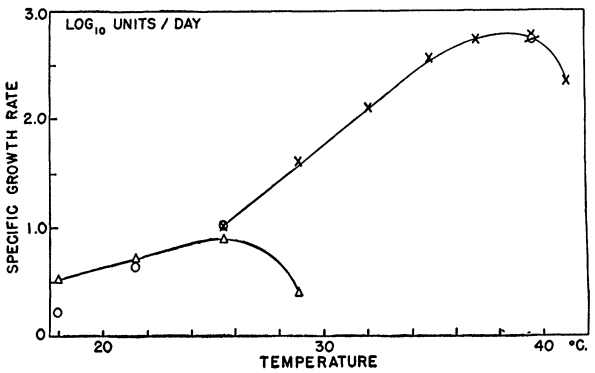

FIG. 1. Growth rate as a function of temperature. △, *Chlorella pyrenoidosa* (Emerson's strain) at 1600 ft-c; ×, Tx 71105 at 1600 ft-c; O, Tx 71105 at 500 ft-c; ø, Tx 71105 at 2800 ft-c.

agent, ethylene diamine tetracetic acid, as previously described (*3*). The cultures were aerated with 4% carbon dioxide in air, illuminated at 500 ft-c by fluorescent lamps, and incubated at 32° C. After several serial transfers, samples were plated out on agar medium. Bacteria-free cultures were obtained from isolated colonies.

Preliminary study of 12 strains isolated at 32° C indicated that one of these (Tx 1105) would show rapid and continued growth at 39° C. After repeated culture at 39° C, a second plating-out yielded a second series of isolates. One of these (Tx 71105) was selected for further study, preliminary results of which are presented here. Subsequently a third series of isolates was obtained via accumulation cultures at 39° C. Among 61 original strains established there are significant differences in growth rate and in the tendency of the cells to clump in liquid cultures. Cells of the various strains are not readily distinguishable from the Emerson strain of *Chlorella pyrenoidosa* in size, morphology, or mode of reproduction; at this time we prefer to regard them as strains of a species of Chlorella.

Tx 71105 and the Emerson strain of *C. pyrenoidosa* have been submitted to a comparative study of the effect of temperature on the growth rate at light saturation. Growth in test tube cultures in photothermostats (as described above for the accumulation cultures) was determined in terms of the optical density measured by an Evelyn colorimeter with 600 mμ filter. The logarithm of the optical density plotted against time yields a straight line, the slope of which (k) is the specific growth rate. For *C. pyrenoidosa* the method yields a value for k of 0.85 ± 0.05 $\log_{10}$ u/day at 25° C, as compared to a value of 0.87 determined by the more elegant method of an automatic dilution device.

Light-saturation of growth of *C. pyrenoidosa* could be achieved by two banks of two 20-w daylight fluorescent lamps placed on each side of the photothermostat and delivering about 500 ft-c to opposite sides of the test tube cultures. This arrangement was adequate also for Tx 71105 at temperatures below 26° C. At higher temperatures two banks of tungsten lamps delivering 1600 ft-c or 2800 ft-c to opposite sides of the cultures were used to obtain light-saturation.

Cultures for the growth measurements were inoculated from 3 units of a continuous-culture apparatus which contained Tx 71105 at 25° and 39° C and *C. pyrenoidosa* at 25° C. Test tube cultures held at the experimental temperature were continued by serial transfer until the growth rate remained constant over two successive attempts.

The results are presented in Fig. 1. The less extensive data for *C. pyrenoidosa* indicate a temperature optimum at 25°–26° C. In spite of repeated attempts we were unable to train *C. pyrenoidosa* to maintain a stable growth rate at temperatures higher than 30° C. The temperature optimum for Tx 71105 lies at about 39° C. A stable growth rate for this strain could not be obtained above 41.2° nor below 25.5° at 1600 ft-c. Reduction of the light intensity to 500 ft-c permitted a stable growth rate down to at least 21.5° C.

