under identical conditions. Each chromatogram was rated according to the number and intensity of spots. The results, which are plotted in Fig. 1, can be summarized, as follows:

1) The chromatograms of the urine of schizophrenic patients (pre-diet) generally showed a greater than normal number and intensity of spots.

2) The chromatograms of the urine of patients on the test diet, which was lacking in phenylalanine, tyrosine, and tryptophan, showed a reduction in the number and intensity of spots to normal or near-normal range.

3) The chromatograms of the urine of patients on the control diet, which contained all the aromatic amino acids, showed no such decrease in color.

4) The color in the chromatograms of patients on the test diet dropped sharply at first, but, after about 16 days, some color began to reappear. It is not known why this occurred, but it may be significant that the patients' nitrogen excretion dropped at the same time.

5) The results with patients whose diet was lacking only tryptophan (case C) or only phenylalanine and tyrosine (case D) allow no definite conclusions regarding the origin of the chromogens, but they suggest that the greater part of the color is derived from phenylalanine and tyrosine metabolites.

The chromatograms were analyzed for the frequency of occurrence of each spot during the various test periods (Table 1). In general, the spot-by-spot analysis confirmed the findings listed here. Of the 13 colored areas particularized, nine appear much more frequently in the urine of schizophrenics than in the urine of normal individuals; two may not appear at all in normal chromatograms. All but one of the "abnormal" spots showed a significant decrease in frequency during the period of test diet.

The patients showed little or no physical change during the experiment. There was no significant variation in weight or blood nonprotein nitrogen. The patients were kept under psychiatric observation and given electroencephalograms before, during, and after the test period. Minor changes were noted in patients A, B, and C, but these were not considered to be significant; no change was noted in patient D. It is felt that the lack of significant clinical change during the brief span of this experiment does not influence the hypothesis that schizophrenic hallucinations may be of biochemical origin. The patients were all chronic cases with well-established abnormal behavior patterns. The refrac-

Table 1. Spot-by-spot analysis of chromatograms and estimated daily intake of aromatic amino acids. The effect of the various dietary regimens on the aromatic excretion pattern may be obtained from comparison of the figures given in the various columns; changes of less than 10 percent should not be considered significant.

Color and R_f*	Frequency of occurrence (%)						
		Test patients		Control patients			
		Cases A + B		Cases C + D		Case C	Case D
	Normals (18 cases)	Pre-diet (10 days)	Test diet (24 days)	Pre-diet (10 days)	Control diet (16 days)	Test diet + tyrosine and phenylalanine (8 days)	Test diet + tryptophan (8 days)
Brown to yellow (R_f 0–0.1)	80	93	42	77	47	87	75
Brown (R_f 0.1–0.2)	40	93	15	57	33	66	25
Yellow (R_f 0.1–0.2)	20	64	34	28	31	71	42
Yellow or orange (R_f 0.2–0.3)	20	86	44	71	60	87	87
Yellow to brown (R_f 0.3–0.35)	0	67	25	40	37	55	30
Red or pink-brown (R_f 0.3–0.4)	10	79	27	72	91	90	40
Yellow to brown (R_f 0.38–0.45)	15	86	40	70	57	75	66
Yellow (R_f 0.55–0.6)	80	98	62	74	46	75	71
Pink (R_f 0.56–0.66)	80	100	43	89	61	86	43
Yellow-brown (R_f 0.62–0.72)	0	50	9	53	50	50	25
Orange (R_f 0.72–0.78)	92	97	89	75	67	72	7
Red to brown (R_f 0.85)	20	87	28	41	65	12	63
Yellow (R_f 0.85–0.9)	20	80	83	100	62	78	20
Estimated daily intake in grams							
Histidine		1.1	1.8	1.1	1.8	1.8	1.8
Phenylalanine		2.4	< 0.2	2.4	7.0	5.0	0.2
Tyrosine		1.9	< 0.2	1.9	5.6	4.0	0.2
Tryptophan		0.7	< 0.1	0.7	2	0.1	5.5

* Reduced to an R_f of 0.5 for the urea spot.

toriness of chronic schizophrenia to treatment with Frenquel and reserpine (*5*) suggests that changes may take place which are not readily reversed.

The chromatographic work does point to a physiological alteration in schizophrenia which can be controlled by means of a suitable diet. It remains for future work to establish whether such an alteration is of basic importance to the disease.

PATRICK L. MCGEER
EDITH GRAEF MCGEER
JOSEPH E. BOULDING
Department of Neurological Research, Faculty of Medicine, University of British Columbia, Vancouver, and *Crease Clinic of Psychological Medicine, Essondale, British Columbia*

References and Notes

1. M. K. Young, Jr., *et al., Univ. of Texas Publ. No. 5109* (1951), pp. 189–197; I. Sano, *Folia Psychiat. et Neurol. Japonica* 8, 218 (1954).
2. P. L. McGeer, E. G. McGeer, W. C. Gibson, *Science* 123, 1029 (1956).
3. We wish to thank the laboratory and dietetic staff of the Provincial Mental Hospital and of Crease Clinic; without their cooperation this project would have been impossible.
4. L. I. Woolf *et al., Brit. Med. J.* 1955I, 57 (1955); L. E. Holt, Jr., *et al., Proc. Soc. Exptl. Biol. Med.* 48, 726 (1941).
5. F. Rinaldi *et al.*, paper presented before the American Psychiatric Association, Atlantic City, May 1955.