Preliminary manometric studies on Tx 71105 at 39° C have yielded maximum values of about 5 mm^3 O_2/mm^3 cells/hr for respiration and 100 mm^3 O_2/mm^3 cells/hr for photosynthesis, although in the latter case we are not certain that light-saturation was attained. This is the highest rate of photosynthesis per unit quantity of cell material of any organism so far observed.

References

1. PRINGSHEIM, E. G. *Pure Cultures of Algae.* Cambridge: University Press, 82 (1946).
2. FISHER, A. W., JR., WALSH, W. A., and PETTENGILL, K. H. In J. S. Burlew (Ed.), *Algal Culture: From Laboratory to Pilot Plant,* Carnegie Institution of Washington Pub. 600, Chap. 17 (in press).
3. MYERS, J., PHILLIPS, J. N., and GRAHAM, J. R. *Plant Physiol.,* **26**, 539 (1951).

Manuscript received August 14, 1952.

Metabolism and Removal of Ca^{45} in Man[1]

Judith Bellin and Daniel Laszlo[2]

Division of Neoplastic Diseases, Montefiore Hospital, New York

This report deals with the metabolism and removal of Ca^{45} in man, as studied by the administration of a single tracer dose of high specific activity radiocalcium. Although such data are available for animals (*1–9*), none has been reported for man. From the rate of disappearance of Ca^{45} from the blood stream, its excretion, and its uptake and removal from bone, information on calcium metabolism may be obtained without disturbing the calcium homeostasis.

The sodium salt of ethylene diamine tetracetic

[1] This study has been supported in part by a grant from the National Cancer Institute of the National Institutes of Health, USPHS, and in part by a Contract AT(-30-1)-880 with the U. S. Atomic Energy Commission.

[2] The assistance of Leonard Woidowsky and Agnes Hausinger is gratefully acknowledged.

TABLE 1

TISSUE ANALYSES*

	Ca (g%)	Ca^{45} $\left(\frac{\mu c}{g} \times 10^{-3}\right)$	Specific activity ($\mu c/g\ Ca^{40}$)	N (g%)	P (g%)	P corr† (g%)	$\frac{Ca}{P\ corr†}$
Rib	18.31	12.6	0.069	3.55	8.29	8.05	2.28
Femur							
Cortex	22.57	5.9	0.026	2.44	9.71	9.54	2.37
Spongiosa	19.01	9.0	.047	3.96	9.25	8.98	2.06
"Tumor"	15.30	22.7	.148	6.00	9.04	8.63	1.69
Spongiosa adjacent to tumor	18.33	18.8	.103	7.07	9.45	8.96	1.95
Vertebra‡	9.53	5.31	0.056	7.88	5.63	5.09	1.69
							$\frac{N}{P}$
Liver‡	0.40	0	—	12.25	0.81	—	15.1
Kidney‡	0.60	0	—	10.38	0.75	—	13.9

* Patient M. A. Analyses of tissues obtained at autopsy. Patient received 57.6 μc Ca^{45} 14 days prior to death.
† P corr = Total P—P which is accounted for by nitrogen (N : P = 14.6).
‡ = Tissues dried but not defatted.

acid (EDTA), a strong chelating agent, forms complexes with calcium and other metallic ions (*10–12*). Marked excess calcinuria without lowering of serum calcium levels was observed in man following the slow intravenous administration of Na-EDTA (*13*), illustrating the demineralizing effect of this salt. The rate of such skeletal demineralization was studied by infusing Na-EDTA following the equilibration of a dose of radiocalcium.

In another series of experiments (not reported here) the injection of the calcium salt of EDTA induced an excess excretion of Ca^{45} and of metals (Pb, Y) deposited in bone (*14, 15*). Such data may indicate the quantity of calcium and of metallic ion available for surface exchange.

The studies were performed on patients[3] under controlled conditions of the metabolic ward, while they were on a low calcium diet. Analyses of food and of excreta were performed as previously described (*16*). Upon the completion of the balance studies a tracer dose of $Ca^{45}Cl_2$[4] (0.75–100 μc/kg body weight) was injected rapidly into the antecubital vein of the fasting patient. Blood samples were taken at short intervals from the femoral artery through a Cournand needle. Urine samples were obtained through an indwelling catheter at frequent intervals; the bladder was rinsed at the completion of each collection. Stool collections were made daily. On 3 days following the injection of radiocalcium, blood and urine were frequently assayed for Ca^{45}.

When the levels of Ca^{45} approximated an equilibrium in the serum and excreta, the sodium salt of EDTA was infused intravenously. Four g of the salt in 500 ml of 5% glucose in water was infused over a 6-hr period on 3 consecutive days.

Calcium 45 was determined in serum and urine on unashed samples and in stool on ashed samples. Occasionally, the presence of larger amounts of organic materials necessitated the dry-ashing of the urine. In one instance, bone obtained at autopsy was analyzed. The bone was first defatted by extraction with alcohol and ether and then dried to constant weight; weighed aliquots were ashed and analyzed for calcium, Ca^{45}, and other constituents (Table 1). Calcium 45 was precipitated as the calcium oxalate with 15 mg stable calcium added as carrier, and the precipitate was collected onto planchettes using especially constructed centrifuge tubes. The precipitate was air-dried and counted in a Q-gas flow counter. Correction for self-absorption was made by drying the precipitate to weight constancy and by using an absorption curve previously determined. A standard was prepared by adding a known amount of Ca^{45} to 1 ml of serum containing no activity and precipitating the Ca^{45} in the manner described. The lowest level of activity that could be determined was 0.0001 μc, with reproducibility of ±5%.

The information obtained on the metabolism of Ca^{45} by this technique is illustrated by the following example. A patient with multiple myeloma and primary hyperparathyroidism was given 57.6 μc of Ca^{45}. The metabolic balance studies disclosed a rapid rate of demineralization, with an average daily urinary calcium excretion of 334 mg. Fig. 1 shows the rapid disappearance of Ca^{45} from the circulation and its prompt excretion in the urine. Five min after the administration of Ca^{45} only 26% of the dose was found in the circulation when calculated on the basis of a plasma volume of 2500 ml.[5] Thereafter, the disappearance rate gradually decreased; 4.3% of the injected dose was present after 24 hr. The radiocalcium appeared rapidly in the urine. Within 30 min 0.64%, and after 24 hr 7.4%, of the injected dose had been excreted. The feces collected for 2 days after the injection contained 0.8% of the dose. Near

[3] Authorization for the use of tracer doses of Ca^{45} in cancer patients only was granted through the Subcommittee on Human Applications, by the Isotope Division of the U. S. Atomic Energy Commission.

[4] $Ca^{45}Cl_2$ in a solution containing 42 μc/mg calcium was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tenn.

[5] This rapid decline is attributable to equilibration of the injected dose with calcium in the extracellular fluid space.

equilibrium was reached in the blood 2 days after the injection. In 14 days a total of 40.4% of the administered dose was accounted for: 0.9% remained in the serum, 32.7% had been excreted in urine, and 6.8% in the stool. Thus, 34.6 μc was retained in the tissues.