20 December 1955

Induced Spawning in the Indian Catfish

In the course of an investigation of the relationship between structural changes in the pituitary and the development of ova in the common Indian catfish, *Heteropneustes* (*Saccobranchus*) *fossilis* (Bloch), we have attempted to induce spawning under laboratory conditions (*1*). This work is preliminary to a wider project designed to bring about the spawning of other major Indian carps (*Labeo, Cirrhina, Catla*) in confining waters. Thus restricted, either these species may become gravid but fail to ovulate, or they may discharge unfertilized eggs (*2*). So far as we are aware, no attempt has previously been made in India to induce spawning of food fishes by injecting fresh (or acetone dry) pituitaries of the same species. Injections of mammalian pituitary preparations result in ova that are not viable (*3*).

Heteropneustes breeds in large tanks just after the monsoon showers. Ghosh and Kar (*4*) report that the active phase of the ovary extends from late April to July, but we have collected gravid females in August and September (1955). These were taken from a local tank (Bellandur, area 893 acres) from which all of our supply comes. The occurrence of gravid fish in our collections after the normal breeding season is doubtless due to the delay in the break of the monsoon. Weights of the gravid fish ranged from 70 to 135 g. They were held in aquaria (60 by 30 by 33 cm) that were supplied with city water from overhead cisterns, and the water was renewed daily. No additional aeration was provided, and all experiments were conducted at room temperature (24° to 25°C). The animals were not fed during the experimental period.

After a series of replications, it was found that a single injection of one-quarter of a fresh pituitary from a gravid *Heteropneustes* homogenized in 0.5 ml of standard Holtfreter solution was quite effective.

The injections were administered intramuscularly, since it was difficult to give intraperitoneal injections without damaging the egg-laden ovisacs. Controls injected with an equal quantity of Holtfreter solution did not spawn. Only specimens weighing more than 80 g were utilized, since it was found that smaller animals did not always respond to the injections.

Because spawning usually happened during the night, we have not been able to compute the time lapse necessary for the gravid females to spawn after each injection. However, our experiments indicate that at least 12 to 14 hours must elapse before the injected females spawn in aquaria. Eggs stripped from such females the following morning were fertilized in sperm suspension in Holtfreter solution and were cultivated both in Holtfreter solution and city water, since spring water was not easily accessible to us. Development proceeded normally in both media, and hatching took place within 32 to 34 hours.

L. S. RAMASWAMI
B. I. SUNDARARAJ*
Department of Zoology, Central College, Bangalore, India

References and Notes

1. We wish to express our thanks to B. R. Seshachar, Central College, for kind encouragement.
2. S. L. Hora, *Proc. Nat. Inst. Sci. India* 11, 303 (1945).
3. A. Husain, *ibid.* 11, 320 (1945).
4. A. Ghosh and A. B. Kar, *Proc. zool. Soc. Beng.* 5, 29 (1952).
* Research scholarship, Ministry of Education, Government of India.

28 December 1955

Postirradiation Protection of Rabbits by Injection of "Splenic" Plasma

Jacobson (*1*) has attributed the "protection" of animals by shielding of the spleen during irradiation to a "humoral factor" elaborated by the spleen. It has also been shown that injection of certain tissue homogenates (of spleen or bone marrow, for example) soon after irradiation is similarly protective. However, it seems that the presence of cells or cell fragments in the injected material is necessary to bring about protection. If the postulated humoral factor is elaborated by the spleen, it should be present in higher concentration in the blood leaving the spleen. Therefore, plasma of blood taken from the splenic vein should exhibit a protective action.

Table 1. Comparative data in series I and II.

No. of animals	Plasma injection* (cm^3)	30-Day survival (%)	Avg. survival (day)
First series			
27		22	14.4
13	6 A	54	22.2
10	6	20	16.0
15	3†	20	14.4
Second series			
25		4	8.9
25	6 A	24	14.4
25	6 B	18	12.2

* Intraperitoneal, except where otherwise indicated.
† Intravenous. Eight rabbits received A plasma and one B plasma. The other six had been injected before it was thought necessary to distinguish between A and B plasma.

Such an experiment has been carried out using adult male chinchilla rabbits (*2*). In essence, heparinized blood was obtained from a nonirradiated donor rabbit as follows. The distal gastric coronary vein and artery were ligated at the stomach wall and then severed beyond the ligature on the spleen side. Blood from the splenic vein and artery was allowed to flow into a test tube in ice water through a polyethylene tube. This "splenic blood" is really a mixture of blood from the splenic vein, the splenic artery, and the proximal gastric coronary veins. The first 35 cm^3 of blood thus collected constitutes the A sample and the second 35 cm^3 the B sample. Each sample was centrifuged for 20 minutes at 2300 rev/min, and the plasma was separated.

After centrifugation of the splenic blood, approximately 18.0 cm^3 of plasma was carefully removed by pipette for injections. After the three 6.0-cm^3 doses of plasma had been injected, a residual drop of the plasma was placed on a slide, covered with a cover slip, and examined microscopically. No cells were found in the specimens examined. In one case, a more significant search for cells in splenic plasma was made as follows. Measured amounts of the plasma were drawn into erythrocyte- and leukocyte-diluting pipettes, and then the plasma