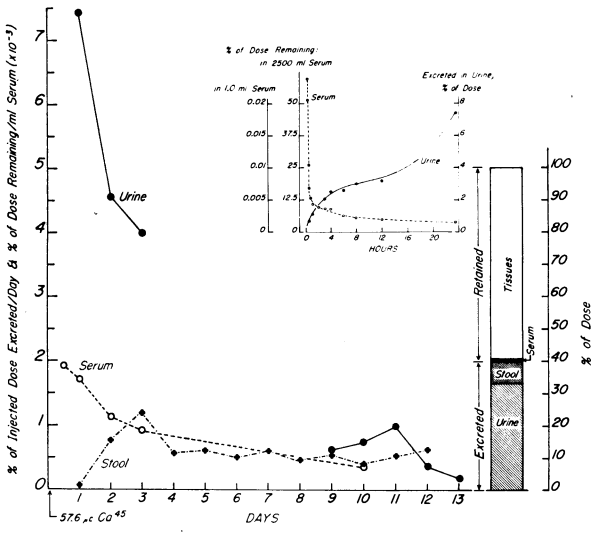

FIG. 1. Patient M. A. Serum levels, urine and fecal excretion of Ca^{45} following intravenous injection of 57.6 μc. Bar diagram shows the distribution of the dose at the time of death.

Fourteen days after the administration of radiocalcium the patient died of an intercurrent fulminating pneumonitis. Table 1 represents the analysis for Ca^{45} of samples of bone and of tumor and other tissues obtained at autopsy. Significant amounts of Ca^{45} were found in all bone samples and in the tumor. None was found in the liver and kidneys. The specific activity of 4 of the bone samples varied from 0.026 to 0.069 μc Ca^{45}/g Ca, but the concentration of Ca^{45} was considerably higher in the myelomatous tissue and in the adjacent spongiosa. The Ca^{45} content of the skeletal mass, estimated[6] on the basis of this bone analysis is in fair agreement with the amount of Ca^{45} retained in the body.

The removal of the administered Ca^{45} induced by the infusion of Na-EDTA[7] is illustrated in Fig. 2; 57.6 μc Ca^{45} was administered to a patient with prostatic cancer and osteoblastic metastases. On 3 days preceding the infusion of Na-EDTA, urine samples were analyzed daily at 6-hr intervals. In contrast to samples from the patient mentioned above, these showed an extremely low excretion of both stable and radioactive calcium. Fifteen days after the administration of the isotope, 4 g Na-EDTA was infused for 6 hr on 3 consecutive days. The daily urine collections, fractionated as above, showed the excretion of both calcium and Ca^{45} increased more than tenfold to 0.2% of the dose/day. The maximum excretion was noted during and shortly after the completion of the infusion. Twenty-four hr later, the excretions were only slightly above the preinfusion levels. There were no changes in the oxalate-precipitable calcium content of the serum.

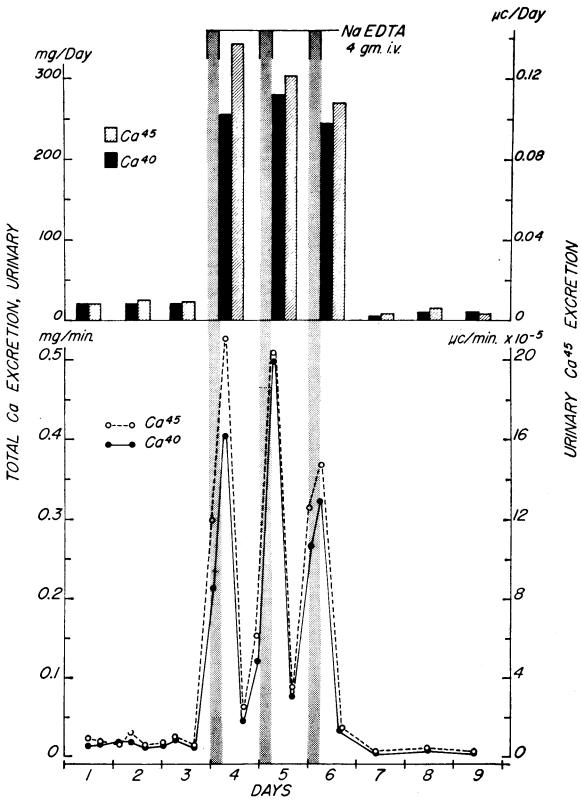

FIG. 2. Patient E. S. Urinary Ca^{40} and Ca^{45} excretions following intravenous infusion of 4 g/day of the sodium salt of ethylene diamine tetracetic acid.

In the experiment illustrated in Fig. 1 and Table 1, it was shown that Ca^{45} rapidly disappears from the blood stream, appears in the excreta, and is deposited in bone. The rate at which these processes take place may vary depending on the state of bone metabolism, and may represent a more useful indicator of skeletal activity than the criteria heretofore employed—such as metabolic balance studies and the calcium-tolerance test (*18*). Indeed, in a series of experiments, the sensitivity of such measurements was indicated (*19*). From the fraction of Ca^{45} recovered from the excreta following the intravenous and/or oral administration of Ca^{45}, the endogenous fecal calcium and the rate of utilization of ingested calcium may be estimated.

The excess excretion of Ca^{45} which follows the administration of Na-EDTA is shown in Fig. 2. Since the ratio of the excreted Ca^{45}/Ca remains unchanged,

[6] The total body calcium of a normal adult of 70 kg body weight is estimated to be 1160 g (*17*). The body calcium of this patient was estimated to be 976 g; the total amount of Ca^{45} retained in the skeleton, calculated on the basis of the analysis of 4 bone samples with the average specific activity of 0.05 μc/g calcium, was 48.8 μc; the actual amount retained was 34.6 μc.

[7] The EDTA was supplied by Riker's Laboratories, Inc., Los Angeles, Calif. The authors thank George L. Maison for his kind cooperation.

it may be assumed that either the radiocalcium was removed from a homogeneous pool or that its deposition and removal occurred in the same area. Induced excess excretion of Ca^{45} and of other metals was also found after the administration of Ca-Na-EDTA. These experiments will be reported in detail elsewhere (*19, 20*).

References

1. CAMPBELL, W., and GREENBERG, D. M. *Proc. Natl. Acad. Sci. U. S.*, **26**, 176 (1940).
2. COPP, D. H., *et al. Metabolic Interrelations,* 3rd Conf. New York: Josiah Macy, Jr. Fdn., 226 (1951).
3. COPP, D. H., AXELROD, D. T., and HAMILTON, T. G. *Am. J. Roentgenol. Radium Therapy,* **58**, 10 (1947).
4. GORDONOFF, T., and MINDER, W. *Intern. Z. Vitaminforsch.*, **23**, 16 (1951).
5. HAMILTON, T. G. *Radiology,* **49**, 325 (1947).
6. NORRIS, W. P., and KISIELSKI, W. *Cold Spring Harbor Symposia Quant. Biol.*, **13**, 164 (1948).
7. PECHER, C. *Univ. Calif. (Berkeley) Pub. Pharmacol.*, **12**, 117 (1942).
8. UNDERWOOD, E., FISCH, S., and HODGE, H. C. *Am. J. Physiol.*, **166**, 387 (1952).
9. THOMAS, R., *et al. Am. J. Physiol.* (in press).
10. POPOVICI, A., *et al. Proc. Soc. Exptl. Biol. Med.*, **74**, 415 (1950).
11. BAUER, R. O., *et al. Federation Proc.*, **11**, 321 (1952).
12. FOREMAN, H. *Rept. Univ. Calif. Radiation Lab.*, **683**, 38 (1952).
13. SPENCER, H., *et al. J. Clin. Invest.*, **31**, 1023 (1952).
14. RUBIN, M., GIGNAC, S., and POPOVICI, A. Spring meeting, Am. Chem. Soc., Milwaukee, Wis. (Apr. 1952).
15. FOREMAN, H. *Rept. Univ. Calif. Radiation Lab.*, **806**, 42 (1950).
16. LASZLO, D., *et al. J. Am. Med. Assoc.*, **148**, 1027 (1952).
17. SHERMAN, H. C. *Chemistry of Food and Nutrition,* 5th ed. New York: Macmillan, Chap. 12 (1937).
18. SCHILLING, A., and LASZLO, D. *Proc. Soc. Exptl. Biol. Med.*, **78**, 286 (1951).
19. HART, H., and LASZLO, D. *Science* (in press).
20. BELLIN, J., *et al.* In preparation.

Manuscript received August 8, 1952.

Agglutinin Linkage and Antibody Globulins

G. W. G. Bird

Blood Transfusion Department, Armed Forces Medical College, Poona, India

Linkage of anti-A and anti-B isoagglutinins in some O sera was demonstrated by Landsteiner and Witt (*1*). Similar linkage can be demonstrated in some cases between "nonspecific cold agglutinins" which occur normally in most sera and cold agglutinins acting specifically at higher temperatures. A typical example out of nine such sera is given below.

An A_2B serum containing the "extra" agglutinin alpha$_1$ (anti-A_1) was repeatedly absorbed in the cold with O cells. Considerable loss of the anti-A_1 resulted.

Titer before absorption:	256
Titer after absorption:	16

Thirty sera containing isoagglutinins were similarly absorbed in the cold with O cells. There was no loss of isoagglutinin titer in any of these sera.

Linkage may therefore exist between isoagglutinins and isoagglutinins, and between cold agglutinins and cold agglutinins, but not between isoagglutinins and cold agglutinins. *It appears that linkage can only take place between antibodies in the same globulin fraction.*

This would be supported by the biochemical findings of Cohn (*2*) who showed that isoagglutinins do not belong to the γ-globulin fraction, to which cold agglutinins are believed to belong (*3*). The absorption experiments of Crawford and Mollison (*4*) also indicate that isoagglutinins and cold agglutinins belong to different globulin fractions. By absorption of anti-globulin sera with sensitized red cells from cases of hemolytic anemia, or with cells that have been exposed to incomplete anti-Rh, or to normal cold antibodies, they were able to prepare sera which would no longer agglutinate the type of cells used for absorption but could agglutinate one or more of the other types.

The work of Crawford and Mollison also suggests that the auto-antibodies of hemolytic anemias differ from the normal cold auto-antibodies. This would be in keeping with the different mode of origin of the two types, which are thought to arise from auto-immunization and heterogenetic bacterial stimuli, respectively. It would be difficult to confirm this by agglutinin-linkage studies, because the various red cells used for absorption would act in a similar manner upon both these types of antibodies.

Wiener (*5*) has emphasized that there is a slender line of demarcation between isoagglutinins and cold agglutinins. This is undoubtedly so, but there does seem to be a difference in their behavior when absorbed in the cold with O cells.

References

1. LANDSTEINER, K., and WITT, D. H. *J. Immunol.*, **11**, 221 (1926).
2. COHN, E. J. *Ann. Internal Med.*, **26**, 341 (1947).
3. STATS, D., *et al. Proc. Soc. Exptl. Biol. Med.*, **53**, 188 (1943).
4. CRAWFORD, H., and MOLLISON, P. L. *Lancet,* **2**, 955 (1951).
5. WIENER, A. S. *Blood Groups and Transfusions.* Springfield: Thomas (1946).

Manuscript received August 22, 1952.

Motor Nerve Filament Block Produced by Botulinum Toxin[1]

Vernon B. Brooks

Department of Physiology, McGill University, Montreal, Canada

Burgen, Dickens, and Zatman (*1*) have shown that during the neuromuscular paralysis produced by botulinum toxin (type A) excitation of a motor nerve releases no acetylcholine (ACh) from its muscular terminals. This finding could be explained by assuming either that the toxin blocks motor nerve terminals just proximal to the site of ACh-release, or that it interferes with the process of release itself. Experiments were carried out to decide between the two alternatives, using the cat's gracilis muscle *in situ* (*2*) and the guinea pig's excised serratus muscle

[1] This work will be reported in full at a later date. The project was supported by a grant from the Defence Research Board of Canada.